Promotor:
Prof. dr. ir. Pieter Spanoghe
Department of Crop Protection, Laboratory of Crop Protection Chemistry
Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium

Members of the examination committee:
Prof. dr. ir. Nico Boon (Chairman)
Department of Biochemical and Microbial Technology
Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium

Prof. dr. ir. Guy Smagghe
Department of Crop Protection, Laboratory of Agrozoology
Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium

Prof. dr. ir. Sven Mangelinckx
Department of Sustainable Organic Chemistry and Technology
Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium

Prof. dr. ir. Dany Bylemans
General director Pcfruit vzw, Sint-Truiden, Belgium
Guest prof at the Department of Biosystems, Division of Crop Biotechnics
Faculty of Science and Technology, KU Leuven, Leuven, Belgium

Prof. dr. Pascal Van Der Voort
Department of Inorganic and Physical Chemistry, Center for Ordered Materials, Organometallics and Catalysis (COMOC)
Faculty of Science, Ghent University, Ghent, Belgium

Dean:
Prof. dr. ir. Marc Van Meirvenne

Rector:
Prof. dr. Anne De Paepe

- Caroline De Smedt -

Zeolites as potential plant protection agents

Thesis submitted in fulfilment of the requirements for the degree of Doctor (PhD) in
Applied Biological Sciences

Dutch translation of the title:

Zeolieten als potentiële gewasbeschermingsmiddelen

Cover picture:

Illustration of an apple treated with zeolite

De Smedt, C. (2016). Zeolites as potential plant protection agents. PhD Thesis. Ghent University, Belgium.

ISBN-number: 978-90-5989-897-4

In loving memory of my grandmother, Andrea Van Overloop (1934-2013).

DANKWOORD

Het laatste wat ik hier nu neerpen zal het eerste (en misschien wel het enige) zijn wat gelezen wordt. Daarom wil ik mijn brein nog een laatste keer pijnigen om alles uit de kast te halen.

Toen ik in 2005 aan de opleiding industrieel ingenieur begon zou ik eens goed hebben gelachen als iemand me zou zeggen dat ik ooit nog zou doctoreren. De voorbeeldige studente bleek dan ook niet in mij te zitten. Toch werd in mijn laatste jaar mijn aandacht getrokken voor de verschillende milieuaspecten. Of was het eerder de schrik om te gaan werken? De schrik om volwassen te worden en mijn studentenleven achter me te laten? Wat de echte reden ook mag geweest zijn, de roeping om voor bio-ingenieur te studeren kon ik niet negeren. Twee super jaren werden dit, waarbij ik opnieuw heel veel toffe mensen heb mogen leren kennen. Tot slot heb ik dan ook alles van de academische wereld mogen ervaren. Na de hogeschool en de universiteit kwam het mailtje van mijn toenmalige promotor met de vraag of ik al toekomstplannen had. DOCTOREREN!!! Wat een kans toch?

Beginnen doe ik dan ook graag met mijn promotor, die mij deze unieke kans heeft aangeboden en reeds van in het begin in mij geloofd heeft. Pieter, of beter Prof. dr. ir. Pieter Spanoghe, een oprechte dank u voor uw steun en vertrouwen. Zonder u had ik deze ervaring niet kunnen beleven.

Ook een speciaal woord van dank aan de voorzitter en leden van de examencommissie voor het kritisch nalezen van dit manuscript. Prof. Nico boon, Prof. Guy Smagghe, Prof. Sven Mangelinckx, Prof. Dany Bylemans en Prof. Van Der Voort, dankzij jullie bijdrage zijn we erin geslaagd om dit werk tot een hoger niveau te tillen. Bedankt voor jullie tijd en nuttige suggesties.

A very big thanks to all the partners of the ECOZEO project. It has been a real pleasure meeting all of you and learning from your knowledge and expertise. When you visit Belgium, we will definitely pass the bar ‘Dulle Griet’ again to taste some lovely Belgian beers.

Natuurlijk zouden deze 3.5 jaar maar eenzaam geweest zijn moest ik dit alleen hebben moeten beleven. Daarom wil ik mijn bureaucollega’s oprecht bedanken voor het lachen en gieren. De babbels, mopjes, dansjes en stoten zullen me altijd bijblijven. Davina en Kim, bedankt voor deze toffe momenten en toch ook wel voor de soms serieuze momenten, waarbij ik op jullie steun kon rekenen. Davina, wij hebben het begin en einde van ons doctoraat samen kunnen beleven. Bedankt voor de vele steun en hulp gedurende al die jaren.

Onze bureauburen, Michael en David, die ik toch wel vaak heb lastiggevallen met mijn vele vragen en verhalen mag ik dan zeker ook niet vergeten. Michael, bedankt voor de altijd opnieuw oppeppende babbel en de vele nuttige tips (vaak samen met een hapje en drankje).

Tevens wil ik alle andere collega's en ex-collega's aan het labo Fytofarmacie hartelijk bedanken; niet alleen voor de dagelijkse steun, maar ook voor de vele leuke en gezellige koffiepauzes. Claudine en Lilian, heel erg bedankt voor jullie hulp en steun in het labo. Bjorn, Elvina, Shirley, Seblework, Wumbei, Chachra, Edelbis, Ilse en Jean Pierre, bedankt voor alle mooie momenten waar jullie allemaal hebben toe bijgedragen. Tot slot wil ik ook graag Veerle bedanken voor haar ontzettend goede hulp met mijn Tuta's.

Niet te vergeten zijn mijn vrienden die me door deze jaren volop gesteund hebben. Mijn trouwe 'Hellestraatse gang' staat hierbij toch op nummer 1 ☺. Jullie zijn vrienden voor het leven en hebben altijd 100% rekening met mij gehouden. Ik kan me voorstellen dat ook jullie mijn labo-verhalen na al die jaren toch wel al eens beu waren, maar toch heb ik altijd op jullie steun en vele lieve woorden kunnen rekenen. Mijn middelbare vrienden Stephanie, Jorien, Laurens en Kristof, bedankt om deel uit te maken van mijn leven. Ook jullie hebben me hier altijd in gesteund, wat ik toch echt wel heel erg apprecieer. Industrieel ingenieurs Hannelore, Liesbet en Lotje, wat ben ik jullie zo dankbaar voor de zalige momenten die we tijdens onze studies beleefd hebben. Wat hebben wij toch wat afgelachen en jawel ook geweend ☺. Mijn bio-ingenieursmeisjes mag ik toch ook wel niet vergeten. Al snel werd ik in jullie groepje opgenomen, waarvoor ik jullie tot op vandaag heel dankbaar ben. Dankzij jullie heb ik vele mooie herinneringen aan deze toch wel korte periode en daar zullen we zeker nog een goede karmeliet op drinken. Joeri, jij bent een apart geval vermits jij mij al vanaf onze opleiding industrieel ingenieur bent gevolgd (of omgekeerd ☺). Daarom wil ik u aan u toch wel een apart dankwoordje richten aangezien jij mij echt op elk moment super hebt bijgestaan. Ik zal je hiervoor speciaal komen bezoeken in Singapore met een extra valies 'met je weet wel wat'. Badmintonners, merci voor al de zalige en ontspannende momenten (al dan niet afhankelijk of ik moet enkelen).

Het moet toch wel gezegd worden dat dit doctoraat er nooit was gekomen zonder de vele steun van het thuisfront. Woorden schieten te kort om mijn dankbaarheid te kunnen uiten. De afgelopen jaren waren met vallen en opstaan. We hebben de meest dierbare mensen in ons leven moeten laten gaan. Maar samen staan we sterk en kunnen wij alles aan. Ook daar ben ik jullie zo dankbaar voor. Ik heb geen nonkels en geen tantes, geen neefjes en geen nichtjes, maar wel een pracht van een broer die iedereen vervangt. Mijn grote broer, waar ik zo naar opkijk, bedankt voor het nalezen en verbeteren van dit werk. Het is eindelijk zover, we kunnen naar Engeland gaan om samen over het gras te lopen ☺.

Kjell, mijn engeltje, wat ben ik u toch ook zo dankbaar. In al die jaren heb jij naar mij geluisterd, heb jij mij gesteund en verdragen in al mijn stressmomenten. Weekends heb jij mij meegeholpen in het labo. Eveneens mocht je alle klusje opknappen waar ik op het einde geen tijd meer voor had. Ook zonder u had ik hier vandaag niet gestaan. Ik zie u zo graag ❤ !!!

Tot slot wil ik graag mijn ervaringen van de afgelopen maanden met jullie delen:

SAMENVATTING

Gewasbeschermingsmiddelen (GBM) hebben enerzijds nut voor de maatschappij, aangezien ze de mogelijkheid bieden om insecten, onkruid en ziekten te bestrijden en zo gewasopbrengsten aanzienlijk te verhogen. Dankzij het gebruik van GBM is de landbouwproductie de voorbije 30 jaar met 82% gestegen, waardoor de sterk toegenomen wereldbevolking van voedsel kon worden voorzien. Anderzijds kunnen GBM schadelijk zijn voor mens, dier en natuur omwille van hun (eco)toxiciteit, hun mogelijke bioaccumulerende eigenschappen en hun mogelijks hormoonverstorende effecten. Daarbij komt nog dat de verblijftijd van gewasbeschermingsmiddelen (persistentie) in het milieu kan variëren van enkele dagen tot meerdere jaren.

Het is dus belangrijk om het verbruik van conventionele chemische GBM te verminderen en meer uit te kijken naar alternatieve oplossingen met een lagere impact op mens en milieu. Om dit in praktijk te realiseren, werden reeds een aantal richtlijnen opgesteld door de Europese Gemeenschap. De EU richtlijn 91/414/EEC, betreffende het op de markt brengen van GBM, zorgde ervoor dat veel schadelijke werkzame stoffen van de markt verdwenen. De nieuwe EU richtlijn 2009/128/EC, ter verwezenlijking van een duurzaam gebruik van GBM, stelt dat niet-chemische methoden van gewasbescherming prioriteit moeten krijgen ten opzichte van chemische middelen.

Een mogelijk alternatief voor de conventionele chemische GBM is het gebruik van zeolieten. Zeolieten zijn microporeuze kristallijne mineralen die vanwege hun unieke poreuze eigenschappen een groot aantal toepassingen kennen. Ook in de landbouw worden ze al toegepast, namelijk als bodemverbeteraar, meststof, additief in veevoeder, dragermateriaal voor GBM en voor het verwijderen van geuren uit stallucht. De nadruk van dit onderzoek ligt meer op het gebruik van zeolieten tegen plantenziekten en insectenplagen. Aangezien zeolieten nog niet gebruikt worden voor dit doeleinde, wordt in **Hoofdstuk 1** een algemeen overzicht gegeven van de verschillende eigenschappen van zeolieten en waarom ze toegepast kunnen worden tegen ziekten en plagen.

Er zijn heel wat zeolieten op de markt, waaronder natuurlijke en synthetische zeolieten. Het is dan ook heel belangrijk om een doordachte keuze te maken welke zeoliet het meest geschikt is voor deze toepassing. In **Hoofdstuk 2** wordt zowel een theoretische als experimentele selectie van zeolieten beschreven.

De theoretische selectie was vooral gebaseerd op de eigenschappen van zeolieten. Met het oog op het gebruik van zeolieten in de landbouw werd vooral gekeken naar natuurlijke types. Ook de samenstelling is hierbij van groot belang. Enkel zeolieten die gebalanceerd werden met uitwisselbare kationen essentieel voor de plant, werden geselecteerd. Andere eigenschappen, waaronder adsorptievermogen van water, carrier vermogen van GBM of andere stoffen, werden ook in rekening gebracht. Hiervoor is het vooral belangrijk dat de zeolieten beschikken over grote ($6Å < \theta < 9Å$) tot extra grote ($\theta \geq 9Å$) poriën. Ook de hydrofobiciteit is van belang. Deze kan worden bepaald op basis van de Si/Al verhouding waarover de zeolieten beschikken. Hoe hoger deze waarden, des te hydrofober de zeoliet en des te beter de kans tot sorptie van materialen uit waterige oplossingen. Tot slot werd bij de finale theoretische selectie ook de toxiciteit en de beschikbaarheid op de markt in rekening gebracht. Op basis van deze selectie kwamen 6 natuurlijke zeoliet types in aanmerking om verder getest te worden, namelijk de zeolieten gecodeerd als BEA, CHA, FAU, HEU, MFI en MOR.

Het tweede deel van Hoofdstuk 2 beschrijft de experimentele selectie van de uit de theoretische selectie gevonden zeolieten. De beperkte beschikbaarheid van sommige natuurlijke zeolieten leidde er toe om voor die zeolieten een synthetische tegenhanger te kiezen. Eerst en vooral werd gekeken naar de mogelijkheid van de zeolieten om verschillende GBM en water te adsorberen. Slechts 2 types zeolieten, BEA en FAU, vertoonden goede resultaten. Vervolgens werden deze twee types zeolieten verder gescreend op vlak van fytotoxiciteit. Uiteindelijk werd van elk type slechts 1 zeoliet geselecteerd, die verder in het onderzoek gebruikt werden. Alle resultaten werden vergeleken met de zeoliet gebruikt in het ECO-ZEO project, zeoliet 4A (LTA), en de natuurlijke equivalent kaolin, in de gewasbescherming beter bekend als commercieel formuleringsproduct ‘Surround WP’.

Tot slot werd in dit hoofdstuk een case-study uitgewerkt waarbij de adsorptie eigenschappen van zeolieten vergeleken werden met die van andere adsorbents, waaronder metal organic frameworks (mofs), actieve kool en harsen. Ondanks de goede resultaten voor de zeolieten, is actief kool nog steeds het meest aangewezen materiaal om bestrijdingsmiddelen te sorberen.

Om de geselecteerde producten te kunnen toepassen, dienen ze eerst geformuleerd te worden. In **Hoofdstuk 3** wordt de zoektocht naar een goede formulering beschreven aan de hand van verschillende fysico-chemische testen. Drie formuleringstypen werden getest, waaronder een olie dispersie, een suspensieconcentraat en een spuitpoeder. De resultaten van de experimenten toonden aan dat de olie dispersie en het spuitpoeder de beste eigenschappen vertoonden. Na afwegen van een aantal voor- en nadelen werd geopteerd de geselecteerde zeolieten als spuitpoeder in formulering te brengen.

In de volgende hoofdstukken wordt het effect van de zeolieten op plantenziekten, insectenplagen getest, alsook de gunstige effecten van zeolieten voor de planten zelf.

In België zijn de appeloogst en tomatenteelt heel belangrijk. Daarom werd er voor geopteerd het effect van zeolieten te testen op de belangrijkste ziekten en plagen die hierop voorkomen. **Hoofdstuk 4** beschrijft die effecten op de plantenziekten *Venturia inaequalis (appel)* en *Botrytis cinerea* (tomaat). Het effect op groei-inhibitie werd zowel nagegaan op agarplaten als op de planten zelf. De test uitgevoerd op agarplaten resulteerde in een grotere groei-inhibitie in vergelijking met de test uitgevoerd op planten. Uit de bekomen resultaten kon ook afgeleid worden dat de schimmelgroei op de agarplaten voornamelijk werd geinhibiteerd door de geformuleerde zeolieten. Dit effect werd niet waargenomen bij de test uitgevoerd op de planten. Toch leidde deze conclusie tot de vraag of het wel degelijk de zeolieten zelf waren die voor het effect zorgden, of eerder de hulpstoffen aanwezig in de formulering.

Hulpstoffen zijn stoffen die meestal inactief zijn op zich, maar die de werking van de werkzame stof verbeteren door de fysische en chemische eigenschappen van het GBM mengsel te modificeren. Toch bleken de hulpstoffen, aanwezig in de geteste formuleringen, een actieve rol te spelen. Om dit aan te tonen, werd het effect van de verschillende hulpstoffen op groei-inhibitie nagegaan op agarplaten. Hieruit kon duidelijk worden besloten dat een aantal hulpstoffen effect hadden. Vervolgens werd een bacteriële genprofileringsassay uitgevoerd. Deze test bestond uit 14 transgene *E. coli* stammen, waarvan 13 stammen single copy chromosomale inserties bezaten van verschillende *promotor::lacZ* fusies. De promotors behoorden tot verschillende toxicologische responsmechanismen, zoals DNA schade, membraanschade, oxidatieve stress, osmotische stress en proteïne verstoring. Het *lacZ* gen dat hier als reportergen werd gebruikt, codeerde voor β-galactosidase en kon op een eenvoudige en snelle manier colorimetrisch worden gedetecteerd. Na het uitvoeren van deze test was het overduidelijk dat elke formulering over één of meerdere hulpstoffen beschikte die de werking van de zeoliet ten goede beïnvloedde. In een normale formulering van een GBM is dit effect van hulpstoffen op de werking niet opvallend. De hoge toepassingsdosis van de zeolieten, tot 20000 mg.l^{-1}, versterkte dit effect en verklaart deze observatie.

Hoofdstuk 5 behandelt het effect van zeolieten op insectenplagen, waaronder *Tuta absoluta* en *Leptinotarsa decemlineata*. Toxiciteitstesten werden op de eitjes van *T. absoluta* en op de larven van beide insecten uitgevoerd, alsook keuzetesten voor *T. absoluta*. De resultaten vertoonden weinig tot geen effect van de zeolieten. Enigszins werd toch groei-inhibitie opgemerkt bij *L. decemlineata*.

Verder werd nagegaan of de zeolieten positieve effecten uitoefenden op de behandelde plant zelf. **Hoofdstuk 6** beschrijft de mogelijke invloed van de zeolieten op de plantengroei, drooggewicht, wateropname en fotosynthese. Ondanks het feit dat weinig tot geen effecten waargenomen werden voor plantengroei, drooggewicht en wateropname, sprong de fotosynthese er wel bovenuit. Dit was hoofdzakelijk het geval bij de behandelde appelbomen, waarbij een duidelijke trend waargenomen kon worden. Na de behandeling van de appelbomen met de zeolieten werd een stijging in fotosynthese waargenomen die na twee weken terug begon af te nemen.

Als besluit kan gesteld worden dat de zeolieten toch een aantal positieve kenmerken vertoonden. Vooral op vlak van adsorptie, schimmelbestrijding en fotosynthese. Deze vaststellingen worden uitgebreid besproken in **Hoofdstuk 7**, alsook enkele voor- en nadelen van het gebruik van zeolieten in de landbouw, gecombineerd met enkele suggesties voor verder onderzoek.

SUMMARY

Plant protection products (PPPs) appear to have beneficial effects for the society, since they offer the ability to control insects, weeds and diseases, and as such induced a significant increase in crop yield. Due to PPPs, agricultural production has been increased by 82% over the past 30 years, which allowed foreseeing enough food for the highly increased world population. However, PPPs can be harmful to human, animal and the environment due to their (eco)toxicity and their bioaccumulative and hormone disrupting properties. In addition, the residence time of PPPs (persistence) in the environment varies from a few days to several years.

Therefore, it becomes really important to reduce the use of conventional chemical PPPs and to look for alternative solutions having a lower impact on humans and the environment. In order to achieve this, some guidelines have been drawn up by the European Community. The EU directive 91/414/EEC, concerning the availability of PPPs on the market, has resulted in the withdrawal of many harmful active substances from the market. The new EU directive 2009/128/EC, to achieve a sustainable use of PPPs, states that non-chemical methods of plant protection should be given priority over chemical agents.

A possible alternative to conventional chemical PPPs is the use of zeolites. Zeolites are microporous, crystalline minerals and are used for a great number of applications due to their unique porous properties. Zeolites are already used in agriculture, e.g. as a soil improver, fertilizer, animal feed additive, carrier material for PPPs and for the removal of bad odours in animal stables. The focus of this research is more on the use of zeolites against plant diseases and insect pests. Since zeolites are not used for this purpose yet, **Chapter 1** presents a general overview of the different properties of zeolites and why they can be used against diseases and pests.

There are many zeolites on the market, including natural and synthetic zeolites. It is therefore very important to determine which zeolite is the most suitable for this application. **Chapter 2** describes both a theoretical and experimental selection of zeolites.

The theoretical selection was based on the properties of zeolites. Considering the use of zeolites in agriculture, natural zeolite types were considered as the main focus. Next to that, the composition is of great importance. Only zeolites were selected that have been balanced by exchangeable cations essential to plants. Other properties, such as the adsorption of water, carrier effect for PPPs or other substances, were also taken into account. Therefore, zeolites having large ($6Å < \theta < 9Å$) to extra-large ($\theta \geq 9Å$) pores were considered. The hydrophobicity

of zeolites is also important. This property can be determined based on the Si/Al ratio of the zeolites. Higher Si/Al ratio provides more hydrophobic zeolites with improved probability to adsorb materials from aqueous solutions. Finally, the toxicity and the availability of zeolites on the market were also taken into account in the theoretical selection. Based on this selection, six natural zeolite types were considered for further testing, i.e. the zeolites coded as BEA, CHA, FAU, HEU, MFI and MOR.

The second part of Chapter 2 describes the experimental selection of the theoretically selected zeolites. However, the limited availability of some natural zeolites eventually led to the selection of their synthetic counterpart. As primary experimental selection criterion, the capability of zeolites to adsorb different PPPs and water was considered. Only two types of zeolites, i.e. BEA and FAU, showed good adsorption results. Next, these two types of zeolites were further evaluated with respect to their phytotoxicity. Eventually, only one zeolite of each zeolite type was selected and used during this study. All results were compared with the zeolite used in the ECO-ZEO project, zeolite 4A (LTA), and the natural equivalent kaolin, better known as the commercial plant protection product 'Surround WP'.

The final part of Chapter 2 describes a case study comparing the adsorption properties of zeolites with those of other adsorbents, including metal organic frameworks (MOFs), activated carbon and resins. Despite the good results of the zeolites, activated carbon is still found to be the most preferred PPP adsorbing material.

In order to apply the selected zeolites, they firstly need to be formulated. **Chapter 3** describes the exploration for a suitable formulation by means of different physicochemical tests. Three formulation types were tested, i.e. an oil dispersion, a suspension concentrate and a wettable powder. As a result of these tests, the oil dispersion and the wettable powder formulations provided the best characteristics. After consideration of advantages and disadvantages, a wettable powder formulation of the selected zeolites was used.

In the following chapters, the effect of zeolites on plant diseases and insect pests were tested, as well as the beneficial effects of zeolites for the plants themselves.

In Belgium, the apple and tomato harvest are very important. Therefore, it was decided to test the effect of zeolites on the main pests and diseases of these crops. **Chapter 4** describes the effects on the plant diseases *Venturia inaequalis* (apple) and *Botrytis cinerea* (tomato). The effect on growth inhibition was examined on agar plates as well as on the plants themselves. A higher growth inhibition was noticed for the test on agar plates compared to the test on the plants. Also, it could be deduced that the fungal growth on the agar plates was mainly inhibited by the formulated zeolites. This effect however was not observed in the test carried out on the plants.

Considering the obtained results, one could question whether the effect was caused by the zeolites themselves, or by the adjuvants present in the formulation. Adjuvants are usually considered to be inactive as PPP, but improve the activity of the active substance by modifying the physical and chemical properties of the PPP mixture. However, the adjuvants present in the tested formulations did seem to play an active role. In order to verify this, the effect of these adjuvants on the growth inhibition was examined on agar plates, from which it became clear that some of them showed an effect. Subsequently, a bacterial gene profiling assay was performed using 14 transgenic *E. coli* strains, including 13 strains with single copy chromosomal inserts of different promoter::LacZ fusions. These promoters belonged to different toxicological response mechanisms, e.g. DNA damage, membrane damage, oxidative stress, osmotic stress and protein denaturation. The lacZ gene, used as a reporter gene, encoded a β-galactosidase and could be easily and fastly detected using a colorimetric assay. The results of this test illustrated clearly that each formulation contained one or more adjuvants influencing the activity of the zeolite. In a typical formulation of a PPP, this effect of adjuvants is not noticeable. The high application dose of the zeolites in this study, i.e. up to 20000 mg.l^{-1}, enhanced this effect and explains this observation.

Chapter 5 deals with the effect of zeolites on insect pests, including *Tuta absoluta* and *Leptinotarsa decemlineata*. Toxicity tests were performed on the eggs of *T. absoluta* and on the larvae of both insects, as well as choice tests for *T. absoluta*. The results showed little or no effect of the zeolites. Nevertheless, some growth inhibition was noted for *L. decemlineata*.

Subsequently, tests were performed to investigate potential beneficial effects of zeolites on the treated plant itself. **Chapter 6** describes the possible effect of zeolites on plant growth, dry weight, water uptake and photosynthesis. Despite the fact that little or no effects were observed for plant growth, water uptake and dry weight, some effects were noticed for the photosynthesis. A clear trend could mainly be observed for the treated apple trees, i.e. an increase of photosynthesis was observed after treating the apple trees with the zeolites, followed by a decrease after two weeks.

As conclusion, zeolites did show some positive properties, in particular with respect to adsorption, fungicides and photosynthesis. These findings are discussed in **Chapter 7**, as well as some advantages and disadvantages of the use of zeolites in agriculture, combined with some suggestions for further research.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

Abbreviation	**Description**
1D	one-dimensional
BBU	basic building unit
BDDT	Brunauer, Deming, Deming and Teller
BET	Brunauer-Emmet-Teller
BGPA	bacterial gene profile assay
CAGR	compound annual growth rate
CC/AGW	Climate Change/Anthropogenic Global Warming
CEC	cation exchange capacity
COF	covalent organic framework
CPB	Colorado potato beetle
CpGV	Cydia pomonella granulovirus
DAD	diode array detection
DDT	dichlorodiphenyltrichloroethane
DLP	double log polynomial
DNA	deoxyribonucleic acid
EU	European Union
FDA	Food and Drug Administration
FTC	framework type code
GAC	granular activated arbon
GRAS	Generally Recognised As Safe
GUS	groundwater ubiquity score
HPLC	high performance liquid chromatography
IPM	integrated pest management
IUPAC	International Union of Pure and Applied Chemistry
IZA	International Zeolite Association
LCD	largest cavity diameter
LED	light-emitting diode
LHC	light harvesting complex
LSF	liquid-state fermentation

Abbreviation	**Description**
MOF	metal organic framework
NASA	National Aeronautics and Space Administration
OD	oil dispersion
ONP	O-nitrophenol
ONPG	O-nitrophenyl-β-D-galactopyranoside
PAC	powder activated carbon
PAF	porous aromatic framework
PAR	photosynthetically active radiation
PBU	primary building unit
PDA	potato dextrose agar
PLD	pore limiting diameter
POEA	polyethoxylated alkylamine
PPP	plant protection product
PRI	pesticide risk indicator
PSII	photosystem II
PVDF	polyvinylidene fluoride
RNA	ribonucleic acid
RuBisCo	ribulose-1,5-bifosfaat carboxylase oxygenase
SBU	secondary building unit
SC	suspension concentrate
SEM	scanning electron microscope
SME	small and medium sized enterprises
SSF	solid-state fermentation
TMDS	1,1,3,3-tetramethyldisilazane
TRL	Technology Readiness Levels
UV	ultraviolet
UVB	ultraviolet B
UVC	ultraviolet C
WP	wettable powder
XRD	X-ray powder diffraction
XRF	X-ray fluorescence

LIST OF SYMBOLS

Symbol	Description	Unit
A	mean seed germination or root length of the control	mm
A_n	net photosynthesis	μmol $CO_2.m^{-2}.s^{-1}$
A_T	Temkin isotherm equilibrium binding constant	$l.g^{-1}$
a_w	water activity	-
B	mean seed germination or root length of the test sample	mm
b_0-b_3	empirical constants	-
B_D	Dubinin-Radushkevich isotherm constant	$mol^2.J^{-2}$
B_T	heat of adsorption	$J.mol^{-1}$
b_T	Temkin isotherm constant	-
C_e	equilibrium plant protection product concentration	$mg.l^{-1}$
C_f	final plant protection product concentration	$mg.l^{-1}$
C_i	initial plant protection product concentration	$mg.l^{-1}$
C_L	larval weight gain of the larvae in the control treatment	g
CMC	critical micelle concentration	$mg.l^{-1}$
C_r	colony radius of the control	cm
C_s	plant protection product saturation concentration	$mg.l^{-1}$
DI	disease incidence	%
DLP_m	double log polynomial moisture	g.g $solids^{-1}$
DS	disease severity	%
D_w	dry weight	G
E	transpiration rate	mmol $H_2O.m^{-2}.s^{-1}$
EC_{50}	effective concentration which causes 50% growth inhibition	$mg.l^{-1}$
EC_{90}	effective concentration which causes 90% growth inhibition	$mg.l^{-1}$
E_D	mean free energy of adsorption	$kJ.mol^{-1}$
ED	effective dose	$mg.l^{-1}$
ε	Dubinin-Radushkevich isotherm constant	-
F	force	$mN.m^{-1}$

Symbol	Description	Unit
F_0'	minimal fluorescence level	µmol photons.m^{-2}.s^{-1}
F_m'	maximum light fluorescence level	µmol photons.m^{-2}.s^{-1}
F_v'	actual variable fluorescence	µmol photons.m^{-2}.s^{-1}
F_w	fresh weight	G
g	gravitational acceleration	m.s^{-2}
G_c	seed germination percentage of the control	%
GI	germination index	%
G_s	seed germination percentage of the test sample	%
g_s	stomatal conductance	mol H_2O.m^{-2}.s^{-1}
I	inhibition of fungal growth	%
IG	inhibition of seed germination	%
IR	inhibition of root growth	%
k_1	First-order adsorption rate constant	hour^{-1}
k_2	Second-order adsorption rate constant	hour^{-1}
K_F	Freundlich isotherm constant	mg.g^{-1}
K_{foc}	adsorption coefficient	mg.l^{-1}
K_L	Langmuir isotherm constant	l.g^{-1}
K_{oc}	organic carbon partition coefficient	l.kg^{-1}
K_{ow}	octanol-water partition coefficient	-
L_c	root elongation of the control	Mm
L_s	root elongation of the test sample	Mm
m	weight	G
MM	molar weight	g.mol^{-1}
n	Freundlich exponent	-
N_c	number of eggs laid on the control plant	-
n_i	number of leaves in infection class i	-
N_I	number of infected leaves	-
N_t	number of eggs laid on the treated plant	-
OAI	oviposition activity index	-
OD	optical density	-
P	morality of the treated insects	%
P_0	mortality of the insects in the control treatment	%
q_D	maximum sorption capacity	mg.g^{-1}

Symbol	Description	Unit
q_e	adsorbed concentration at equilibrium time	$mg.g^{-1}$
$q_{e,calc}$	calculated adsorbed concentration at equilibrium time	$mg.g^{-1}$
$q_{e,exp}$	experimental adsorbed concentration at equilibrium time	$mg.g^{-1}$
q_m	maximum sorption capacity	$mg.g^{-1}$
q_t	adsorbed concentration at time t	$mg.g^{-1}$
R	ideal gas constant	$J.K^{-1}.mol^{-1}$
R_1	droplet radius of the diluted formulation	Mm
R^2	determination coefficient	-
R_2	droplet radius of distilled water	Mm
RH	relative humidity	%
R_L	Langmuir isotherm separation factor	-
S_0	Energy level for an electron at ground state	eV
S_1	Energy level for an electron at the excited state of red light	eV
S_2	Energy level for an electron at the excited state of blue light	eV
S_{BET}	Specific surface area by the BET method	$m^2.g^{-1}$
S_{water}	water solubility	$mg.l^{-1}$
T	absolute temperature	K
T_{air}	air temperature	°C
T_l	total numer of leaves	-
T_L	larval weight gain of the treated larvae	G
T_{leaf}	leaf temperature	°C
T_r	colony radius of the test plate	Cm
V	Volume	L
v	highest infection class value	-
VPD	vapour pressure deficit	kPa
WHC	water holding capacity	-
W_{plate}	weight of the Wilhelmy plate	Kg
$WUE_{biomass}$	water use efficiency based on the biomass	$g.kg^{-1}$
$WUE_{instantaneous}$	water use efficiency based on the transpiration rate	$\mu mol\ CO_2.mmol\ H_2O^{-1}$
$WUE_{intrinsic}$	water use efficiency based on the stomatal conductance	$\mu mol\ CO_2.mol\ H_2O^{-1}$
α	contact angle	°

Symbol	Description	Unit
γ	surface tension	$N.m^{-1}$
θ	zeolite pore size	Å

GENERAL INTRODUCTION AND THESIS OUTLINE

This general introduction describes the framework in which this doctoral dissertation was conducted. It provides background information about the needs and the solutions in agriculture and how the ECO-ZEO project aimed to progress beyond the state-of-the art.

1 RATIONALE

Plant protection products (PPPs) are applied to manage and prevent diseases, pests, weeds, etc. Due to PPPs, agricultural production has been increased by 82% over the past 30 years (UNFPA, 2007). However, the use of PPPs have caused numerous unforeseen problems, e.g. poisoning of applicators, farmworkers and consumers, animal mortality, disruption of natural biological control and pollination, groundwater contamination and development of resistance of pests to PPPs (Isman, 2006).

To improve the sustainability of agriculture and to avoid resistance development against the receding number of active ingredients remaining on the market, alternatives are needed. This research is based on the use of zeolites as possible alternative.

Zeolites represent a broad range of microporous, crystalline aluminosilicates of natural or synthetic origin. Generally, their structure can be considered as an inorganic polymer built from $[SiO_4]^{4-}$ and $[AlO_4]^{5-}$ tetrahedra (primary building units, PBUs) linked by the sharing of all oxygen atoms. A pure silica (SiO_2) solid framework is uncharged. When some of the Si^{4+} in the silica framework is replaced by Al^{3+}, the +3 charge on the aluminium makes the framework negatively charged, which is compensated by the presence of extra-framework cations (counterions), located together with water, to keep the overall framework neutral (Payra and Dutta, 2003). Connecting small units of several tetrahedral (up to 16) provides the formation of secondary building units (SBUs), i.e. chain- or layer-like units. Subsequently, more complex building units can be formed, i.e. characteristic subunits and cages/cavities that recur in several framework types.

2 PROBLEM STATEMENT

The world population is continuously increasing. Since 1950 till 2000, there has been an increase from 2.5 billion to 6.1 billion and it is expected that by the year 2050, the world population will be about 9.1 billion. Presently, the world population is rising with an annual rate of 1.2%, i.e. 77 million people per year (Carvalho, 2006). However, this global population daily needs food to survive. A significant progress has been made since 1960 towards improving the nutrition and securing the food for human beings. The world gross agricultural production has grown more rapidly than the world population, with an average positive increase in the production of food per capita (Klassen, 1995).

Agriculture is not only crucial for food but also for fodder, fibre, fuel and pharmaceutical production. Half of the EU's land area is devoted to farming activities. As a result, the agricultural sector is responsible for a large share of pollution and natural resource depletion. Recent regulations at European level stress the necessity to improve the efficiency in water consumption, reduce the threat of pests/diseases and fertiliser leachates, prevent the increase in soil salinity and support agrobiodiversity. A bundle of policies, regulations and initiatives are being promoted in order to upgrade the sustainability and safety standards. Due to pressure coming from consumers and legislation, European farmers will also have to become more eco-efficient, reduce the negative impact on the environment and deliver healthier agricultural products. Considering all the above, there is a strong necessity for new, environmentally friendly and efficient products and systems that play a leading role in the current environmental, economic and societal context, but also in mitigation strategies for Climate Change/Anthropogenic Global Warming (CC/AGW) scenarios (ECOZEO, 2012).

3 THE SOLUTION (ECO-ZEO PROJECT)

The acronym ECO-ZEO stands for developing a pool of novel and eco-efficient applications of zeolites for the agricultural sector. The ECO-ZEO project started in March 2012 and ended in March 2016. It was supported by the Seventh Framework Programme of the European Commission and aimed at the development of a new pool of green PPPs (efficient, environmentally friendly and sustainable). Such green products deliver a wide range of beneficial effects in terms of water efficiency, control of pests and diseases, reduction in PPP use, increase of crop yield and tolerance to abiotic stress. The novel compositions will be applied as a suspension on the surface of the leaves and fruits, thus forming a coating that will trigger physiological mechanisms in the plant as well as effects against the stressors, and as such leading to the claimed benefits.

The multidisciplinary consortium (**Figure 1**) of ECO-ZEO was composed of 10 partners from 8 different countries (European Union and associated countries). An alliance was made between 4 research organizations, 4 small and medium sized agro-biotech enterprises (SMEs), PPP industry and the important contribution of end users represented by an association of fruits and vegetables producers.

Figure 1. Geographic view of the ECO-ZEO consortium.

4 THESIS OBJECTIVES AND THESIS OUTLINE

According to the European directive 2009/128/EC, which establishes a framework to achieve a sustainable use of PPPs, farmers must reduce the risks and impacts of PPP use on the environment and human health. This directive promotes the use of integrated pest management and different techniques such as non-chemical alternatives.

Nowadays, growers use kaolin to help control damage to fruits and vegetables from insects, mites, fungi, bacteria, and to protect against sunburn and heat stress. This naturally occurring clay has been used for many years in foods, cosmetics, and other materials. Based on widespread usage and extensive toxicity studies, kaolin is considered safe for humans and the environment.

Zeolites are just like clay minerals composed of aluminosilicate, but differ in their crystal structure. Therefore, zeolites may also play a beneficial role in agricultural applications. This leads to the overall question of this study: “Can zeolites be used as potential plant protection agents?” In order to narrow down this main research question, different hypotheses (H) and research questions (RQ) were formulated.

I. Literature review

The research started from hypothesis 1 (**H1**): "Zeolites exhibit certain properties that can be useful in crop protection". In order to check H1, **Chapter 1** investigates the available literature with the aim to answer following research questions (RQ):

RQ1.1: Are zeolites already used in agriculture?
RQ1.2: Can zeolites be used as plant protection agents for controlling pests and diseases?
RQ1.3: What are the possible effects after using zeolites as a coating polymer?
    RQ1.3.1: on plants
    RQ1.3.2: on pathogen/insect behaviour
    RQ1.3.3: on soils
RQ1.4: Can zeolites be used as carriers?
    RQ1.4.1: of plant protection products
    RQ1.4.2: of semiochemicals or plant extracts
    RQ1.4.3: of microorganisms
RQ1.5: Is there a risk of toxicity due to the use of zeolites as a coating polymer?
    RQ1.5.1: to plants
    RQ1.5.2: to the environment
    RQ1.5.3: to humans

II. Product selection

The ECO-ZEO project focuses on the use of Zeolite 4A, the synthetic counterpart of kaolin. However, there are many different zeolites on the market. At present, there are 231 zeolite frameworks identified and 46 naturally occurring zeolite frameworks are known. As a result of the large number of zeolites, it can be hypothesized (**H2**) that: "Next to Zeolite 4A, there are more zeolites on the market that can be used in plant protection". **Chapter 2** verifies this hypothesis by first answering following research question:

RQ2.1: What structural features are required in order to use zeolites in agriculture?

Part A of Chapter 2, answering this question, focuses on the theoretical selection of natural zeolites that can be used in agriculture, taking into account different properties like their extra-framework cations, pore size, hydrophilic character and toxicity.

After a first selection of zeolite structures in Part A of Chapter 2, new research questions were put forward as beneficial to plant protection:

RQ2.2: Can these zeolites be used as carrier of active ingredients?
RQ2.3: What is the water sorption capacity of these zeolites?
RQ2.4: What is the risk that these zeolites are phytotoxic?

Part B of Chapter 2 gives an answer on these research questions based on experimental results. The performed tests take into account the adsorption and water holding capacity of the zeolites, as well as their phytotoxicity.

Finally, in addition to this, a case study was performed in order to compare the adsorption properties of zeolites to those of other adsorbents, based on:

RQ2.5: Are zeolites better adsorbents in comparison with other adsorbents such as activated carbon, metal organic frameworks (MOFs) and resins?

III. Product development

In order to confirm the theoretical insights acquired for zeolites and to further increase our understanding of the role of these zeolites in agriculture, the necessity for experimental validation arose. Because a crop protection product is rarely used or applied in its pure form, the technical grade compound was formulated. This hypothesis (**H3)**: "Zeolites cannot be used in their pure form in agriculture", led to the first research question:

RQ3.1: Which formulation type exhibits the best physicochemical properties?

Galenika Fitofarmacije, an industrial partner in the ECO-ZEO project, was in charge of the product development. This company developed three different types of formulations of Zeolite 4A in function of the project, i.e. oil dispersion, suspension concentrate and wettable powder.

Based on a few relevant physicochemical properties, i.e. evaporation, surface tension, spreading, deposition and rainfastness, the most appropriate formulation for this research was selected in **Chapter 3**. Based on this outcome, Galenika Fitofarmacije also developed formulations of the zeolites selected in Chapter 2.

IV. Product application

Chapter 4, **Chapter 5** and **Chapter 6** focus entirely on the biological effects of the selected zeolites (Chapter 2) and their formulations (Chapter 3) against fungi, pests and plants, respectively. The selection of target crops in this research (apple and tomato) was based on their water demand, vulnerability to pests, higher added value and extensive production. The selected pests and diseases, identified in the project, were *Venturia inaequalis and Cydia pomonella* for apple trees and, *Botrytis cinerea and Tuta absoluta* for tomato plants. In order to compare the observed results from this PhD study with the ECO-ZEO project results, it was decided to test the same diseases and pests.

In **Chapter 4**, hypothesis 4 (**H4**) was tested: "The selected zeolites have fungicidal activity". An answer is presented on the following research question in Part A of Chapter 4:

RQ4.1: Do zeolites exhibit similar fungicidal activity on agar plates and plants?

In order to answer this question, inhibition tests together with disease incidence and severity assessments of *Venturia inaequalis* (Apple) and *Botrytis cinerea* (Tomato) were carried out. These results, however, have led to a new research question, which is discussed in Part B of Chapter 4:

RQ4.2: Was the observed antifungal activity caused by the zeolites themselves, or by the adjuvants present in the formulation?

This question was answered by comparing test results of two different tests. First, inhibition tests against *Venturia inaequalis* (Apple) and *Botrytis cinerea* (Tomato) were performed. This consisted of the same tests as carried out for the zeolites, but now testing the different adjuvants that were present in the zeolite formulations. Additionally, a bacterial gene profiling assay was performed in order to evaluate these adjuvants at the toxic mode of action level, i.e. DNA damage, oxidative stress, membrane damage and general cell lesions.

Additionally, it can be hypothesized that zeolites also have other biological effects. In **Chapter 5**, hypothesis (**H5**) was tested: "The selected zeolites have insecticidal activity".

Perhaps the most widely recognized type of insect damage to plants is chewing insect damage. Chewing damage is caused by insects with mouth parts consisting of two opposing mandibles. Insects with chewing mouth parts are responsible for ragged leaves, foliage consumption, and mining in leaves, stems and trunks of plants.

In order to verify this hypothesis, toxicity tests were carried out against *Tuta absoluta* (Tomato). Since the cultivation of *Cydia pomonella* (Apple) failed, a small bioassay on *Leptinotarsa decemlineata* was provided. These chewing insects led to the following research questions:

RQ5.1: Do these zeolites desiccate *Tuta absoluta* eggs?
RQ5.2: Do these zeolites repel *Tuta absoluta females*?
RQ5.3: Do these zeolites irritate *Leptinotarsa decemlineata* larvae?

Subsequently, **Chapter 6** evaluates the effect of zeolites on apple trees and tomato plants. The hypothesis (**H6**): “The selected zeolites have beneficial effects on plants” led to following research questions:

RQ6.1: Do these zeolites have an effect on plant growth?
RQ6.2: Do these zeolites have an effect on plant biomass production?
RQ6.3: Do these zeolites have an effect on water use of plants?
RQ6.4: Do these zeolites have an effect on plant photosynthesis?

All these research questions were answered by performing experiments on apple trees and tomato plants.

Conclusions

Finally, in **Chapter 7**, general conclusions and future perspectives are provided based on the results obtained in this study. A graphical overview of the different research chapters is presented in **Figure 2**.

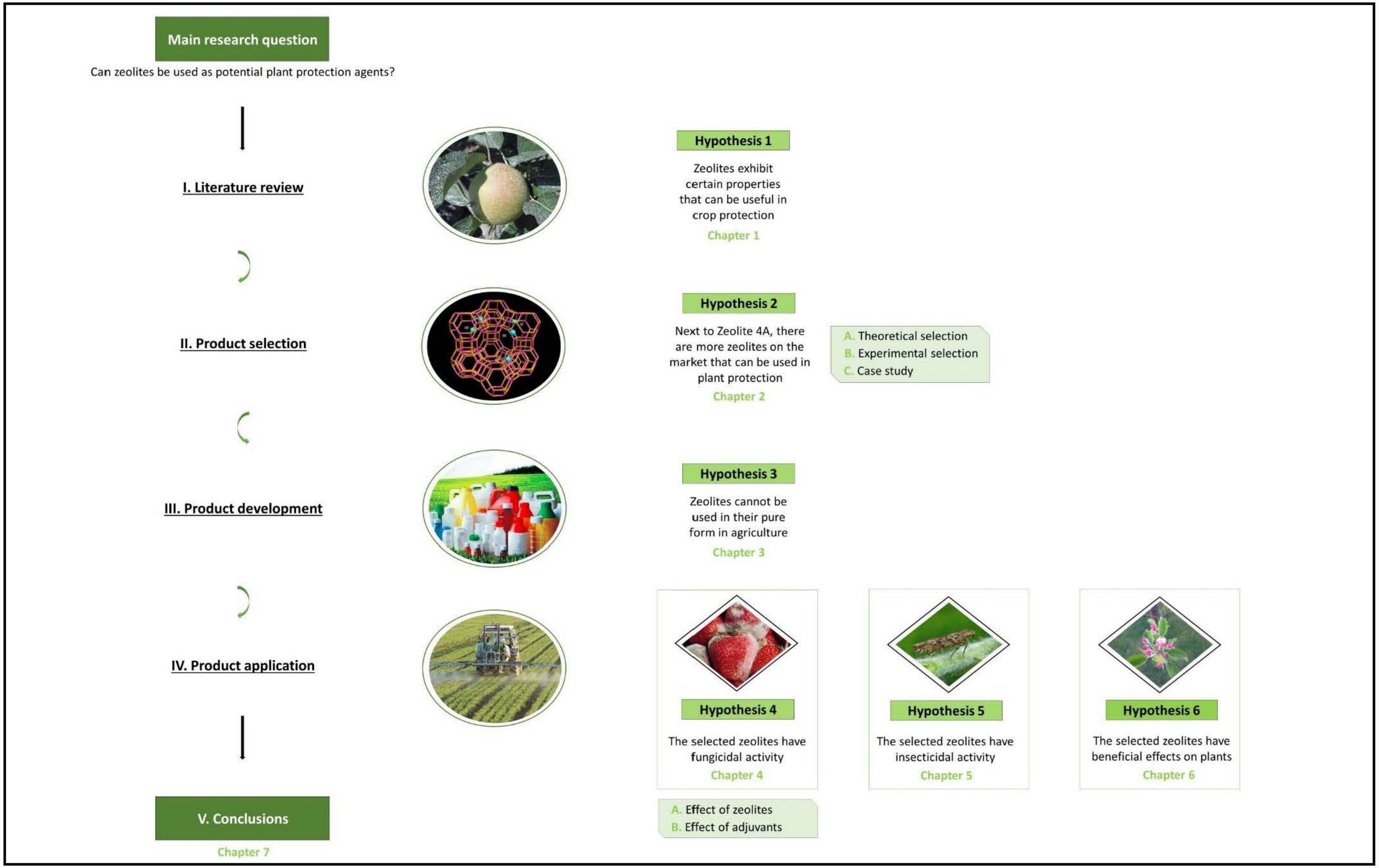
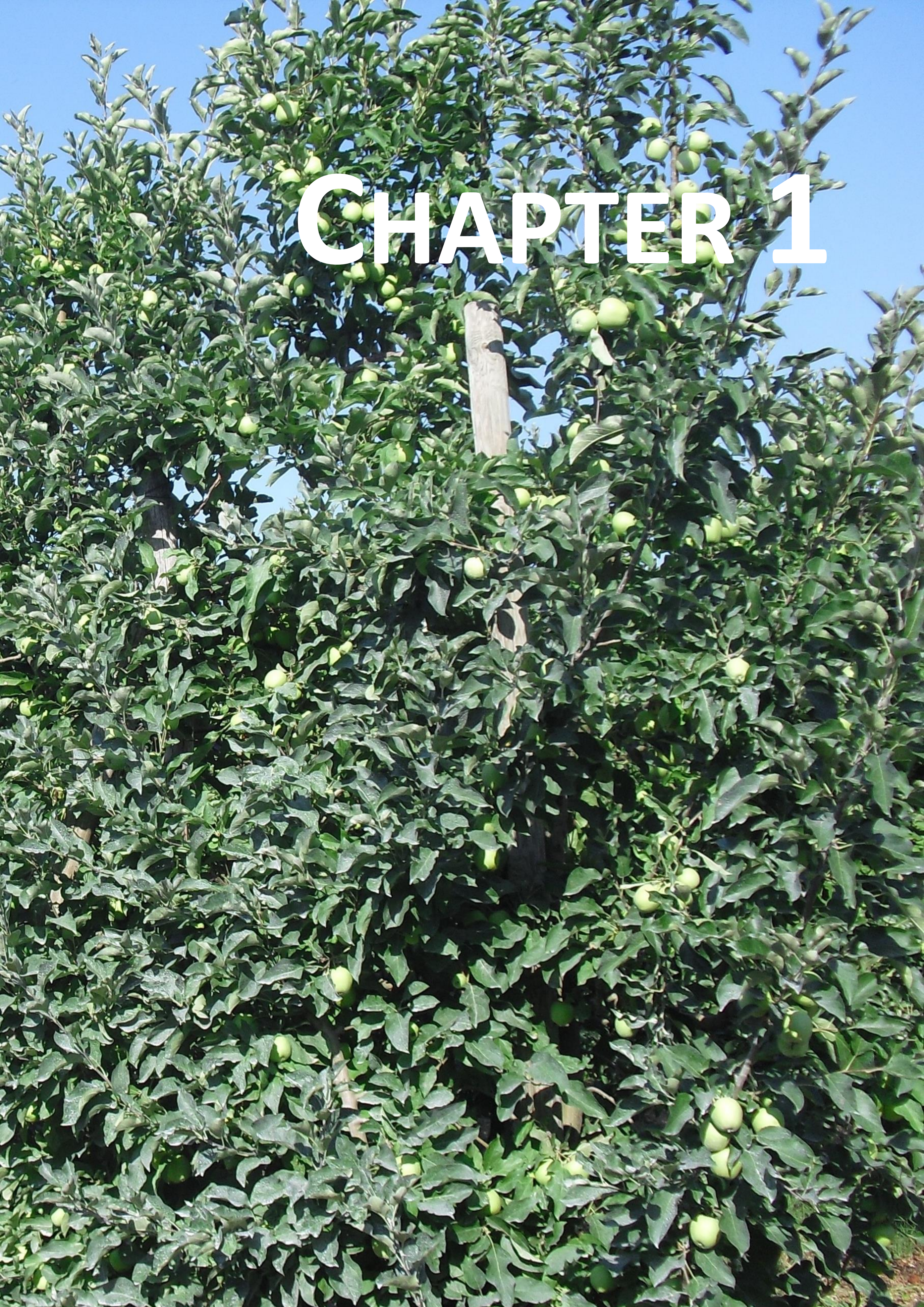


Figure 2. Overview of the different research chapters.

> *"Live as if you were to die tomorrow.*
> *Learn as if you were to live forever."*
> **-Mahatma Gandhi-**

CHAPTER 1



Chapter 1

LITERATURE REVIEW

In the following chapter, an overview is presented of the basic properties of zeolites and their potential role in control of agricultural insect pests and plant diseases.

1 INTRODUCTION

The usage of PPPs to manage diseases, pests, weeds, etc., has become a common practice around the world (Mogul et al., 1996; Ravier et al., 2005; Chevillard et al., 2012). Environmental pollution and ecological issues, however, make it necessary to look for alternatives, such as other organic agrochemicals or the controlled release of PPPs, including a reduction in the amount of active ingredients.

Current research is based on the use of nanoparticles and their potential role in agriculture to reduce the negative impacts of environmental stresses on crop plants, to suppress diseases and to protect crops from insect pests (Kahn and Damicone, 2008). This approach has been applied by making use of dust applications. However, such material has some drawbacks - see the discussion of kaolin-based particle films used on plant surfaces in Section 2.

1.1 BASIC CHARACTERISTICS OF ZEOLITES

Zeolites are crystalline solid structures made of silicon, aluminium and oxygen that form a framework with cavities and channels. The zeolite structure may be represented by the formula:

$$M_{x/n}\,[(AlO_2)_x(SiO_2)_y]\ .\ wH_2O$$

where M is an alkali or alkaline-earth cation (Na, K, Li and/or Ca, Mg, Ba, Sr), n is the cation charge, w is the number of water molecules per unit cell, x and y are the total number of tetrahedra per unit cell and the ratio y/x usually has values ranging from 1 to ∞ (Payra and Dutta, 2003; Georgiev et al., 2009).

Every zeolite material is classified by the framework type to which it belongs. The framework type does not take into account the elements present in the zeolite structure, just the connectivity (topology) of the framework. It defines the size and shape of the pore openings, the dimensionality of the channel system, the volume and arrangement of the cages and the types of cation site available (McCusker and Baerlocher, 2001, 2007). The chemical formulas and structure types of some important natural and synthetic zeolites are presented in **Table 1-1** and **Figure 1-1** (Ibrahim, 2004; Baerlocher et al., 2007; Wang et al., 2009). More detailed information on the framework structure of zeolites is provided in Chapter 2.

Table 1-1. Chemical formula and structure of some important zeolites (Baerlocher et al., 2007).

Zeolite	Chemical formula	Structure type	Channel dimensions	Volume (Å^3)	Symmetry
		Natural zeolites			
Chabazite[a]	$\|(K,Na,Ca_{0.5})_4(H_2O)_{12}\|$ $[Al_4\ Si_8O_{24}]$	CHA	3D	2391.59	Rhombohedral
Clinoptilolite	$\|(K,Na,Ca_{0.5})_6(H_2O)_{20}\|[Al_6\ Si_{30}\ O_{72}]$	HEU	2D	2054.84	Monoclinic
Mordenite	$\|Na_2,Ca,K_2)_4(H_2O)_{28}\|$ $[Al_8Si_{40}O_{96}]$	MOR	1D	2827.26	Orthorhombic
		Synthetic zeolites			
Zeolite A[a]	$\|Na_{12}\ (H_2O)_{27}\|_8\ [Al_{12}Si_{12}\ O_{48}]_8$	LTA	3D	1693.24	Cubic
Zeolite L	$\|K_6Na_3\ (H_2O)_{21}\|$ $[Al_9Si_{27}\ O_{72}]$	LTL	1D	2153.11	Hexagonal
Zeolite Y	$\|(Ca,MgNa_2)_{29}\ (H_2O)_{240}\|$ $[Al_{58}Si_{134}\ O_{384}]$	FAU	3D	14428.77	Cubic

[a] The scanning electron micrograph and framework are shown in **Figure 1-1**.

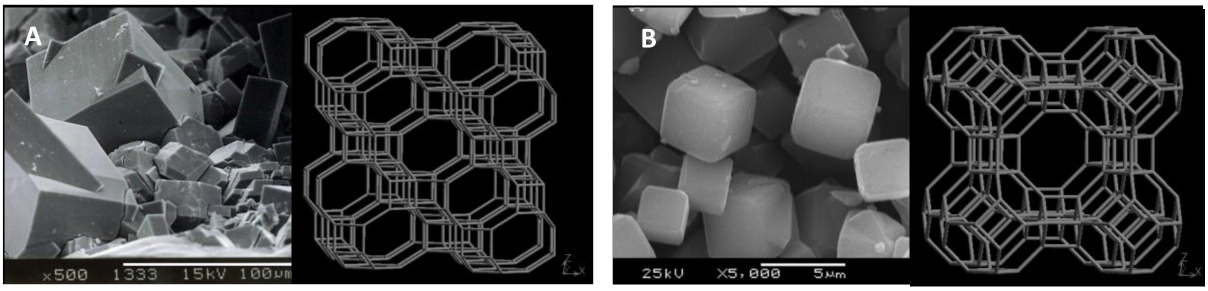


Figure 1-1. Scanning electron micrographs and frameworks of (A) natural zeolite 'chabazite' and (B) synthetic zeolite 'zeolite A' (Ibrahim, 2004; Baerlocher et al., 2007; Wang et al., 2009).

1.2 APPLICATIONS OF ZEOLITES

Owing to their unique physical and chemical properties, zeolites are used for a great number of applications in different domains. In industry, zeolites are well known and commercially used as separation agents, ion exchangers, adsorbents, as fillers in paints, paper and plastics, etc. (Al-Dwairi and Al-Rawajfeh, 2012). Also, the use of zeolites for environmental applications is attracting new research interest, mainly owing to their properties and significant worldwide occurrence. Application of natural zeolites for water and wastewater treatment, focused on ammonium and heavy metal removal, has been realised and is still a promising technique in environmental cleaning processes (Caputo and Pepe, 2007; Wang and Peng, 2010). In addition to these applications, zeolites also have their medical applications, for example as detoxicants, vaccines and agents in haemodialysis, bone formation, etc. (Pavelic and Hadzija, 2003).

Furthermore, zeolites have been widely used in agriculture for the removal of bad odours in animal stables and for their soil-improving properties (e.g. increase in water-holding capacity and nutrient adsorption, and decrease in levels of heavy metals or radionuclides in contaminated soils). Studies have verified that phytoremediation processes can be performed with the aid of zeolites. In a mixture with compost, zeolites have been shown to promote plant species growth and to increase, at the same time, the accumulation of metals in the aerial part of the plant. When composted together with poultry manure, zeolites become ammoniated and enhance the soil microbial population (Leggo et al., 2006; Ramesh et al., 2010; Lai et al., 2012; Tulod et al., 2012). In combination with fertilisers, zeolites may help to buffer soil pH levels. After a few years of zeolite action in the soil, the zeolite increases crop yields and is used as fertiliser itself (Ramesh et al., 2010).

Natural zeolites can also be added as dietary additives to animal food in order to neutralise the negative effects of mycotoxins. Controlled release of inputs is being employed extensively in agriculture to deliver active substances such as PPPs and fertilisers. Zeolites are attractive candidates as carriers to immobilise these crop protection products and nutrients first, before slow release can take place (see Section 2.5) (Mumpton, 1999; Mravec et al., 2005; Ramesh et al., 2010).

Note that the aforementioned list of applications is not exhaustive, and that zeolites can also be used for applications in other domains as well. However, the main emphasis of this review is on the use of zeolites in agriculture, and more specifically on the use of zeolites as PPPs against pests and diseases.

In the following sections, an overview is presented of the different properties of zeolites for their usage in crop protection. These properties will demonstrate that zeolites are also able to form a good particle film for controlling pests and diseases. The most important characteristics for an effective particle film on plant tissues, summarised in **Table 1-2**, are taken into account (Glenn and Puterka, 2005).

Table 1-2. Characteristics of the effectiveness of particle film technology on plant tissues (Glenn and Puterka, 2005).

	Characteristics
1.	The formulation contains chemically inert mineral particles
2.	The particle diameter < 2µm
3.	The formulation spreads well and creates a uniform film
4.	The porous film does not interfere with gas exchange from the leaf
5.	The technology alters insect/pathogen behaviour on the plant
6.	The particle film is easy removable from harvested commodities
7.	Ultraviolet (UV) and infrared (IF) radiation is excluded, while it transmits photosynthetically active radiation (PAR)

2 ZEOLITES: A GOOD PARTICLE FILM FOR CONTROLLING PESTS AND DISEASES?

Particle film technology may be defined as a synthesis of combined knowledge on mineral technology, insect behaviour and photo-chemistry. It aims to control pests and diseases of plants. A particle film is a microscopic layer of mineral particles attached to the plant surface. An example of a scanning electron microscope (SEM) image of a particle film is shown in **Figure 1-2** (Glenn, private communication).

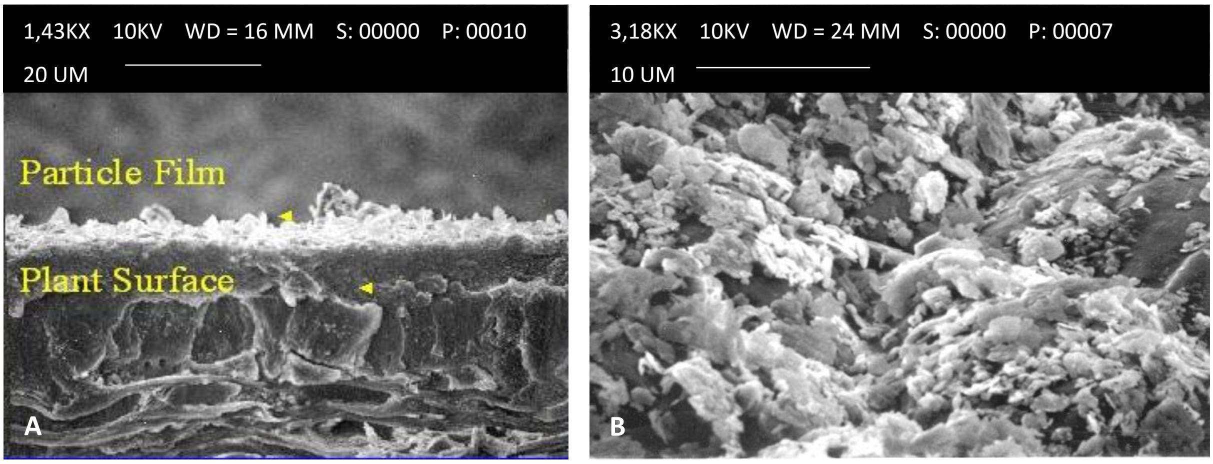


Figure 1-2. Scanning electron micrographs of (A) a leaf and a particle film and (B) kaolin on the upper surface of an apple leaf (Glenn, private communication).

This technology has proven to be a viable alternative to synthetic PPPs for managing arthropod pests and diseases of agricultural crops (Glenn et al., 2001; Glenn and Puterka, 2005). The use of particle films on plant tissues is aimed at preventing most of the negative effects that occur with the current application of PPPs. It might deliver a wide range of beneficial effects in terms of water efficiency, control of pests and diseases, reduction in PPP use, increase in crop yield and tolerance to abiotc stress (Glenn and Puterka, 2005).

In the 1920s, dusts were increasingly applied and even preferred to liquid sprays (Glenn and Puterka, 2005). Current particle film technology is based on kaolin, a white clay mineral $\{[(Al_2O_3)(SiO_2)_2].2H_2O\}$, also called aluminosilicate (Puterka et al., 2000). The hydrophobic kaolin particle M-96-018 was the first prototype of particle film technology that was applied as a dust on trees in order to make the plant surfaces repellent and to suppress anthropod pests and diseases (**Figure 1-3**) (Glenn et al., 2001).

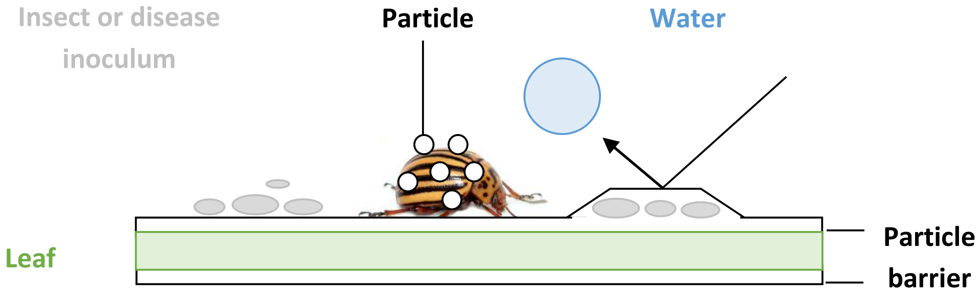


Figure 1-3. Mechanisms of arthropod pest and disease suppression in plants (Glenn et al., 2001).

This dust coating was water repellent, prevented diseases and arthropod infestations, favoured a lower oviposition rate and reduced the survival of insects. Nevertheless, the drift associated with dusting operations and the lack of adhesion to the plant made M-96-018 dust applications impractical. The need for an easier formulation led to the development of M-97-009. A formulation of this hydrophilic kaolin particle combined with a non-ionic spreader sticker, M-03, was just as effective as M-96-018 in controlling pests and diseases, with improved formulation properties, i.e. ease of mixing, adhesion, spreading and rainfastness. In 1999 this product became commercially available under the name Surround® WP crop protectant (BASF, Research Triangle Park, NC; previously Engelhard Corp., Iselin, NJ) (Glenn and Puterka, 2005). An example of a kaolin-based particle film on crops is illustrated in **Figure 1-4** (Godfrey et al., 2005).

Figure 1-4. Kaolin sprayed onto crops forms a mineral-based particle film repelling insects and preventing feeding (Godfrey et al., 2005).

Zeolites are just like clay minerals composed of aluminosilicate, but differ in their crystal structure (**Table 1-3**) (Mumpton, 1999; wang et al., 2006; Ramesh et al., 2010). Therefore, zeolites may also play an increasing role in a wide range of agricultural applications. However, the use of zeolites as a biofilm matrix for controlling various pests and diseases needs further research.

Table 1-3. Mineralogy of kaolin and zeolite (Mumpton, 1999; Wang et al., 2006; Ramesh et al., 2010).

KAOLIN	ZEOLITE
Aluminosilicate (Hydrated)	Aluminosilicate (Hydrated)
↓	↓
Phyllosilicates (Two-dimensional) = Parallel sheets	**Tectosilicates** (Three-dimensional) = Framework
↓	↓
Clay mineral group	Zeolite family

2.1 CHEMICALLY INERT MINERAL PARTICLES

Unlike typical agricultural chemicals, mineral particle films such as kaolin and zeolite are very stable solids and resistant to extreme environmental conditions, i.e. they resist high temperatures and high pressures, they do not resolve in water and they do not oxidize in the air. As a consequence, zeolites have no direct biochemical or physiological effect on the plant or pest. Instead, particle films provide activity through their physical properties, such as particle size, shape, surface area, etc. (Nielsen et al., 2000; Lalancette et al., 2005). The chemical and thermal stability of a zeolite is generally high, but depends on the dealumination of the framework. Zeolites with low Si/Al ratios are the least stable zeolites (Celik et al., 2010; van den Bergh et al., 2010).

2.2 IDEAL GRANULOMETRY OF ZEOLITES

Zeolites are commonly fine polycrystalline powders with an average particle size of several micrometres. The different end-uses of silica, for example in the production of paper, paints, etc., depend upon the particle size distribution. A coarse-particle-size silica has very different physical and optical properties compared to a fine-particle silica. Thus, depending on the particular application, it is better to use fine particles instead of coarse particles, and vice versa. The particle size is a critical factor in particle film technology (Harvey and Lagaly, 2006). More than 70% (w/w) of the particles should be smaller than 2 µm. The effectiveness of zeolites against insects generally increases when the particle size decreases to an ideal size of 1-2 µm because of improved adherence to the insect cuticle (see Section 2.4.2) (Glenn et al., 2001).

In recent years, the synthesis of nanocrystalline zeolites has received much attention (Zhang et al., 2007a). The reduction in particle size of zeolites from micrometre to nanometre scale has led to substantial changes in their properties. Previous studies revealed that the particle size and morphology of the zeolite crystals play an important role in their applications in the areas of catalysis and separation (Miller et al., 2004). Nanostructured zeolites below 100 nm have a larger external and internal surface, a higher surface energy and a shorter channel in comparison with the conventional microsized zeolites (Mintova and Valtchev, 1999; Jung et al., 2004). A nanocrystalline zeolite with a crystal size of 50 nm has an external surface area of >100 $m^2 \cdot g^{-1}$. For comparison, a 500 nm zeolite crystal has less than 10 $m^2 \cdot g^{-1}$ of external surface area. The increased external surface of nanocrystalline zeolites results in enhanced adsorption capacity and additional surface area available for adsorption and reaction of molecules (Song et al., 2004a, 2004b; Tosheva and Valchev, 2005).

2.3 PLANT-SURFACE-ORIENTED CROP PROTECTION

In order to understand how crops are grown and protected against pests and diseases, it is important to focus on the general aspects of plant physiology and PPP application.

2.3.1 PLANT GROWTH

Plants are essentially autotrophic, photosynthetic organisms, with basic requirements of light, CO_2, water and nutrients (P/K/N/O) (Lovatt, 2014). The three most important physiological phenomena that are basic to plant growth and development are photosynthesis, respiration and transpiration (Holding and Streich, 2013).

2.3.2 DIFFERENT STEPS OF PLANT PROTECTION PRODUCT APPLICATION

Nowadays, both systemic and non-systemic products are used in agriculture. Systemic products are taken up by the roots and transported throughout the plant, while non-systemic products generally control a pest or disease as a result of direct contact (Bognolo, 2000). Just like kaolin, zeolites will be applied as a non-systemic product (McBride, 2000).

Conventional PPP application comprises movement of the spray, starting from the spray equipment to the molecular site of action on the target plant. It is a very complex process involving several different steps. The major steps, together with some important influencing factors, are presented in **Table 1-4** (Steurbaut et al., 2001a).

Below, these different steps will be explained using the example of a fungicide application, as this involves the most extended pathway. Insecticide (and herbicide) applications, on the other hand, would not cover all the steps presented in the scheme in **Table 1-4** (Steurbaut et al., 2001a). Given that the use of zeolites will only influence the spraying, it is expected that they will have an impact on steps 1 to 6 of the application process.

Formulation and dispersion stability of the active ingredient(s) in the spray solution (steps 1, 2 and 3). As many active ingredients are hydrophobic and consequently do not easily dissolve in water, PPP formulations usually contain some specific adjuvants (e.g. dispersion agents) in order to obtain a spray mix suitable for tank mixing (Nikolov et al., 2002). Surfactant impregnation is also commonly employed in order to change the hydrophilic/hydrophobic properties of zeolites (Wang and Peng, 2010).

Spray droplet formation and aerial transport to the target (step 4).

Physical properties of the spray liquid, such as viscosity, density, temperature, etc., may affect the droplet size distribution of a spray. The droplet size distribution during atomisation is very important because it affects (1) the biological activity and (2) the spray drift (droplets that are too small are prone to drift away to adjacent fields and non-target areas) (Gaskin et al., 2005; Spanoghe et al., 2007). The optimum droplet size depends on the content of the droplet, the amount of active ingredient in the droplet and the type of application, i.e. as an insecticide, a herbicide, a fertiliser, etc. (Spanoghe, 2005). Studies of Skuterud et al. (1988) showed that, when applying contact products such as zeolites, it is important to use fine (60 µm) or mediate (60-200 µm) droplets.

Spray deposition: wetting and spreading properties on treated leaf surfaces (step 5).
Droplet velocity is known to be a factor affecting impaction; it determines whether a drop is being either retained or reflected. Deposition of droplets on crop canopies is a very complex subject. Generally, the epicuticular wax on a leaf acts as a substantial barrier to wetting (Holloway, 1970). Water alone tends to bead up and roll off the leaf, which can make spray applications ineffective. Because surfactants have the ability to reduce the surface tension of water and to induce a surface tension gradient, they enable spray solutions to wet waxy leaf surfaces more effectively, thereby increasing the amount of spray retained on the leaf. Enhancing droplet spread increases its potential coverage and can result in an increased biological activity (Nikolov et al., 2002). The final coverage is also affected by the spray type. High volume applications can result in product run-off, which leads to considerable losses. On the other hand, low-volume spraying leads to very poor coverage of the leaf surface and results in insufficient biological activity and hence loss of efficacy (Gaskin et al., 2000, 2002). Adding an appropriate surfactant will reduce the contact angle and enhance the degree of leaf coverage, which will improve crop protection (Holloway, 1970). In general, a good coverage becomes very important when using non-systemic products, such as zeolites (Spanoghe, 2005). This is because only the parts of the leaf surface covered with the product have a toxic effect. New growth is also unprotected growth, which makes it necessary to reapply the zeolite formulation (McBride, 2000).

Physical form and adhesion properties of the leaf deposits (step 6).
Increased spreading will tend to decrease the dose of active ingredient needed per unit area. According to their concentration and composition, adding adjuvants produces either solid, gel or liquid deposits. Once the droplets on the leaf surface are dry, it is Important that the physical form of the deposit is such that the active ingredient is (1) uniformly distributed on the leaf and (2) has become rainfast. This phase is greatly affected by the leaf's epicuticular waxy layer, cuticle age and composition, the environmental conditions and the variability in plant species (Hall et al., 1997). Areas of low rainfall are most adaptable to this technology, because the applied zeolite, just like kaolin, will eventually get washed off all crops by rain. This will lead to a situation where the plant is unprotected again and the zeolite formulation will have to be reapplied, which causes an increase in costs (McBride, 2000).

Penetration into and translocation in the leaf (steps 7 and 8).
Plant uptake is also affected by the leaf and fruit surface wax, cuticle age and composition and species variability. Transport of the active ingredient through the plant cuticle is determined by three processes: (1) absorption into the cuticle, (2) diffusion through the cuticle and finally (3) desorption from the cuticle (Spanoghe et al., 2007). Translocation is the transport of the agrochemical from the initial absorption site to other parts of the plant and can occur either

via the phloem or via the xylem or both (Norris, 1969). However, these steps are mainly of importance when using systemic products, which have some additional barriers to overcome before they reach the pest or the disease. This is not the case when zeolites are used, as contact products will not penetrate into the plant and will not be transported (Spanoghe, 2005).

Penetration and transport in the fungal cell (steps 9 to 11).

Only certain steps mentioned in **Table 1-4** apply to foliar treatments with current PPP formulations, and some of them are very specific. For fungicides, extra important steps are involved in the process that can influence the final activity dramatically. These extra steps will include possible phytotoxic side effects, specific demands for fungal cell penetration and a range of other interactions that may interfere with these steps, such as the behaviour of infected plants to a treatment, the effect on resistance development of the fungus, the treatment type and the location of the biochemical site of action in the fungal organism (Rogiers et al., 2005; Ryckaert et al., 2007). The fungus is not actively controlled by the zeolite formulation, because contact products cannot penetrate into the fungal organism. Nevertheless, zeolites can have a reducing effect on spore germination (see Section 2.4.2) (Puterka et al., 2000; Glenn et al., 2001).

Table 1-4. Different steps in the pathway of a fungicidal spray solution from spray nozzle to the aerial part of the plants (Steurbaut et al., 2001a).

1. **Active ingredient**

⇓ + surfactant (emulsifier, ...)

2. **Formulation**

⇓ dispersing agent

3. **Spray solution**

⇓ dynamic surface tension viscosity

4. **Spray droplet formation with transport to target**

⇓ wetting and adhesion, spreading properties retention or reflection

5. **Impaction and contact on leaf surface**

⇓ sticking properties, rain fastness, evaporation, physical form of deposit, humefactant effects, UV-protection

6. **Formation of deposit** **TOTAL FUNGICIDAL EFFECT**

⇓ transcuticular penetration, stomatal infiltration

7. **Penetration into the leaf** ⇒ ⇒ ⇒ ⇒ ⬂

⇓ *systemic activity*, translocal activity ***contact activity***
⇓

8. **Translocation in the plant**

⇓ membrane penetration ⇓

9. **Penetration into fungal cell** ⇐ ⇐ ⇐ ⇐ ⬃

⇓ hydrophilic-lipophilic properties

10. **Transport and complexation with site of action**

⇓ reaction with site of action

11. **Fungicidal activity**

2.4 EFFECTS OF ZEOLITES THAT OCCUR AFTER APPLICATION

Various coating polymers are used to reduce water losses, protect plant surfaces against invading microorganisms and prevent the development of certain plant diseases. These coating polymers used as protective barriers are non-phytotoxic, permeable to gases and resistant to changing environmental conditions and penetration of solar irradiation (Zekaria-Oren and Eyal, 1991). The following sections describe whether these effects are also valid when zeolites are used as particle films on plants.

2.4.1 EFFECTS OF ZEOLITES ON THE PLANT

Photosynthesis enhancement by zeolites on crops

Zeolites are able to adsorb carbon dioxide (CO_2) molecules and release them slowly into the environment (Jaramillo and Chandross, 2004; Montanari and Busca, 2008). When zeolites are spread on plant leaves, they may (this is not yet proven) increase the amount of CO_2 near the stomata, which could induce a higher photosynthesis rate for plants using both C_3 and C_4 carbon fixation. In particular, C_3 plants, such as apple, orange, tomato, grape, etc., take advantage of this increase (Cleland et al., 1998; Ainsworth and Rogers, 2007). A higher concentration of CO_2 by applying zeolites may increase the velocity of carboxylation by competitively inhibiting the oxygenation reaction, increasing the efficiency of net carbon CO_2 uptake by decreasing photorespiratory CO_2 loss (Long et al., 2004). Because of the increased CO_2 concentration, the efficiency of light usage increases in net CO_2 uptake, which results in increased growth and an increased rate of production of leaf area. Furthermore, the water usage decreases because of a lower transpiration rate, which further accelerates leaf development (**Figure 1-5**) (Long et al., 2004). In the literature, conflicting data have been observed on this subject using kaolin. Grange et al. (2004) found a reduction in photosynthetic rates of individual leaves owing to a reduction in light because of a 20-40% increase in reflection and decreased absorption. Wünsche et al. (2004) observed that, in spite of a reduction in photosynthetic rates of individual leaves, there was no decrease in canopy photosynthesis. Glenn et al. (2003) noted an increase in canopy photosynthesis. Rosati et al. (2007) conducted a study on this and demonstrated that kaolin application does reduce photosynthesis of individual leaves, but increases the canopy photosynthesis, which explains the increased yield.

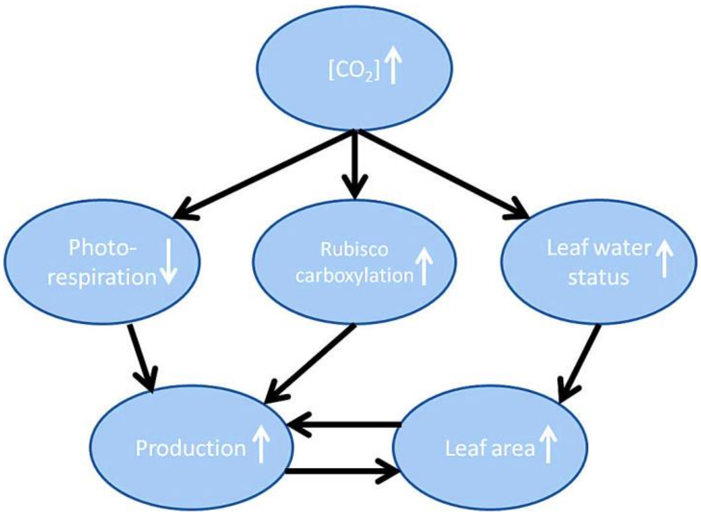


Figure 1-5. Overview of direct effects of increasing CO_2 concentration on C_3 plant production (Long et al., 2004).

Heat stress and sunburn of zeolites on crops

It is known that the affinity of Rubisco (the enzyme responsible for carbon fixation in plants) for CO_2 and the solubility of CO_2 relative to O_2 both decrease with rising temperature. Therefore, the relative ratio of carboxylation to oxygenation is reduced when the temperature increases (Ainsworth and Rogers, 2007). By coating the plants with zeolite, the plant leaf temperature can potentially be diminished, caused by increasing leaf reflectiveness (whiteness) of infrared radiation. Similar experiments are already executed with kaolin. Tests indicate a higher leaf carbon assimilation rate and a reduced canopy temperature in grapefruit and apples (Glenn et al., 2003; Jifon and Syvertsen, 2003; Glenn and Puterka, 2005). That explains why this product is labelled for reduction of heat stress and sunburn on several crops (Kahn and Damicone, 2008). Kaolin cools tissues and protects plants from extreme heat and ultraviolet radiation by increasing leaf reflectance and reducing transpiration rate (Sotelo-Cuitiva et al., 2011). Experiments with kaolin demonstrated that leaf temperature increases linearly with increasing light intensity. This effect was observed with and without the use of a coating, but the leaf temperature was significantly lower ($P < 0.001$) after application of the coating. Abou-Khaled et al. (1970) have determined that leaves of dwarf orange trees (*Citrus sinensis cv. Valencia*), rubber plants (*Ficus elastica*) and kidney bean plants (*Phaseolus vulgaris*) are cooled approximately 4°C by the reflecting material (**Figure 1-6**) (Abou-khaled et al., 1970). This effect contrasts with the tendency of antitranspirants to raise leaf temperatures. The lowered temperature results in a 25% reduction in transpiration, which improves water-use efficiency. Subsequently, this effect also has a positive influence on yield and fruit quality. Heat stress is recognised as the main cause of the reduction in tomato yield and fruit quality worldwide. Cantore et al. (2009) have illustrated that applying kaolin to tomato plants increases the marketable yield by as much as 21% owing to a 96% reduction in sunburned fruit, a 79% reduction in fruit damaged by tomato fruit worm and a 9% increase in fruit mean weight.

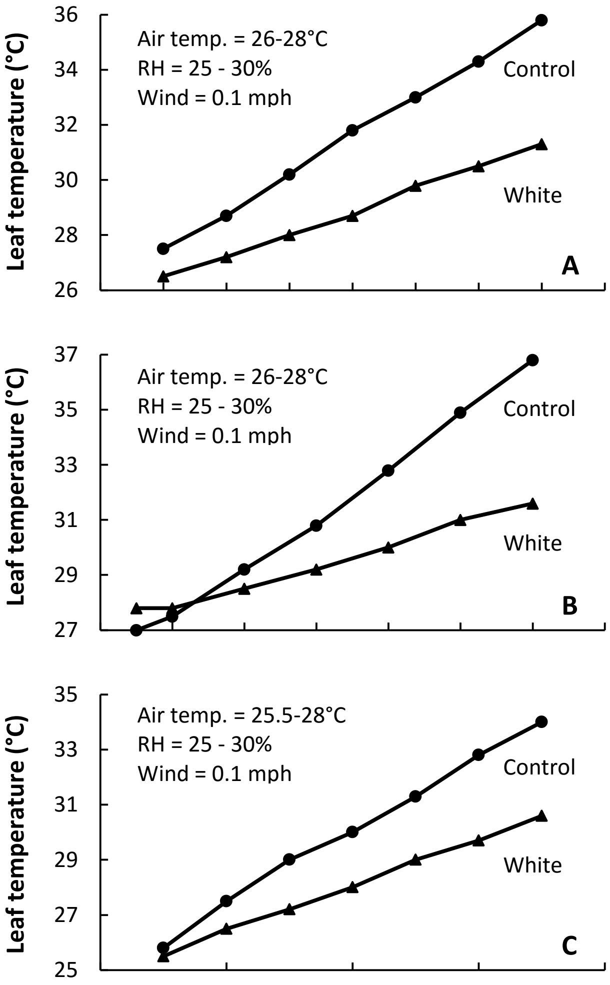


Figure 1-6. Effect of white reflecting material on the temperature of (A) orange leaves (*Citrus sinensis cv. Valencia*), (B) rubber plant leaves (*Ficus elastica*) and (C) bean leaves (*Phaseolus vulgaris*) (Abou-Khaled et al., 1970).

Water sorption capacity of zeolites

In addition to the reduction in heat stress, zeolites may also be used to reduce water stress. The adsorption selectivity of zeolites for water (H_2O) is greater than any other molecule (Lalancette et al., 2005). This is shown by an adsorption capacity that may reach up to 30% by weight of the zeolite without any volume modification (Eriksson, 2008). These polymers form a film over the stomata, increasing resistance to water vapour loss (Reddy, 2012). By absorbing condensing water and eliminating free water on the plant surface, zeolites will serve as a physical barrier to liquid water. This barrier will prevent the formation of the liquid film of water that is required by many fungal and bacterial pathogens for disease propagule germination (Lalancette et al., 2005). The water sorption behaviour of a sorbent depends on many factors, such as the structure and the chemical composition of the material, the presence of charged species, the type of framework structure and the hydration level. The key physical property of every adsorbent is the surface hydrophobicity (Sakuth et al., 1998).

The hydrophobicity of zeolitic adsorbents can be varied by changing the silicon to aluminium ratio. In general, zeolites are highly hydrophilic sorbents owing to their electrostatic charged framework and the abundance of extra-framework cations. Almost all of the zeolites (especially the high-aluminium zeolites) show type I water sorption isotherms (**Figure 1-7**), which indicate a high affinity for water at a low partial pressure (Ng and Mintova, 2008). The water sorption capacity generally is also proportional to the size of the pores, because of the highly polar surface within the pores. That is why aluminosilicate zeolites with larger pores have a higher capacity for water. Zeolites can be placed according to pore size into the following categories: extra-large-pore zeolites (θ ≥ 9Å), large-pore zeolites (6Å < θ < 9Å), medium-pore zeolites (5Å < θ < 6Å) and small-pore zeolites (3Å < θ < 5Å), depending on the access to the inner part using 8-, 10- or 12-atom oxygen rings respectively (Melo et al., 2012).

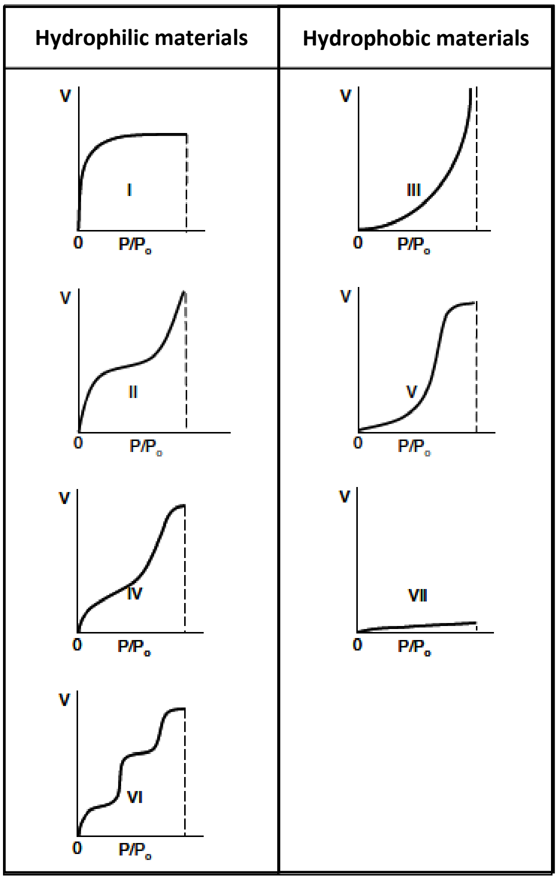


Figure 1-7. Adsorption isotherms classified according to IUPAC: type I: very hydrophilic material; type II: hydrophilic material; type III: hydrophobic/low hydrophilic material with weak sorbent-water interactions; type IV: hydrophilic material; type V: hydrophobic/low hydrophilic material with weak sorbent-water interactions; type VI: hydrophilic material with multiple sorbent-water interactions and stepwise sorption; type VII: very hydrophobic material (Ng and Mintova, 2008).

2.4.2 EFFECTS OF ZEOLITES ON PATHOGEN/INSECT BEHAVIOUR

Fungicide properties of zeolites

Film-coating polymers have been reported to provide additional protection against various foliar pathogens (Zekaria-Oren and Eyal, 1991). Aluminium-rich zeolites are often used as desiccants. This is due to their high concentration of hydrophilic active sites, which can enhance the water sorption capacity and hydrophilicity (Ng and Mintova, 2008). Their high affinity for water is another potential advantage of zeolites over kaolin. Just like kaolin, the zeolite coating creates a barrier that prevents disease inoculums from directly contacting the leaf surface (**Figure 1-8**). Each type of microbial organism (bacterium, yeast or fungus) needs water to grow and to develop. The availability, rather than the amount, of moisture is an impediment (such as the pH or the temperature) to avoiding or promoting its development (Fontana and Campbell, 2004). Scott (1953, 1957) showed that microorganisms have a limiting water activity level below which they will not grow. The water activity, and not the water content, determines the lower limit of available water for microbial growth. Percival and Boyle (2009) showed that, just like kaolin, zeolites could provide protection against apple scab (*Venturia inaequalis*) by preventing a liquid film. By absorbing condensing water, the zeolites prevent the formation of the liquid film of water that is required for many fungal and bacterial pathogens for disease propagule germination (Puterka et al., 2000; Glenn et al., 2001).

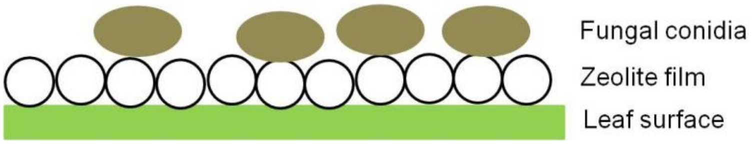


Figure 1-8. Schematic overview of a zeolite film creating a barrier against fungal germlings.

Insecticide properties of zeolites

Besides their function as desiccants against microbial organisms, zeolites are also effective against insect (Puterka et al., 2000; Walters, 2006). They can partially remove the insect’s outer cuticle (epicuticule) through abrasion by hard non-sorptive particles. Since it is not likely that large insect epicuticular lipid molecules enter the zeolite’s inner channels, the insect’s outer cuticle can also partially be removed by absorption and adsorption of the lipid molecules onto sorptive particles of a zeolite surface. This adsorption will take place on silica-rich zeolites, which have hydrophobic properties. Both processes induce rapid water loss from the insect’s body and cause death by desiccation. Consequently, there is an inverse relationship between insect mortality and relative humidity (Glenn et al., 1999). Tests done with kaolin clay particles showed that pest insects, including psyllids, aphids, fruit flies and thrips, have a lower oviposition rate (Knight et al., 2000; Glenn et al., 2001; Mazor and Erez, 2004; Larentzaki et al., 2008; Leskey et al., 2010). The results also showed that the hatch rate of eggs covered with the particle film decreases, larval development is interrupted and mortality is higher for leaves on which the pest insects are exposed to the particle film. The particles also attach to the insect's body, inducing a tactile deterrence that can lead to disruption of the insect's behaviour to such a degree that it is unable to feed and eventually starves (Glenn et al., 2001). Moreover, the layer of particle film covering the leaves and fruit reduces the attractiveness of visual cues and prevents insects from recognising and finding plant parts on which they lay eggs (Leskey et al., 2010). However, particle films also induce negative effects, as some pest insects are able to thrive on leaves sprayed with particle films, while the presence of natural enemies is reduced. Marko et al. (2008) illustrated that, while a kaolin-based particle film application reduces many insect pests on apple trees, including the codling moth (*Cydia pomonella*), the apple sawfly (*Hoplocampa testudinea*) and several weevils, leafhoppers and scales, the infestation levels of other pest insects increase. Leaves covered with kaolin promote a severe infestation of the woolly apple aphid (*Eriosoma lanigerum*) and reduce the abundance of polyphagous predators and parasitoids. Also it was noted that some weeks after the treatment the number of predaceous coleopterans was low.

2.4.3 EFFECTS OF ZEOLITES ON THE SOIL

Soil water retention of zeolites

When zeolite is lost during application or washed off the leaves by rainfall, it can still have a positive effect on the soil composition. When water is supplied adequately, plants are prodigal in their water usage because only roughly 5% of water uptake is used for their growth and development, while the remaining 95% is lost on transpiration (Moftah and Al-Humaid, 2004). Actively growing plants transpire each hour a weight of water equal to their fresh leaf weight in arid and semi-arid regions. This makes it necessary to find ways to use the available water economically (Moftah and Al-Humaid, 2005). Zeolites form a permanent water reservoir and provide prolonged moisture in dry periods, which helps plants to withstand drought. Amendment of sand with zeolite increases available water to the plants by 50% (Ramesh et al., 2010).

Cation exchange capacity of zeolites

Zeolites are also one of the most efficient cationic exchangers. Their cationic interchange capacity is 2-3 times greater than other types of mineral found in soils (Perez-Caballero et al., 2008). That is why zeolites are widely used as slow-release fertilisers that increase nutrient retention capacity. Because natural zeolites are not acidic but marginally alkaline, their use with fertilisers may also help to increase (buffer) soil pH levels (Ming and Boettinger, 2001). Zeolites in soils exchange sodium (Na^+) and potassium (K^+) cations for ammonium (NH_4^+) (Li, 2003). Ammonium and potassium charged zeolites have shown their ability to increase the solubilisation of phosphate (PO_4^{3-}) and the capture of nitrate (NO_3^-). In addition, the inclusion of negatively charged nitrate ions promotes the uptake of positively charged nutrient ions, such as magnesium, calcium and potassium. All of this simultaneously contributes to a reduction in contamination, a reduction in the amount of fertiliser to be applied and an improvement in crop yield (Perez-Caballero et al., 2008; Ramesh et al., 2010). A number of examples of zeolites used as fertilisers were given by Mumpton (1999). By using clinoptilolite-rich tuff as a soil conditioner, significant increases in the yields of wheat (13-15%), eggplant (19-55%), apples (13-38%) and carrots (63%) were reported when 1.6-3.2 t.zeolite.ha^{-1} was used. The addition of NH_4^+-exchanged clinoptilolite in greenhouse experiments resulted in 59% and 53% increases in root weight of radishes in medium- and light-clay soils respectively.

2.5 CARRIER EFFECT OF ZEOLITES

One of the major concerns in the use of organic compounds, such as herbicides, fungicides and PPPs, in agronomy and horticulture is their leaching into groundwater. As most of these organic compounds are too large to enter the zeolite framework, the high adsorption capacity of zeolites makes it possible to control the rate of diffusion of molecules in and out of the micropores and thus control the release of adsorbed active ingredients (Colella, 2007). The use of controlled-release formulations can, in many cases, supply the active ingredients at the required rate, thus on the one hand reducing the amount of chemicals needed for pest control and on the other hand decreasing the risk to the environment. Controlled release of PPPs and other organic agrochemicals can, in many cases, permit safer, more efficient and at the same time more economical crop protection (Gerstl et al., 1998).

2.5.1 PLANT PROTECTION PRODUCT CARRIER EFFECT OF ZEOLITES

Sopkova and Janokova (1998) were able to enclose the solid form of the synthetic pyrethroid insecticide supercypermetrine in the natural zeolite clinoptilolite. They used an experimental set-up to indicate the enclosure and stabilisation of the insecticide in the mineral. The pyrethroid was gradually released from the zeolite, demonstrating that the mineral can be used as a reservoir for the insecticide for a longer time. Moreover, the insecticide was better protected against photolysis and early release, which ensured better protection of the environment against an excess of the chemical.

The external surface activity of zeolites can be modified in such way that minerals can be exploited as carriers of different products, including herbicides, fungicides, insecticides and growth regulators (Colella, 2007). Zhang et al. (2006) modified the surface of zeolite Y by silylation with 1,1,3,3-tetramethyldisilazane (TMDS). This modification narrowed the pores of the zeolite after the mineral had been loaded with the herbicide paraquat because of ion exchange. Slow release of paraquat in TMDS-modified zeolite Y is obtained by slower diffusion of paraquat through the blocked ‘windows’ at the zeolite-surface interface, while the pore interior is not modified. This surface alteration is an ideal solution for modifying zeolites to carry products that benefit from slow release.

2.5.2 SEMIOCHEMICAL/PLANT EXTRACT CARRIER EFFECT OF ZEOLITES

Semiochemicals can be defined as chemicals emitted by living organisms (plants, insects, etc.) that induce a behavioural or a physiological response in other individuals. These compounds can be classified into two groups according to whether they act as intraspecific (pheromones) or interspecific (allelochemicals) mediators (**Figure 1-9**) (Heuskin et al., 2011).

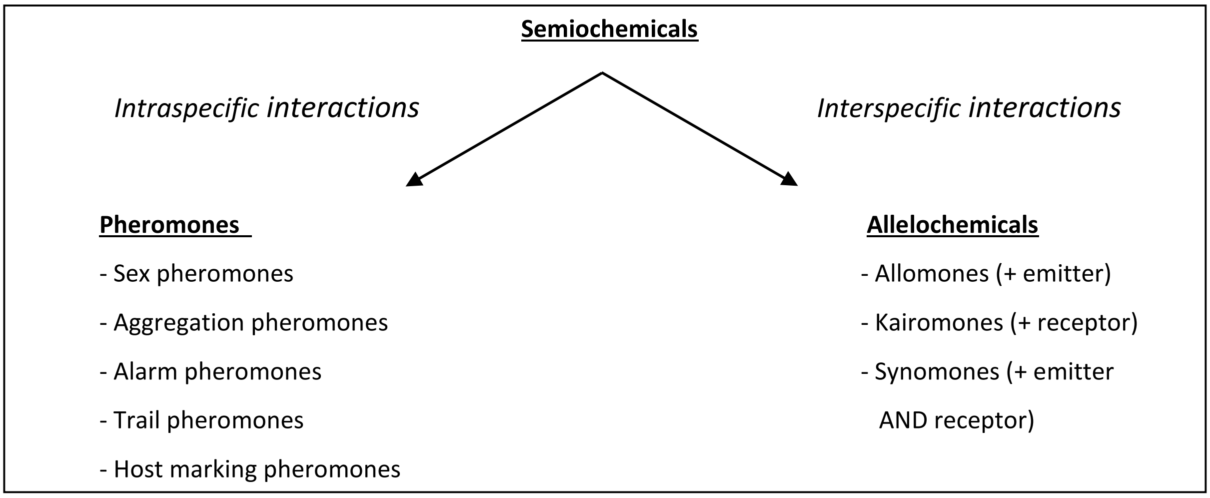


Figure 1-9. Semiochemicals (Heuskin et al., 2011).

Munoz-Pallares et al. (2001) examined zeolites with different pore diameters for use as dispensers for pheromones. They showed that zeolites are able to decrease initial pheromone emission rates and thus significantly reduce pheromone losses. Zeolites are also used for the controlled emission of semiochemicals in order to contribute to environmental management of agricultural pests and diseases. These active ingredients can play a major role, as they induce interference of the insect’s perception: the behaviour of insects towards the plant (depending on the range of colours, odours and textures they can perceive) and the behaviour of insects towards each other (depending on sex pheromones) (Heuskin et al., 2011).

Kvachantiradze et al. (1999) demonstrated that the natural zeolite clinoptilolite can be used to photostabilise *Bacillus thuringiensis*, a bioinsecticide. Several strains of this environmentally safe entomopathogenic bacterium produce endotoxins that are highly specific against certain insect pests. The main drawback using this PPP is that its biological activity decreases during exposure to solar irradiation (Cohen et al., 1991). Kvachantiradze et al. (1999) and Colella (2007) mixed *B. thuringiensis* with the zeolite, demonstrating that the presence of the zeolite can extend the photostability of the complex by deflecting sunlight and allowing a gradual desorption of the endotoxin by this aluminosilicate mineral (**Figure 1-10**) (Kvachantiradze et al., 1999).

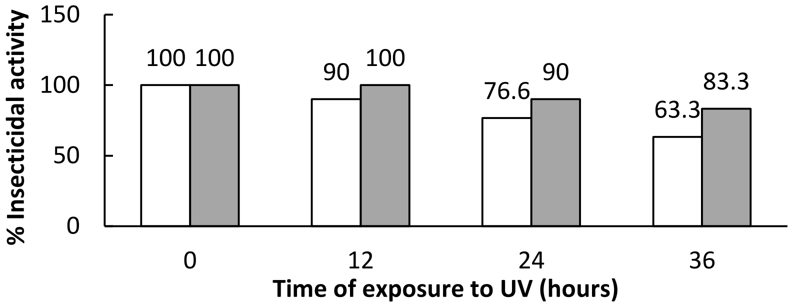


Figure 1-10. Insecticidal activity of unprotected B. thuringiensis affected by solar irradiation (☐) and of the B. thuringiensis-zeolite complex (▣) (Kvachantiradze et al., 1999).

2.5.3 MICROBIOLOGICAL CARRIER EFFECT OF ZEOLITES

In the literature, bacteria, yeasts and fungi are described as promising candidates for development as biological PPPs (Rodgers, 1993; Ooijkaas et al., 1998). In the past, these microorganisms were mainly characterized as planktonic, free-living cells. However, in nature, most microorganisms are found in association with each other or with solid surfaces and form multicellular aggregates called biofilms (Costerton et al., 1999; Wimpenny et al., 2000).

Biofilm formation may facilitate the use of microorganisms as biological PPPs. The biofilm serves as support for the formation and functioning of consortia, as it allows stable cell-cell contact. This is necessary because of the high metabolic fluxes between the cells that occur in synergistic interactions (Nielsen et al., 2000). In addition, the biofilm matrix provides additional protection against environmental stress (Flemming, 1995; Horn and Morgenroth, 2006). Finally, surfaces and biofilms can also serve as sites for the transfer of genetic material (Paul et al., 2005).

In principle, two methods for spore production can be distinguished, i.e. liquid-state fermentation (LSF) and solid-state fermentation (SSF) (Ooijkaas, 2000). SSF, defined as the growth of microorganisms on (moist) solid material in the absence or near-absence of free water, is generally the preferred production method, as most fungi sporulate well on solid substrates. In addition, SSF produces biocontrol agents of better quality than liquid fermentation (Pandey et al., 2008a).

Among several other factors that are important for microbial growth and activity in a particular substrate, particle size and moisture level/water activity are the most critical. Generally, smaller substrate particles would provide a larger surface area for microbial attack and should therefore be considered as a desirable factor. However, excessively small substrate particles may result in substrate agglomeration, which may interfere with microbial respiration/aeration, and may result in poor cellular growth. At the same time, larger particles provide a better respiration/aeration efficiency (owing to increased interparticle space) but provide a limited surface for microbial attack (Pandey et al., 2008a, 2008b).

Zeolite particles represent suitable mineral microhabitats and good carriers for immobilisation of microorganisms (Shindo et al., 2001; Hrenovic and Tibljas, 2002; Weiss et al., 2013). SEM micrographs of a zeolite carrier for yeast cells are shown in **Figure 1-11** (Shindo et al., 2001).

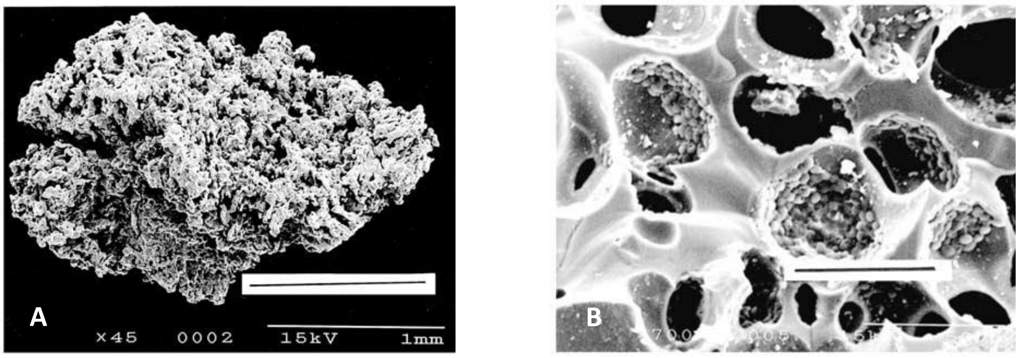


Figure 1-11. Scanning electron micrograph of (A) a natural zeolite carrier and (B) the same carrier with immobilized yeast cells (Shindo et al., 2001).

Another example is the use of zeolites as carriers for fungal colonisation. The use of fungal biological control agents to control plant pathogens has been investigated for more than 70 years; however, research in this area has increased dramatically only in the past 20 years. Over 40 biological control products have been introduced into the market within the past 10 years, but these are used on a very small scale as compared with chemical fungicides (Kaewchai et al., 2009).

3 RISK OF TOXICITY DUE TO THE COATING

3.1 PLANT TOXICITY

Coating plants with particle films is in general not phytotoxic (Puterka et al., 2000; Pasqualini et al., 2002). This particle film can act as an extra barrier against pathogen infections. The coating can also work well as a disguise for both the cues necessary for the development of fungal germlings, as well as for insect pests.

However, a possible disadvantage for plants may be that zeolites used for coating of plants may be washed off by rain showers (Dufour, 2001; Larentzaki et al., 2008). The Na form of some zeolites in the soil may inhibit growth of some plant species. The zeolitic ion exchange ability and selectivity for certain microelements can result in negative effects for plants, by adsorption of essential trace elements such as manganese, zinc, copper, iron and boron from the soil. Even some of the macroelements, such as potassium or mineral nitrogen (as the ammonium ion), can be made unavailable for plant uptake by counteracting selective uptake on zeolite exchange sites (Shindo et al., 2001; Colella, 2007).

3.2 ENVIRONMENTAL TOXICITY

Environmental risk assessments performed on zeolite A, a zeolite made from the natural source kaolin, together with the knowledge that zeolites degrade into natural products over time, indicate that the use of zeolites does not pose a risk to the environment (HERA, 2004; Belviso et al., 2013). Moreover, natural zeolites have the ability to remove soil pollutants and to interact with organic fertilisers (manure) for a modulated transfer of nutrient matter to the soil. Also, the exchangeable cations in zeolites can exert beneficial effects on soil structure stability. Zeolites are able to form aggregate compounds with humic acids, which give stability to the soil structure by avoiding loss by leaching. These humic acid-zeolite aggregates are useful for the reconstruction or remediation of depleted soils (Colella, 2007).

On the other hand, the Na form of zeolite A exhibited a growth inhibition effect towards the most sensitive plant species, *Raphanus sativus*, at test concentrations higher than 900 $mg.kg^{-1}$. In fact, zeolites are considerably less toxic when charged with Ca^{2+}, as toxicity tests showed a lower toxicity by a factor of 67 compared with the Na form (HERA, 2004). Therefore, it may be a consideration to exchange the native Na^{+} ions with Ca^{2+} ions, but taking into account that, when the Na form of zeolite becomes dispersed in water before application, part of the Na^{+} will be exchanged by the soluble Ca^{2+} present in the dispersant (on account of the water hardness) (McArthur and Spalding, 2004).

3.3 HUMAN TOXICITY

The use of zeolites as feed supplements for animals and their medical applications, as previously stated, indicates that zeolites are not harmful to humans (Pavelic and Hadzija, 2003). Material Safety Data Sheets from zeolite products also consider zeolites to be safe (BASF, 2012). The health hazard induced by prolonged and repeated contact of the zeolite powder and watery suspensions with the human skin of the operators is merely some local irritation, i.e. slight to moderate eye irritation. The raw material may reach the lungs through inhalation and has been shown to induce inflammatory reactions in the lung, alveolar and bronchial tissues. Zeolites are also tested for their carcinogenic effects (HERA, 2004). Studies on rats, in this case using clinoptilolite, showed no significant increase in incidence of tumours (Kahler, 2014).

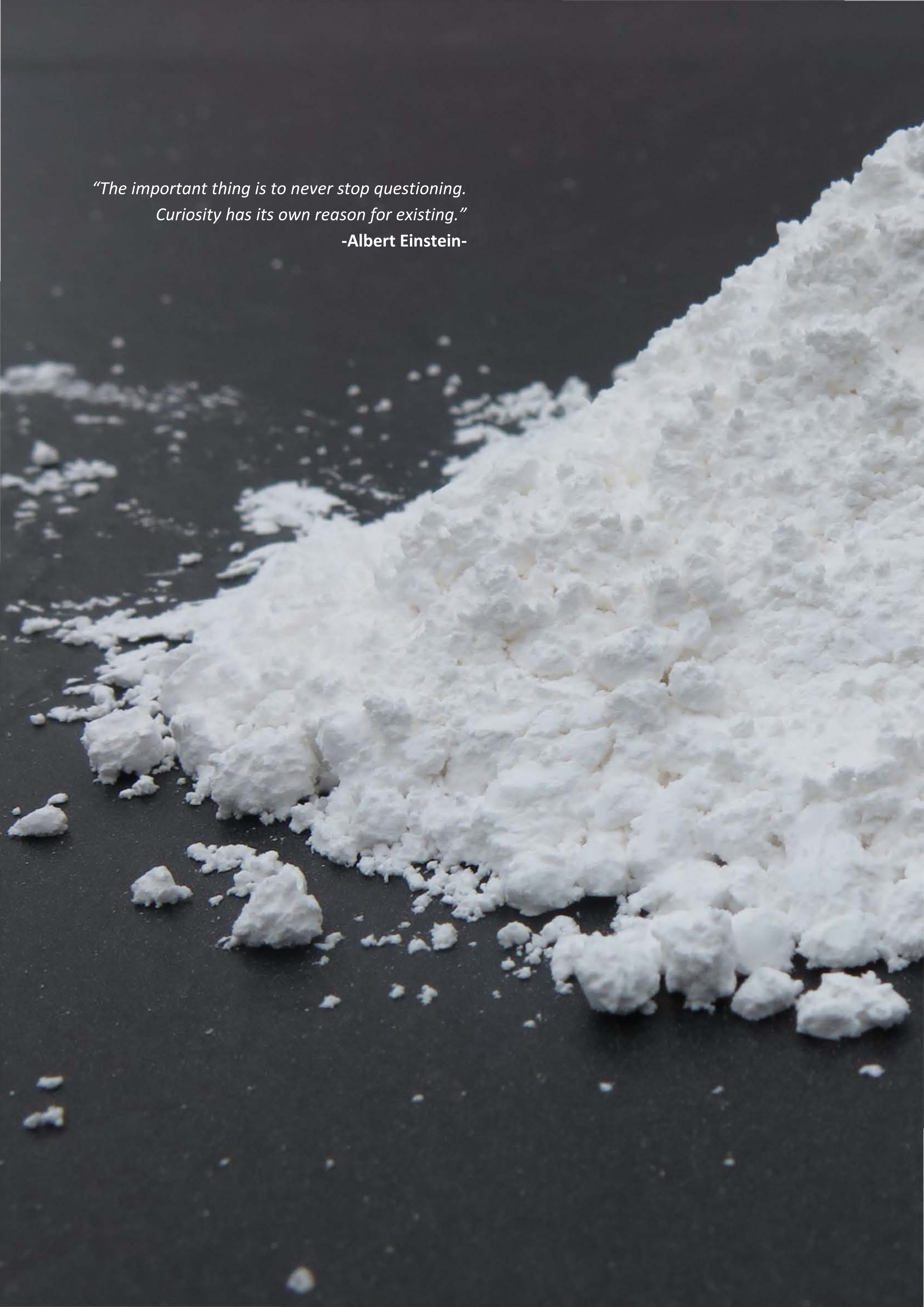
In Europe, zeolite is approved as an anticaking and anticoagulant feed additive (Directive 70/524/EC) for all species or categories of animals, for all feeding stuffs. Synthetic sodium aluminium silicate is also used as a food additive (E 554) (EFSA, 2004). In the United States, according to the United States Food and Drug Administration (FDA), zeolite A is also approved for use as a food additive. The FDA's GRAS (Generally Recognised As Safe) status is also awarded to pure clinoptilolite (potassium-calcium-sodium-aluminosilicate) zeolite products (US-FDA, 2006).

4 CONCLUSION

Once applied on the plant, the zeolite-based product forms a coating that fulfils many functions. The coating will have a double effect regarding water consumption. It may reduce regular evapotranspiration and it may increase photosynthetic efficiency. Particles of zeolite may also protect the surface of the plant from solar UVB/UVC radiation and reduce the superficial temperature. This reduces the risk of ‘sunburn’ injury (and subsequent crop losses), which also increases CO_2 solubility and RuBisCO yield. All these properties result in an increase in crop yield.

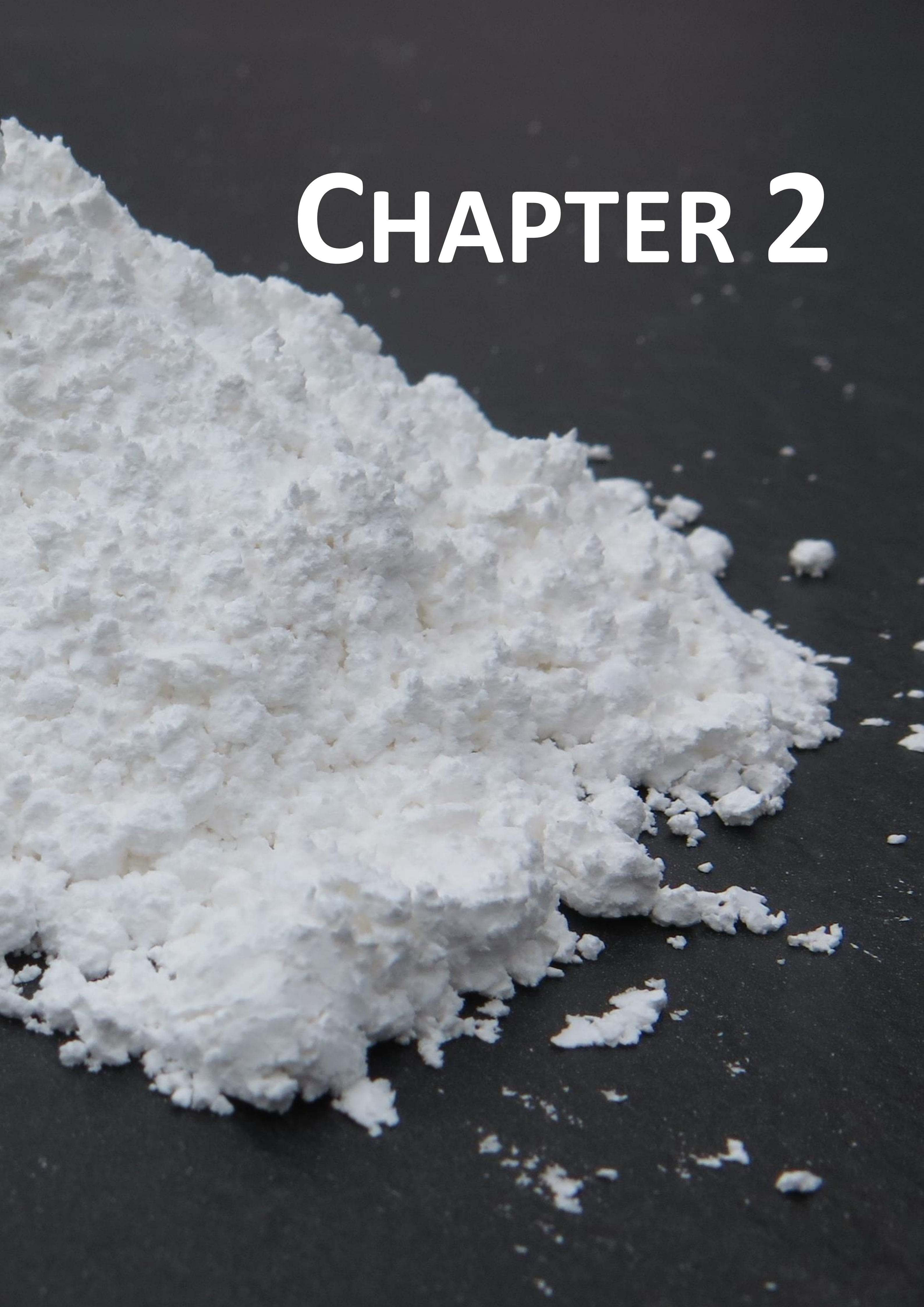
Besides their effects on the plant, these zeolites can also control pests and fungal diseases. The ability of the zeolite to adsorb water molecules from the plant’s surface (drying effect) may create a hostile environment for fungi, larvae and eggs, as the coating acts as a desiccant as soon as condensation takes place. On the other hand, active ingredients will endow the coating with persistent effects against pests. In addition, the coating may protect the plant from adult insects and other phytophagous arthropods, as the colouring and microscopic texture of the plant’s surface are altered.

Finally, when PPPs are released into the environment, most of their quantity is lost before even reaching the intended target. These PPPs can cause harm to human health and the environment. The high adsorption capacity of zeolites for other molecules besides water makes it possible to use them for controlled release. When zeolites are used as a carrier for PPPs, semiochemicals, plant extracts and microorganisms, this slow-release effect ensures a reduced need for these active substances. Whether or not they are combined with active substances, the use of zeolites for crop protection will reduce the amount of used fungicides, insecticides, etc. This will lead to safer, more efficient and more economical crop protection. In addition to the fact that in this manner zeolites are less harmful to the environment, zeolites are also innocuous substances in terms of human impact and toxicity.



“The important thing is to never stop questioning.
Curiosity has its own reason for existing.”
-Albert Einstein-

CHAPTER 2



Chapter 2

SELECTION OF ZEOLITES

As there are many zeolites on the market, including natural and synthetic zeolites, it is therefore very important to determine which zeolite is the most suitable for its application as PPP. The following chapter describes both the theoretical and experimental selection of zeolites. Part A discusses the theoretical selection, while Part B describes the experimental selection of the theoretically selected zeolites from Part A. Additionally, in Part C, a case-study compares the adsorption capacity of zeolites to those of other adsorbents in function of water treatment.

1 GENERAL INTRODUCTION

1.1 BACKGROUND

The term 'zeolite' was mentioned for the first time by the Swedish mineralogist A. F. Cronstedt in 1756, who discovered the so called stilbite. He observed that upon rapidly heating the material, a large amount of steam was obtained (Bogdanov, 2009a). In response to this phenomenon, the material was named 'zeolite', which is derived from the Greek words 'zeo' (boil) and 'lithos' (stone) (Bogdanov, 2009b). Since then, zeolites have been recognized as a separate group of minerals, one of the most abundant on earth.

Zeolites are crystalline, aluminosilicate minerals formed in nature when volcanic rocks and ash layers react with alkaline ground water. Generally, their structure can be considered as an inorganic polymer built from tetrahedral TO_4 units, where T is a Si^{4+} or Al^{3+} ion. Each O atom is shared between two T atoms, as shown in **Figure 2-1**.

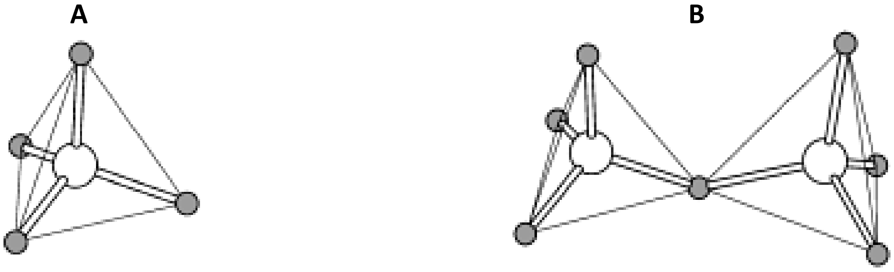


Figure 2-1. (A) TO_4 tetrahedron and (B) TO_4 tetrahedra sharing a common oxygen vertex (Xu et al., 2007).

The negative charge created by the presence of AlO_2^- is balanced by cations that neutralize the charge deficiency. These cations are weakly connected to the framework, which makes them as mobile species present in the pores of the zeolites. That is why these cations can be exchanged with other cations. It makes zeolites able to reversibly adsorb polar molecules (Denayer et al., 2011). Important for this ion exchange property is the Si/Al ratio. A zeolite with equal amounts of aluminium and silicon has each oxygen linked to one Al and one Si, and cavities contain a maximum density of exchangeable cations, which makes them ideal as ion exchangers and adsorbents (Ramesh et al., 2011).

1.2 NOMENCLATURE

There is no systematic nomenclature developed for zeolites and related materials. Information of the different framework types is published in the sixth edition of the Atlas of zeolite framework types and on the internet by the structure commission of the International Zeolite Association (IZA). To each unique structure type, which has been established based on the characteristic X-ray powder diffraction (XRD) patterns irrespective of their chemical composition, the IZA has assigned a code composed of three capital letters (Baerlocher et al., 2007). Review publications containing new tetrahedral frameworks are periodically announced on the IZA web pages and included in the internet version of the Atlas (Bellussi et al., 2012). As of February 2007, 176 zeolite framework types had been confirmed by the Structure Commission in the Atlas, but an update led to 231 framework types.

1.3 CLASSIFICATION

Zeolites are classified according to their framework structure, in addition to their chemical composition that can usually be considered variable. A description of a zeolite structure almost always begins with a description of the framework types and defines the size and shape of the pore openings, the dimensionality of the channel system, the volume and arrangement of the cages, and the types of cation sites available (McCusker and Baerlocher, 2001, 2007). Information on the framework type alone can elucidate many of the observed properties of a zeolite. Nonetheless, the chemical composition of the framework, the nature of the species within the channels, and the type of post-synthesis modification also play a very important role in determining the specific properties of a particular zeolitic material. Precise structural details, such as the nature of the distortion of a framework from ideal symmetry or the exact location of extra-framework species, are often needed to fully understand the properties of a specific zeolite (McCusker and Baerlocher, 2007).

1.4 STRUCTURE

Zeolites have a three dimensional, four-connected framework structure constructed from corner-sharing TO_4 tetrahedra, where T atoms generally refer to Si, Al or P atoms. In some cases, other atoms such as B, Ga, Be, Ge, etc., are also involved. These [SiO_4], [AlO_4] or [PO_4] tetrahedral are the basic structural building units or BBU. In other words, the TO_4 tetrahedra are referred to as the primary building units or PBU of zeolite structures, which are linked together to form secondary building units or SBU (**Appendix A**, **Figure A-1**).

These SBUs consist of n-ring structures, containing n tetrahedra, which refers to the size of the ring and characterizes the pore openings (McCusker and Baerlocher, 2005). In general, zeolites are classified as small-pore, medium-pore and large-pore systems according to the number of pore openings. The small-pore zeolites contain the pore opening enclosed by 8 TO_4 tetrahedra, with a diameter of about 4Å. Medium pore zeolites generally feature a 10-ring pore opening with a diameter of approximately 5,5Å and the large pore zeolites have pore openings formed by 12 TO_4 tetrahedra, with a diameter of about 7,5Å. The zeolites with pore openings comprising more than 12 T-atoms are called extra-large pore zeolites, but they are still rare. It is worth noting that 8-, 10- and 12-rings are common in zeolites and that the largest ring is limited to a 20-ring system (Xu et al., 2007).

Secondary building units can be linked to form chains, channels or cages (**Appendix A**, **Figures A-2**, **A-3** and **A-4**) within the structure (Xu et al., 2007). Chains are one-dimensional polyhedral building units that are frequently found among zeolite structures.

A channel is a pore that is infinitely extended in at least one dimension with a minimum aperture size (n ring) that allows guest molecules to diffuse along the pore. In many zeolites the channels intersect forming two- and three-dimensional channel systems. The dimension of the pore is one of the critical properties of zeolite materials, since this dimension determines the maximum size of the molecules that can enter from the exterior of the zeolite crystal into its micropores.

Cages are polyhedral units whose largest rings are too narrow to allow the passage of molecules larger than water. It is usually considered that 6-rings are the limiting ring size to form a cage. Cavities are polyhedral units that differ from cages by the fact that they contain windows that allow the passage of molecules in and out of the cavity. Cavities should not be infinitely extended and should be distinguished from other units such as pores and channels (Lobo, 2003).

1.5 EVOLUTION FROM NATURAL TO INDUSTRIAL MATERIAL

As mentioned in Chapter 1, zeolites can be natural or synthetic. Natural zeolites were first discovered in cavities and vugs of basalts, and at the end of the 19th century, they were also found in sedimentary rocks. With geological exploration and study on minerals, more and more natural zeolites have been discovered. Up till now, 48 types of natural zeolites have been found. Most zeolites known in nature are of lower Si/Al ratios, since organic structure-directing agents necessary for formation of siliceous zeolites are absent. That is why natural zeolites do not reach the high standards of the industry (Xu et al., 2007).

The period from 1954 to the early 1980s is the golden age for the development of zeolites. Zeolites with low (Si/Al = 1-1.5), medium (Si/Al = 2-5), and high (Si/Al = 10-∞) Si/Al ratios were extensively explored, which greatly facilitated the applications of zeolites and stimulated industrial progress. By the end of 1954, zeolites A and X became the first industrially produced zeolites by means of a hydrothermal crystallization. Despite the fact that many new zeolites were developed, these materials remain of huge academic and commercial interest. Zeolites A and X have the largest amount of cations and are excellent ion exchangers. In 1964, a number of companies in the United States imitated the formation of natural zeolites and produced a series of synthesized zeolites with an intermediate Si/Al ratio, including NaY, mordenite, zeolite L, erionite, chabazite, clinoptilolite, and many others. These zeolites were widely applied in the fields of gas purification and separation, and catalytic processes of petroleum refining and petrochemistry. Finally, zeolites with high Si/Al ratios were developed in the 1960s and 1970s. The best known example is ZSM-5. Even though the Al content is low, the acidity of the zeolites is adequate for hydrocarbon catalysis reactions. In contrast to natural zeolites, the acidity and acid strength of synthetic zeolites can be modified by changing the sample pretreatment, or preparation method, exchanging the cations, modifying the Si/Al ratio or by isomorphous substitution of Al^{3+} and Si^{4+} (Deca, 1998). Thermochemical modifications after the synthesis, such as in the production of zeolite Y, are of technological interest (Xu et al., 2007).

Synthetic zeolites are manufactured on a large scale for industrial use. However, over the past years, the demand for natural zeolites has increased steadily. This is mainly due to the growth in the use of natural zeolites in agricultural products, odour control and environmental applications (Cicala et al., 2001).

1.6 ZEOLITE SYNTHESIS

The synthetic zeolites are commercially more often used than natural zeolites due to the purity of crystalline products and the uniformity of particle sizes. The main advantages of synthetic zeolites over naturally-occurring zeolites are their ability to be engineered with a wide variety of chemical properties and pore sizes and their greater thermal stability.

Zeolite synthesis is well known to be a complex process. The rate of crystallization, types of products formed, and their particulate properties (habit, morphology, and crystal size distribution) depend on a large number of parameters. These parameters encompass the crystallization conditions (temperature, stirring, seeding, and gel aging) as well as composition-dependent parameters (pH, water content, the ratio between framework-forming elements, template concentration, and ionic strength) (Cubillas and Anderson, 2010).

Typically, the laboratory synthesis of zeolites is performed by hydrothermal means (**Figure 2-2**). In this approach, an amorphous silica source, an amorphous aluminum source and a mineralizing agent (most commonly an alkali metal hydroxide) are combined with water in a closed system and heated (between 50-200°C) under autogenous pressure. The hydrothermal reaction is characterized by the gradual transformation of the amorphous material to an approximately equal mass of zeolite crystals (which are recovered by filtration, washing and drying). Prior to this, the reaction mixture may be left for aging for a period of time (hours to a few days) (Cundy and Cox, 2005).

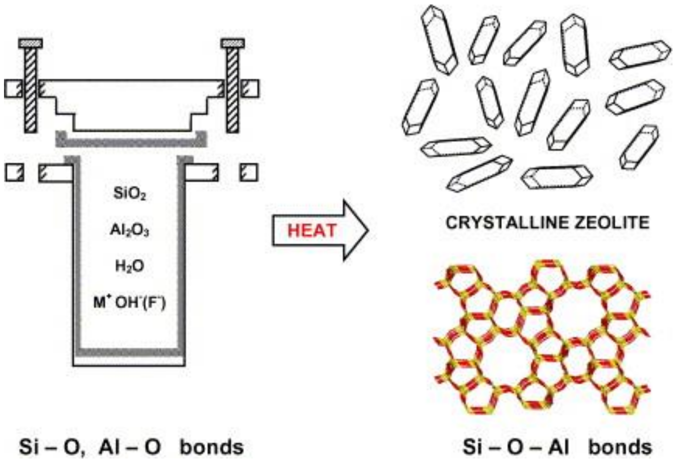


Figure 2-2. Hydrothermal zeolite synthesis (Cundy and Cox, 2005).

The main problem in zeolite research is the availability and cost of raw material, especially the silica source. On the other hand, commercial silica (made of sand), which are available in gel, sol, fumed or amorphous solid, are found to be variable in reactivity and selectivity. The preparation of synthetic zeolites from silica and alumina chemical sources is expensive. Yet, cheaper raw materials, such as clay minerals, natural zeolites, coal ashes, municipal solid waste incineration ashes and industrial slags are utilized as starting materials for zeolite synthesis. The use of waste materials in zeolite synthesis contributes to the mitigation of environmental problems, generally in the field of water purification, by removing heavy metals or ammonium, and therefore turns them into attractive and useful products (Georgiev et al., 2009).

PART A: THEORETICAL SELECTION

In Part A, a theoretical selection of the zeolites was made. The main focus was laid on particular characteristics, such as hydrophobicity, pore size, extra-framework cations and availability. The results of this study contribute to a better understanding of zeolite framework structures and were used for further experimental selection, as described in Part B.

A.1 INTRODUCTION

The large surface area of zeolites, as well as their excellent thermal and chemical stability, makes zeolites suitable materials for a large number of applications. Specific zeolites have to be chosen for a particular use depending on their particular characteristics such as hydrophilic character, pore volume, surface area, pore size, extra-framework cations present in the structure, and cost. The importance of zeolites to agricultural use can be attributed to a unique combination of the following properties, i.e. natural zeolite framework types, extra-framework cations, pore size, hydrophilic character, toxicity and availability.

Although synthetic zeolites have many advantages such as high purity, uniform pore size, and better ion exchange abilities, natural zeolites are more applicable when there are huge demands and fewer quality requirements. The reason is that natural zeolites are often located near the surface of the earth and can be easily exploited and used after some simple treatments, which lead to lower costs and hence lower prices. Therefore, natural zeolites have a good prospect of application, especially in the fields of agriculture and environmental protection (Xu et al., 2007). Since the main objective of this study was to use the zeolites in agriculture, the selection was focused on natural zeolites. Simultaneously, the harm to humans, animals and the environment was taken into account.

With the aim to use zeolites also as a carrier, the capacity of the microporous structure of the zeolite to adsorb molecules of active ingredients was explored. These adsorption characteristics of zeolites are dependent upon the detailed chemical/structural makeup of the adsorbent. The Si/Al ratio, cation type, number and location are particularly influential in adsorption. These properties can be changed by several chemical treatments to improve separation efficiency of raw natural zeolite. Acid/base treatment and surfactant impregnation by ion exchange are commonly employed to change the hydrophilic/hydrophobic properties for adsorption of various ions or organics (Ackley et al., 2003).

A.2 SELECTION OF ZEOLITES BASED ON THEIR PROPERTIES

A.2.1 NATURAL ZEOLITES

Table 2-1 gives an overview of all zeolites on the market in 2016. Each zeolite received a unique code. From all 231 different zeolite framework types, only 46 types are naturally occurring zeolites, all highlighted in green in **Table 2-1**. More details about the framework type codes are listed in **Appendix B** (**Table B-1**).

Table 2-1. Natural and synthetic zeolite framework types.

ABW	ACO	AEI	AEL	AEN	AET	AFG	AFI	AFN	AFO	AFR	AFS
AFT	AFV	AFX	AFY	AHT	**ANA**	APC	APD	AST	ASV	ATN	ATO
ATS	ATT	ATV	AVL	AWO	AWW	BCT	**BEA**	BEC	**BIK**	BOF	**BOG**
BOZ	BPH	**BRE**	BSV	CAN	CAS	CDO	CFI	CGF	CGS	**CHA**	**-CHI**
-CLO	CON	CSV	CZP	**DAC**	DDR	DFO	DFT	DOH	DON	**EAB**	**EDI**
EEI	EMT	**EON**	**EPI**	**ERI**	ESV	ETR	EUO	-EWT	EZT	FAR	**FAU**
FER	FRA	**GIS**	GIU	**GME**	**GON**	**GOO**	**HEU**	IFO	IFR	IFU	IFW
-IFY	IHW	IMF	IRN	IRR	-IRY	ISV	ITE	ITH	ITG	-ITN	ITR
ITT	-ITV	ITW	IWR	IWS	IWV	IWW	JBW	JNT	JOZ	JRY	JSN
JSR	JST	JSW	KFI	**LAU**	**LEV**	LIO	-LIT	LOS	**LOV**	LTA	LTF
LTJ	**LTL**	LTN	**MAR**	**MAZ**	MEI	MEL	MEP	**MER**	**MFI**	MFS	**MON**
MOR	MOZ	MRE	MSE	MSO	MTF	MTN	MTT	MTW	MVY	MWF	MWW
NAB	**NAT**	**NES**	NON	NPO	NPT	NSI	OBW	**OFF**	OKO	OSI	OSO
OWE	**-PAR**	**PAU**	PCR	**PHI**	PON	POS	PSI	PUN	**RHO**	**-RON**	RRO
RSN	RTE	RTH	RUT	RWR	RWY	SAF	SAO	SAS	SAT	SAV	SBE
SBN	SBS	SBT	SEW	SFE	SFF	SFG	SFH	SFN	SFO	SFS	SFV
SFW	SGT	SIV	SOD	SOF	SOS	SSF	-SSO	SSY	STF	**STI**	STO
STT	STW	-SVR	SVV	SZR	**TER**	**THO**	TOL	TON	**TSC**	TUN	UEI
UFI	UOS	UOV	UOZ	USI	UTL	UWY	VET	VFI	VNI	**VSV**	**WEI**
-WEN	**YUG**	ZON									

A.2.2 EXTRA-FRAMEWORK CATIONS

The negative charge in the framework of natural zeolites is, in most cases, balanced by mono and divalent exchangeable cations, such as H, Na, Ca and K. Additionally, other cations may also be present, i.e. Li, Mg, Ba, Sr, Be, Cs, Rb, B, Fe and Mn. However, a high concentration of cations often leads to adverse effects on humans, animals and the environment.

Considering the use of zeolites in agriculture, the essential and toxic elements for plants were taken into account. Plants require a complex balance of mineral nutrients to grow and reproduce successfully. In addition to water (H_2O), oxygen (O_2) and carbon dioxide (CO_2), 14 mineral elements are essential to all plants (Mengel et al., 2001). Among them, nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), sulfur (S) and magnesium (Mg) are required in relatively large amounts (> 1000 $mg.kg^{-1}$ dry weight) and are therefore defined as macro-elements. In contrast, chlorine (Cl), iron (Fe), boron (B), nickel (Ni), copper (Cu), manganese

(Mn), zink (Zn) and molybdenum (Mo) are needed in smaller amounts (< 100 mg.kg^{-1} dry weight) and are called micronutrients or trace elements (DalCorso et al., 2014).

To exclude most negative effects, zeolites containing more than 1% of non-essential cations for plants were eliminated and marked in red in **Table 2-2**. More details about the composition of the different zeolites are listed in **Appendix B** (**Table B-2**).

Table 2-2. Natural zeolite framework types containing only plant essential exchangeable cations.

ANA	BEA	**BIK**	BOG	**BRE**	CHA	**-CHI**	DAC	**EAB**	**EDI**	EON	EPI
ERI	FAU	FER	GIS	GME	GON	GOO	HEU	LAU	LEV	**LOV**	**LTL**
MAR	MAZ	**MER**	MFI	MON	MOR	**NAB**	NAT	NES	OFF	-PAR	PAU
PHI	**RHO**	**-RON**	STI	TER	THO	**TSC**	**VSV**	**WEI**	YUG		

A.2.3 PORE SIZE

The pore size of zeolites is also a very important property that must be taken into account. The arrangement of the structural unit in zeolite frameworks results in the generation of pores and cavities of various dimensions, responsible for the molecular sieving and confinement effects during adsorption of molecules. Considering the use of zeolites as carrier for PPPs, a large pore size is very important. Therefore, zeolites with small and medium pores were excluded and highlighted in red in **Table 2-3**. More details about the pore size of the selected zeolites are listed in **Appendix B** (**Table B-3**).

Table 2-3. Natural zeolite framework types with large and extra-large pores.

ANA	BEA	BOG	CHA	**DAC**	EON	**EPI**	ERI	FAU	FER	**GIS**	GME
GON	**GOO**	HEU	LAU	LEV	MAZ	MFI	**MON**	MOR	**NAT**	NES	OFF
-PAR	PAU	**PHI**	STI	TER	**THO**	**YUG**					

A.2.4 HYDROPHILIC CHARACTER

The key physical property of every adsorbent is the surface hydrophobicity (Sakut et al., 1998). Adsorbents that have a polar surface are called hydrophilic, since they adsorb highly polar molecules such as H_2O. In contrast, adsorbents with a non-polar surface are termed hydrophobic, since they usually adsorb non-polar molecules as there is no electrostatic interaction, which causes water to be held only very weakly on the surface of the adsorbent. Because the physical properties of zeolites are dependent on the aluminium content in the zeolite, the hydrophobicity can be varied by changing the silicon to aluminium ratio.

Based on the Si/Al composition, Flanigen (2001) classified zeolites as follows: low silica/hydrophilic zeolites (Si/Al 1-1.5), intermediate silica/intermediate hydrophobic (Si/Al 2-5) and high silica/hydrophobic zeolites (Si/Al > 10). The zeolitic hydrophilic property increases as the aluminium content in the zeolite framework increases and vice versa. The hydrophobic/hydrophilic nature of zeolites also appears to depend on their framework structure (Cekova et al., 2006).

Generally, zeolites are highly hydrophilic sorbents due to their electrostatic charged framework and the abundance of extra-framework cations. The reference zeolite type LTA, used in the ECO-ZEO project, was a hydrophilic zeolite (Si/Al = 1). In order to compare this hydrophilic zeolite with more hydrophobic zeolites, the low silica or hydrophilic zeolites (Si/Al < 2) were removed from the list and marked in red in **Table 2-4**. Hydrophobic zeolites were also preferred, taken into account the possible use of zeolites as carrier material. More details about the Si/Al ratio of the selected zeolites are listed in **Appendix B** (**Table B-4**).

Table 2-4. Natural zeolite framework types with medium and high Si/Al ratio.

BEA	BOG	CHA	EON	ERI	FAU	FER	GME	**GON**	HEU	LAU	LEV
MAZ	MFI	MOR	NES	OFF	PAU	STI	TER				

A.2.5 OTHERS

A last selection was made based on the crystal and channel system of the zeolite types, as well as on their toxicity and availability properties. More details are listed in **Appendix B** (**Table B-5**). First of all, the toxicity of the zeolite types was taken into account. As a result, ERI and OFF were removed from the list because of their carcinogenic properties. Subsequently, the selection was based on the crystal and channel system of the zeolites.

Zeolite types with a cubic crystal structure include FAU and PAU. Both zeolite types are rare, but the natural zeolite faujasite (FAU) gained attention because it has the same framework topology as Linde X and Linde Y, synthetic counterparts applied as sorbents and catalysts. Faujasite also corresponds to the most open framework of all natural zeolites. For these reasons, FAU was selected and PAU was removed from the list.

After removing ERI and OFF from the list of hexagonal zeolites, GME, LEV and MAZ were also not taken into account because of their limited availability and lack on suppliers.

Subsequently, HEU, LAU, NES and STI are zeolite types with a monoclic crystal structure. Due to the number of zeolites that have this crystal structure, a first selection was made based on their availability. Two zeolites from the framework type HEU and one zeolite from the framework type STI are widely found and used in industry. Because their large-scale presence, these materials will also be cheaper. Heulandite and clinoptilolite belong to the same framework type HEU, with their Si/Al ratio as the only difference. Therefore, clinoptilolite was selected because of its higher Si/Al ratio. Despite STI is widely found and used in industry, it was not taken into account because of difficulties in obtaining small amounts of this zeolite.

Orthorhombic zeolites include framework types BOG, EON, FER, MFI, MOR, TER and the unassigned zeolite tvedalite. The highest Si/Al ratio in natural zeolites was found in MFI, making this zeolite framework type was selected (Schüth, 2001). Additionally, zeolite framework type MOR was the only zeolite type with a one-dimensional (1D) channel system that was widely found and used in industry. Therefore, both MFI and MOR were selected of the zeolite types with an orthorhombic crystal structure.

Finally, zeolite types CHA and BEA were the only remaining zeolite types having a rhombohedral and tetragonal crystal structure, respectively. **Table 2-5** presents an overview of the non-selected zeolite types, all marked in red.

Table 2-5. Final selected natural zeolite framework types.

BEA	**BOG**	CHA	**EON**	**ERI**	FAU	**FER**	**GME**	HEU	**LAU**	**LEV**	**MAZ**
MFI	MOR	**NES**	**OFF**	**PAU**	**STI**	**TER**					

Only natural zeolites were taken into account during the selection because of their use in agriculture. Concerning the availability and lack of suppliers of some zeolite types, it became necessary to order their synthetic counterparts. More information about these zeolites is discussed in Part B of this chapter.

PART B: EXPERIMENTAL SELECTION

After selecting 6 zeolite framework types in Part A, the adsorption and phytotoxic characteristics of these zeolites need to be studied in order to meet the requirements that are discussed and tested in this part.

B.1 INTRODUCTION

As described in Chapter 1, zeolites can be used as a carrier for PPPs, semiochemicals/plant extracts and microorganisms. When zeolites have a low activity as single substance, it can be useful to combine them with other active ingredients. Therefore, the adsorption of PPP on zeolites was considered as a first variable to make an experimental selection.

Adsorption is a process in which molecules from gas/liquid phase (adsorbate) attach to the surface of a solid adsorbent. The general mechanisms of adsorption are physical adsorption and chemisorption (Kolasinski, 2012).

Physical adsorption is driven by weak intermolecular forces, such as Van der Waals forces. It is important to note that physical adsorption does not entail the formation of chemical bonds or electrostatic interactions, i.e. there is no exchange of electrons. These Van der Waals forces include dipole/dipole forces and London forces. Because of these weak forces, physisorption is usually a reversible process. Multilayer adsorption is possible, i.e. solutes adhering onto already sorbed solutes.

In contrast, chemisorption involves the formation of a chemical bond between the adsorbate and the adsorbent. This results in a strong attractive force that cannot easily be countered, which means that chemisorption is essentially a non-reversible process. Due to the need of a chemical bond in chemisorption, only one layer of solutes can attach onto the adsorbent (monolayer adsorption).

However, the dominant adsorption mechanism differs for different types of adsorbents. It was found that hydrophobic interactions, π-π bonds, electrostatic interactions and hydrogen bonds are important mechanisms in the adsorption process (Bo and Baoshan, 2008).

Subsequently, the ability of zeolites to adsorb water was taken into account. Zeolites are able to reduce water stress of plants by increasing resistance to water vapour loss. Additionally, by absorbing water, zeolites can serve as a physical barrier that prevents disease inoculums from directly contacting the leaf surface.

Various authors have related zeolite hydrophobicity to aluminum content, i.e. a higher aluminum content indicates a higher affinity for water (Kawai et al., 1994; Olsen et al., 2000; Bolis and Busco, 2006). Specific mechanisms for zeolite-water interaction are proposed by Bolis and Busco (2006), i.e. electron pair donor-acceptor interactions. This occurs at Lewis acid sites, Brønsted acid sites and silanol sites.

Finally, it is important that the used zeolites do not show phytotoxic properties. One of the basic requirements of any PPP is that the product itself should not harm the crop. This is particularly relevant to the herbicides, but must also be demonstrated for the other PPPs such as fungicides and insecticides. At this stage in the selection of potential zeolite products, these selected zeolites need to be tested to ensure no phytotoxicity. Although zeolites are classified as 'inert', the high application dose of 60 kg.ha^{-1} in the field might lead to phytotoxicity.

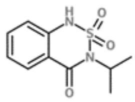
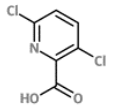
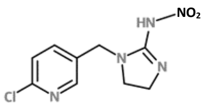
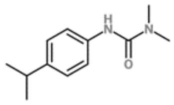
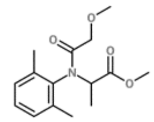
The aim of this study was to evaluate the potential of zeolites to adsorb a number of PPPs from aqueous medium. Therefore, the followinging properties were investigated on several zeolites with different framework structures, i.e. adsorption characteristics including kinetics, isotherms and adsorption energies of five PPPs (bentazon, clopyralid, imidacloprid, isoproturon and metalaxyl-M) with different physicochemical characteristics. The water holding capacity was also measured. Moisture isotherm curves were used to understand the water interactions within materials. Phytotoxicity tests were carried out to assess the potential effects on target crops when depositing zeolites on the leaves and above-ground portions of plants.

B.2 EXPERIMENTAL ANALYSIS

B.2.1 PLANT PROTECTION PRODUCT SELECTION (ADSORBATES)

The studied PPPs were selected based on their physicochemical characteristics and were divided into five categories based on their mobility (K_{oc}) (PPDB, 2014). In addition, the groundwater ubiquity score (GUS) indicator was used, which describes the mobility of the PPPs (Gustafson, 1989). A GUS indicator lower than 1.8 indicates an immobile PPP (De Wilde et al., 2008). One or more PPPs were selected from the mobile category to be used in batch adsorption experiments. Clopyralid (Sigma Aldrich) was selected as a strongly mobile PPP and bentazon (Sigma Aldrich) as a mobile PPP. PPPs selected from the moderately mobile category were imidacloprid (Bayer Crop Protection) and isoproturon (Sigma Aldrich). Metalaxyl-M (Syngenta Crop Protection) was selected as a slightly mobile PPP. The chemical structures of these technical-grade PPPs and their chemical properties are shown in **Table 2-6**. The test solutions were prepared by diluting the stock solutions to the desired concentration of 10 mg.l^{-1}.

Table 2-6. Plant protection product properties.

	Bentazon	Clopyralid	Imidacloprid	Isoproturon	Metalaxyl-M
Molecules					
Purity	97%	100%	99%	99.5%	96.6%
Size (Å)[a]	<3x10x5	<3x7x7	<10x6x5	<3x12x6	<3x12x7.6
Chemical formula	$C_{10}H_{12}N_2O_3S$	$C_6H_3Cl_2NO_2$	$C_9H_{10}ClN_5O_2$	$C_{12}H_{18}N_2O$	$C_{15}H_{21}NO_4$
MM[b] (g mol^{-1})	240.3	192.0	255.66	206.28	279.33
S_{water}[b] (20°C; mg l^{-1})	570	143000	610	70.2	26000
Log K_{ow}[b] (20°C; pH7)	-0.46	-2.63	0.57	2.5	1.71
K_{oc} or K_{foc}[b] (ml g^{-1})	55.3	5.0	225	122	660
GUS	2.59	3.44	3.69	2.61	1.88
pKa	3.28	2.01	-	-	-

[a] All molecular size calculations were performed using the Gaussian09 program (Frisch et al., 2009)

[b] **MM**: Molecular weight; **S_{water}**: Water solubility; **Log K_{ow}**: Octanol-water partition coefficient**; K_{oc} or K_{foc}**: Adsorption coefficient; **pKa**: Acid dissociation constant

B.2.2 ZEOLITE SELECTION (ADSORBENTS)

Six commercially available zeolite structures were selected in Part A of this chapter (Section A.2), including zeolite beta (BEA), chabazite (CHA), zeolite Y (FAU), clinoptilolite (HEU), ZSM-5/silicalite-1 (MFI) and mordenite (MOR). All these zeolites were applied in this study, together with zeolite type LTA, which was used in the ECO-ZEO project. The varying arrangement of the structural unit in zeolite frameworks results in the generation of pores and cavities of various dimensions, responsible for the confinement effects during adsorption of molecules. Two quantities of particular interest for characterizing pores are the largest cavity diameter (LCD) and the pore-limiting diameter (PLD). The LCD corresponds to the maximum of the pore-size distribution and the PLD corresponds to the largest characteristic guest molecule size for which there is a non-zero accessible volume (**Table 2-7**; Baerlocher and McCusker, 2014). These quantities are also plotted in **Figure 2-7**. The utility of such a plot is apparent for the consideration of a chemical reaction. A desirable zeolite would require a PLD larger than all species involved and an LCD larger than the transition state structure (First et al., 2014).

Table 2-7. Accessibility of the different zeolites.

Material name	Framework type code	Crystal structure	Channel structure	Pore volume (cm³ g⁻¹)	LCD (Å)	PLD (Å)
Zeolite beta	BEA	Tetragonal	3D	0.416	6.9	6.7
Chabazite	CHA	Rhombohedral	3D	0.434	8.0	4.2
Zeolite Y	FAU	Cubic	3D	0.506	11.9	6.7
Clinoptilolite	HEU	Monoclinic	2D	0.279	6.6	4.3
Zeolite 4A	LTA	Cubic	3D	0.508	11.7	4.9
ZSM-5/Silicalite-1	MFI	Orthorhombic	3D	0.242	7.0	5.0
Mordenite	MOR	Orthorhombic	1D	0.293	6.5	6.5

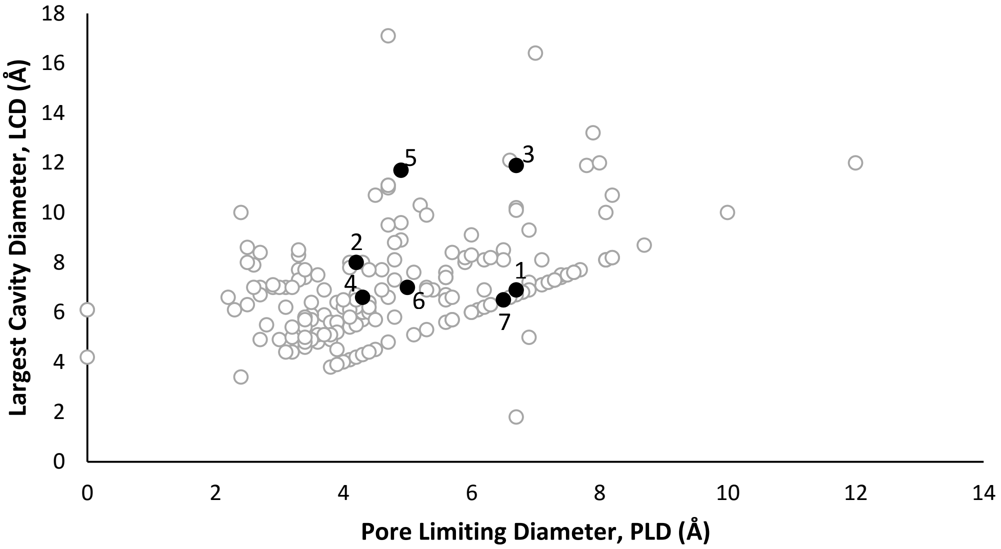


Figure 2-7. Largest cavity diameter (LCD) plotted against pore limiting diameter (PLD) for structures in the 'Database of zeolite structures', with (1) BEA, (2) CHA, (3) FAU, (4) HEU, (5) LTA, (6) MFI and (7) MOR (Baerlocher and McCusker, 2014; First et al., 2014).

B.2.3 ZEOLITE CHARACTERIZATION

The Brunauer-Emmett-Teller (BET) specific surface area of the zeolites was determined using nitrogen adsorption/desorption measurements (**Appendix C**, **Figure C-1**). The isotherms were recorded on a BELSORP Mini II equipment (Bel Japan Inc, Osaka, Japan) at -196°C. The samples were pre-treated at 150°C under vacuum. The results for the different selected zeolites are presented in **Table 2-8**.

X-ray diffraction (XRD) measurements were performed with an ARL X'TRA X-ray diffractometer (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a Cu KR1 tube and a Peltier cooled lithium-drifted silicon solid stage detector. The XRD patterns of the different zeolites showed their characteristic peaks.

Finally, the Si/Al ratio of the samples was determined by X-ray fluorescence (XRF) analysis (**Table 2-8**). This analysis is based on the emission of X-rays by the material when an electron drops down to a vacant level and releases energy. The measurements were performed on an NEX CG (Rigaku) using a Mo-X-ray source.

Table 2-8. Zeolite properties.

FTC		**Si/Al** $_{\text{practical}}$	S_{BET} ($m^2.g^{-1}$) $_{\text{practical}}$	**Product name**
BEA	1	11.84	365	H-BEA-25[1]
	2	15.87	413	H-BEA-35[1]
	3	49.34	348	CP 811C-300[2]
CHA	4	3.08	8[a]	Siliz MFD CH200[3]
	5	3.12	11[a]	Siliz MFD CH70[3]
FAU	6	15.40	661	CBV 720[4]
	7	32.91	642	CBV 780[4]
HEU	8	5.60	21[a]	Slowakije[5]
	9	5.45	23[a]	Turkije[5]
	10	5.58	11[a]	Hungary[6]
LTA	11	1.15	1[a]	Wetstop[7]
	12	1.12	2[a]	Zeolite 4A pH8[7]
MFI	13	13.19	8[a]	TZP-302[8]
	14	30.19	314	H-MFI-90[8]
	15	57.54	198	H-CZP-300[8]
	16	17.83	6[a]	NH_4-CZP-55[8]
	17	/	266	H-CZP-800[8]
MOR	18	6.64	16	H-MOR-14[8]
	19	10.53	34	H-MOR-20[8]

[a] External surface area

Supplier: [1] Clariant, Germany; [2] Zeolyst, America; [3] Somez, France; [4] Zeolyst, Netherlands; [5] Zeoliet, Belgium; [6] Terra Humana, Hungary; [7] FMC, Spain; [8] Clariant, Germany.

B.2.4 ADSORTION EXPERIMENTS

Adsorption experiments, performed in a batch reactor system, were conducted in three ways, namely, dependent on adsorption (percentage), time (kinetic) and concentration (isotherm). The suspensions (adsorbent and PPP aqueous solution) were shaken on an orbital shaker at 150 rpm at room temperature (22°C). The supernatants were then filtered with a syringe filter containing a polyvinylidene difluoride (PVDF) membrane with a pore size of 0.22 µm (Carl Roth, Karlsruhe-Rheinhafen, Germany). The aliquots were injected into the high-performance liquid chromatography (HPLC) column with photodiode array detector (DAD) for PPP concentration measurements. The amounts adsorbed were determined from the initial and final concentrations of the contaminant solution. All tests were carried out in triplicate and control runs containing the PPP but without the addition of an adsorbent were also included.

B.2.4.1 SELECTION BASED ON ADSORPTION PERCENTAGE

Adsorption kinetics and isotherms were not determined for all zeolites, but only for those that best adsorbed the PPPs. For these tests, 0.1 ± 0.001 g zeolite was shaken in 100 ml conical flasks with 20 ml of 10 mg.l^{-1} solution of the respective PPP. The adsorption percentage of PPPs on zeolites was calculated as follows:

$$\text{Adsorption (\%)} = \frac{C_i - C_f}{C_i} \times 100$$

where C_i and C_f are the initial and final PPP concentrations (mg.l^{-1}), respectively.

B.2.4.2 SORPTION KINETICS

The adsorption kinetic study predicts the rate at which adsorption takes place. An amount of 0.1 ± 0.001 g of the respective zeolite was added to 100 ml of 10 mg.l^{-1} solution of the respective PPP in a 250-ml flat-bottomed flask. The samples were collected after 0, 0.25, 0.50, 1, 3, 6, 24 and 48 hours. The most commonly used kinetic models, that is, the pseudo-first-order equation (Lagergren's equation) and the pseudo-second-order equation (Ho equation), were applied to the obtained experimental data. The equations can be described as shown in **Table 2-9**, where k_1 and k_2 are the rate constants of first-order adsorption (hour^{-1}) and second-order adsorption (hour^{-1}), and q_e and q_t are the adsorbed concentration (mg.g^{-1}) at equilibrium and at time t (hour).

Table 2-9. Pseudo-first-order equation (Lagergren) and pseudo-second-order equation (Ho).

	Differential equation	**Integration: q_t=0, t=0 and q_t=q_t, t=t**	**Amount adsorbed (mg g^{-1}) at time t (h)**
1st order eq.	$\frac{dq_t}{dt} = k_1(q_e - q_t)$	$\ln(q_e - q_t) = \ln q_e - k_1 t$	$q_t = q_e(1-e^{-k_1 t})$
2nd order eq.	$\frac{dq_t}{dt} = k_2(q_e - q_t)^2$	$\frac{t}{q_t} = \frac{1}{q_e^2 k_2} + \frac{1}{q_e} t$	$q_t = \frac{t}{\frac{1}{k_2 q_e^2} + \frac{t}{q_e}}$

The adsorbed PPP concentration was calculated as follows:

$$q_e = \frac{(C_i - C_e)V}{m}$$

where q_e represents the adsorbed concentration (mg.g^{-1}), V the volume of PPP solution added (l), m the weight of the adsorbent (g) and C_i and C_e are the initial and equilibrium PPP concentrations (mg.l^{-1}), respectively.

B.2.4.3 SORPTION ISOTHERMS

An adsorption isotherm is a simple description of the performance of an adsorbent for the removal of a given target substance. From this isotherm, the adsorption capacity can be derived, which makes it possible to compare the adsorption capacities of the different adsorbents for pollutants in aqueous solutions. The adsorption isotherms of the PPPs on the zeolites were also measured with the batch equilibration technique. These adsorption isotherms provide information about the surface properties and affinity of the adsorbent. In this experiment, 100 mg zeolite was added to 100 ml of PPP solution at five different concentrations (50, 100, 250, 500 and 1000 mg.l^{-1}). All stock solutions were prepared in distilled water. Adsorption isotherms were obtained by plotting the amount of PPP adsorbed by the substrate (g.kg^{-1}) versus the respective concentration in equilibrium solution (mg.l^{-1}). The experimental adsorption isotherms may conveniently be grouped into six classes according to the International Union of Pure and Applied Chemistry (IUPAC) classification (**Figure 2-8**). The first five characteristic types were originally proposed by Brunauer and his co-workers as the Brunauer, Deming, Deming and Teller (BDDT) classification (IUPAC, 1985).

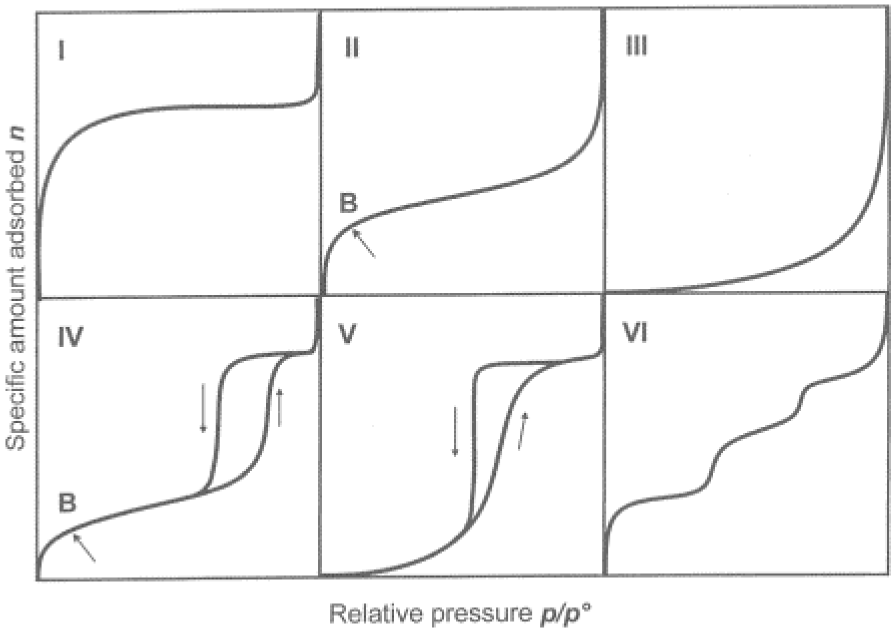


Figure 2-8. The IUPAC classification for adsorption isotherms (IUPAC, 1985).

Type I (Langmuir, **Table 2-10**) isotherm is observed during the adsorption on a microporous solid (pore widths < 2 nm). It represents an adsorption process with a low affinity for the adsorbate and the plateau indicates monolayer coverage. This type of behaviour is typical for chemisorption, where the asymptotic approach to a limiting quantity indicates that all of the surface sites are occupied.

Type II isotherms, observed in physical adsorption, are most frequently encountered when adsorption occurs on non-porous or macroporous powders. The inflection point or knee of the isotherm is called ‘point B’. This point indicates the stage at which monolayer coverage is complete and multilayer adsorption begins to occur.

Type III (Freundlich, **Table 2-10**) isotherms, given by non-porous or macroporous solids, are the characteristic of weak attractive forces. This weakness causes a small uptake in the beginning, but once a molecule has been adsorbed, the adsorbate-adsorbate forces will promote the adsorption of further molecules.

Type IV isotherms occur on mesoporous materials, describing monolayer and multilayer adsorption. The increase in slope indicates an increased uptake of adsorbate as the pores are being filled. The knee, point B, of the type IV isotherm generally occurs near the completion of the first monolayer (i.e. near the onset of multilayer adsorption). A characteristic feature of the type IV isotherm is its hysteresis loop, which is associated with the occurrence of pore condensation. In the simplest case, the initial part of isotherm follows exactly the same path as the corresponding part of type II in a non-porous form.

Type V isotherms are given by microporous or mesoporous solids and show pore condensation and hysteresis. However, in contrast to type IV, the initial part of this adsorption isotherm is very much similar to type III, indicating relatively weak attractive interactions between the adsorbent and the adsorbate.

Type VI isotherms are borderline cases between two or more of the aforementioned types. This stepped isotherm is relatively rare and is associated with layer-by-layer adsorption on a highly uniform surface. The sharpness of the steps is dependent on the system (Rouquerol et al., 2014).

Table 2-10. Isotherm equations.

	Non-linear form	Linear form
Freundlich	$q_e = K_F\ C_e^{1/n}$	$\log q_e = \log K_F + \frac{1}{n} \log C_e$
Langmuir	$q_e = \frac{q_m\ K_L\ C_e}{1+ K_L\ C_e}$	$\frac{1}{q_e} = \frac{1}{q_m\ K_L}\ \frac{1}{C_e} + \frac{1}{q_m}$

where q_e ($mg.g^{-1}$) is the amount of PPP sorbed at equilibrium concentration C_e ($mg.l^{-1}$). K_F ($mg.g^{-1}$) and n are Freundlich constants, representing the maximum sorption capacity and sorption strength or intensity respectively. q_m ($mg.g^{-1}$) and K_L ($l.g^{-1}$) are the Langmuir isotherm constants, representing the maximum sorption capacity and the energy constant related to the heat of adsorption respectively.

The essential features of the Langmuir isotherm may be expressed in terms of the separation factor R_L. Based on the shape of the isotherm of an adsorption system, this dimensionless constant predicts whether the system is favourable or unfavourable (Ghaemi et al., 2011). The value of the separation factor can be interpreted as given in **Table 1-11**.

$$R_L = \frac{1}{1+K_L C_i}$$

Table 2-11. Separation factor based on the shape of the Langmuir isotherm.

Value R_L	Type of adsorption
$R_L > 1.0$	Unfavorable
$R_L = 1.0$	Linear
$0 < R_L < 1.0$	Favorable
$R_L = 0$	Irreversible

B.2.5 HYGROSCOPICITY

A measure of the hygroscopicity of a product is a consequence of the magnitude of the increase or decrease in its water content as a function of relative humidity at a certain temperature. The generation of the moisture isotherms will give more information about the hygroscopic effect of zeolites and describe their relationship with water content. These adsorption isotherms were measured with a fully automated vapour adsorption analyzer (Decagon Devices Inc, Pullman, WA, USA).

The double log polynomial (DLP) model was used to predict the moisture content at a given water activity (a_w) (Decagon Devices, 2015).

$$DLP_m = b_3\chi^3 + b_2\chi^2 + b_1\chi + b_0$$

where DLP_m is the moisture in g.100 solids^{-1} or g.g^{-1} solids, $\chi = \ln[-\ln(a_w)]$ and b_0 - b_3 are empirical constants.

The moisture content value was introduced for calculating weight change at the different a_w values. The water holding capacity (WHC) is the difference between the weight at maximum a_w value and the weight at minimum a_w value divided by the initial weight.

$$WHC = (\text{Weight } a_{w,max} - \text{Weight } a_{w,min}) / \text{Weight}_{\text{initial}}$$

The temperature was 25°C, $a_{w,min} = 0{,}125$ and $a_{w,\,max} = 0{,}900$. The water mass (or weight) is the difference between the weights of the wet and oven-dry samples (24 hours, 105°C).

B.2.6 PHYTOTOXICITY

Zeolites with the best adsorption and water holding capacities were selected en studied for their phytotoxic effects. These results were also compared to the phytotoxic effect of the natural product kaolin. The Phytotestkit® was used to estimate these phytotoxic effects, by measurement of seed germination and root growth of three plant species (Microbiotests Inc, 2008). Phytotestkits® are a variant of Phytotoxkits®, which are used extensively for toxicity testing of contaminated soils, sewages, sludges and sediments (Microbiotests Inc, 2008; Plaza et al., 2005; Czerniawska-Kusza et al., 2006; Oleszczuk, 2008a, 2008b; Samaras et al., 2008; Blok et al., 2009).

B.2.6.1 PLANTS

The three plant species used in the Phytotestkit® setup are *Sorghum saccharatum* (sorghum), *Sinapis alba* (white mustard) and *Lepidium sativum* (garden cress). *Sorghum saccharatum* is an annual grass. It is commonly cultivated as a fodder plant or is used for its grains in food or for industrial purposes, e.g. for beer brewing and production of starch for various purposes (in glue, in paper making, as a food additive, etc.). *Sinapis alba* (white mustard) is an annual plant of the family *Cruciferae*. It is grown as a fodder crop or as green manure. *Lepidium sativum* (garden cress) is an edible herb that is known for its fast germination.

B.2.6.2 PHYTOTESTKIT®

Each kit contains flat transparent test plates that are divided into two compartments. The lower compartment is filled with a foam layer and two layers of filter paper, one thick white filter paper layer and one fine black filter paper, which are soaked with a test solution (20 ml). All zeolites were tested at a concentration of 100, 1000 and 10000 mg.l^{-1}. Subsequently, 10 seeds of each of the three selected plant species were positioned on the plate upon the black filter paper at equal distances from one another upon the black filter paper, near the ridge that divides both compartments. The upper compartment was left empty to allow growth of the shoots. After closing the test plates with a transparent cover, the plates were vertically placed in a holder and put in an incubator at 25°C for an incubation period of 3 days. The length of the incubation period was chosen in function of the speed of germination of the seeds and of the speed of growth of the roots and shoots, which are both plant-dependent. Nine plates were filled per test solution (three plates per plant species, each with 10 seeds) and compared with the results of nine plates filled with distilled water, which served as a blank. **Figure 2-9** shows a view of three stages of the Phytotestkit experimental setup.

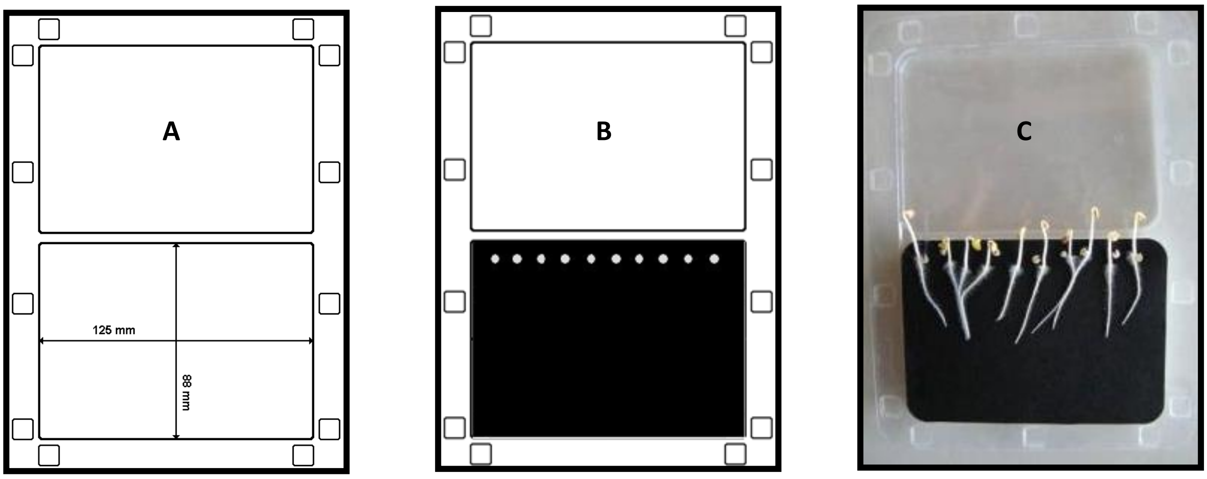


Figure 2-9. Phytotestkit® setup, with (A) empty test plate, (B) test plate with bottom filled with foam layer, parafilm layer, paper layer and 10 seeds and (C) test plate filled with the germinated white mustard seeds after 3 days of incubation.

B.2.6.3 DATA ANALYSIS

After three days of incubation, a digital picture was taken from each plate. The number of germinated plants was counted on this picture and the germination percentage was calculated per plate. Mean root length and shoot length were measured with ImageJ, version 1.48, a freeware image analysis program for digital photos. All root lengths of the treatments were divided by the mean root length of the blank treatment to obtain a relative root length percentage. These relative root length percentages were compared and statistically analysed. A one-sample Kolmogorov-Smirnov test was used to test whether the root lengths in the data groups (1 plant species, 1 treatment) had a normal distribution or not. When this was not the case for a certain group, all comparisons of means of this group with another group were analysed by means of a Mann-Whitney non-parametric test. All normally distributed groups were compared using a one-way ANOVA with a post-hoc test for multiple comparisons. When a Levene's test for homogeneity of variances returned a p-value greater than 0.05, the Tukey HSD-test was used as post-hoc test. When the Levene's test returned a p-value lower than 0.05, the Dunnett T3-test was chosen for comparisons between groups.

Subsequently, the percent inhibition of seed germination (IG) and inhibition of root growth (IR) for the plant were calculated using the formula:

$$\text{IG or IR} = \frac{\text{A-B}}{\text{A}} \,.\, 100$$

where A is the mean seed germination or root length (mm) of the control and B is the mean seed germination or root length (mm) of the test sample.

For a comprehensive interpretation of the data, seed germination and root elongation were combined in a germination index (GI), according to the equation:

$$\text{GI} = \frac{G_S \,.\, L_S}{G_C \,.\, L_C} \,.\, 100$$

where G_s and L_s are the seed germination (percent) and root elongation (mm) of the test sample, and G_c and L_c are the corresponding control values. GI values within the range of 90-110% were classified as no effect/non-toxic, GI values < 90% were classified as inhibition, and GI values ≥ 110 were classified as stimulation (Beltrami et al., 1999; Czerniawska-Kusza and Kusza, 2011).

B.3 RESULTS AND DISCUSSION

B.3.1 ADSORPTION AND HYGROSCOPICITY EXPERIMENTS

B.3.1.1 ADSORPTION PERCENTAGE

An initial evaluation of the different zeolites was made based on their PPP adsorption capacity. The adsorption capacity is represented by the adsorption coefficient (K_{oc}) and the octanol-water partition coefficient (K_{ow}) of the PPPs, which is also closely related to the water solubility (S). The logarithmic value (log K_{ow}) is used to describe the hydrophobicity or polarity of a compound. Values of log K_{ow} < 0 are considered as hydrophilic/polar, log K_{ow} between 0.5 and 2.5 as intermediate hydrophobic/polar and log K_{ow} > 3 as hydrophobic/non-polar (Wang and Liu, 2007). As described in literature, PPPs that have high solubility in water will remain in water and tend to not adsorb onto non-polar zeolites. This is in contrast to non-polar PPPs, which tend to be pushed out of the water (Bansal, 2012).

From the obtained adsorption percentages, shown in **Table 2-12**, it can be seen that clopyralid did not adsorb onto the zeolites. The high water solubility, low K_{oc} and K_{ow} of clopyralid indicate that this polar PPP prefers to stay in the aqueous phase. The combination of these properties might explain the fewer adsorption capacity for bentazon and the better adsorption capacity for imidacloprid, isoproturon and metalaxyl-M (**Figure 2-10**).

Table 2-12. Adsorption percentage (± SD) of the different zeolites (**Table 2-8**) determined at an initial plant protection product concentration of 10 $mg.l^{-1}$ (n=3).

Adsorption (%)		Bentazon	Clopyralid	Imidacloprid	Isoproturon	Metalaxyl-M
BEA	1	69.56 ± 2.46	9.12 ± 1.20	98.78 ± 0.03	100.00 ± 0.00	42.01 ± 0.79
	2	42.77 ± 2.26	3.55 ± 0.49	98.51 ± 0.02	100.00 ± 0.00	55.19 ± 3.15
	3	50.58 ± 2.17	16.94 ± 1.75	98.02 ± 0.04	100.00 ± 0.00	50.97 ± 1.98
CHA	4	9.90 ± 2.94	3.90 ± 0.33	5.78 ± 1.61	11.07 ± 0.32	0.40 ± 0.78
	5	15.50 ± 2.20	2.72 ± 0.01	8.37 ± 1.31	3.70 ± 0.60	24.06 ± 1.59
FAU	6	63.22 ± 1.63	10.67 ± 1.76	99.75 ± 0.02	100.00 ± 0.00	99.84 ± 0.19
	7	100.00 ± 0.00	10.81 ± 1.53	99.72 ± 0.02	100.00 ± 0.00	99.34 ± 0.14
HEU	8	14.53 ± 3.10	4.24 ± 1.13	10.48 ± 0.93	11.49 ± 1.50	51.48 ± 0.36
	9	14.69 ± 2.76	13.60 ± 1.91	7.47 ± 0.85	7.83 ± 1.77	58.91 ± 1.39
	10	14.01 ± 3.39	9.06 ± 1.23	10.08 ± 0.23	7.06 ± 1.09	42.43 ± 0.80
LTA	11	0.23 ± 0.35	0.00 ± 0.00	44.18 ± 1.02	0.00 ± 0.00	64.56 ± 1.88
	12	1.59 ± 1.63	5.04 ± 0.97	7.26 ± 0.95	0.00 ± 0.00	46.53 ± 6.25
MFI	13	15.93 ± 1.04	2.72 ± 0.47	13.89 ± 0.71	7.21 ± 1.87	29.43 ± 1.62
	14	21.74 ± 2.95	3.87 ± 0.54	21.17 ± 0.80	86.10 ± 0.79	36.87 ± 1.34
	15	7.00 ± 0.96	15.02 ± 1.45	8.46 ± 0.88	18.15 ± 1.46	28.62 ± 1.98
	16	22.16 ± 1.94	0.00 ± 0.00	8.95 ± 0.30	8.74 ± 1.83	27.56 ± 1.52
	17	23.06 ± 2.06	0.00 ± 0.00	8.12 ± 3.69	92.77 ± 0.29	27.04 ± 4.12
MOR	18	24.34 ± 4.88	4.04 ± 0.91	8.17 ± 1.05	9.60 ± 1.89	30.11 ± 2.47
	19	22.05 ± 0.58	12.32 ± 0.70	79.48 ± 0.61	94.71 ± 0.39	31.23 ± 4.30

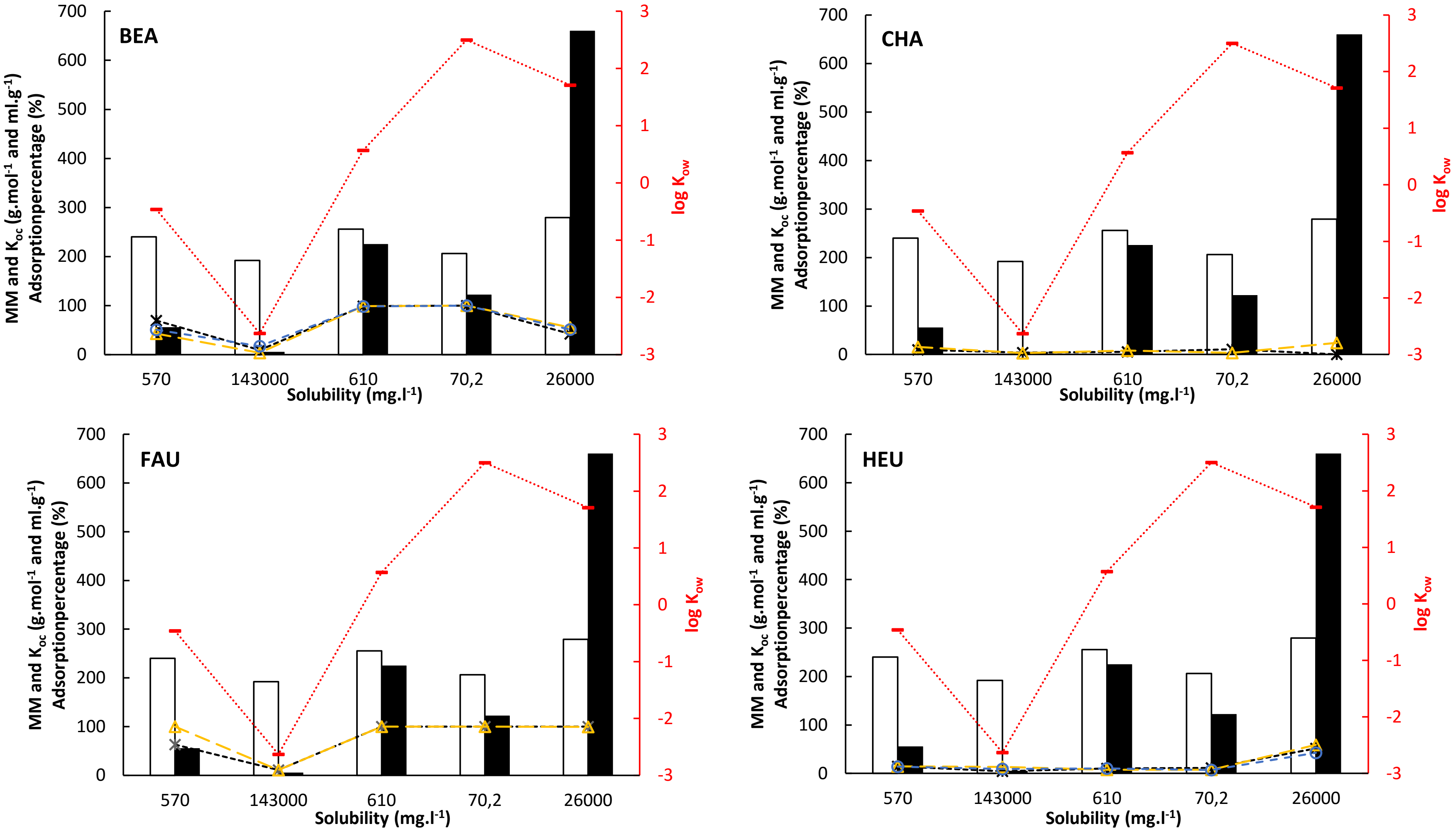
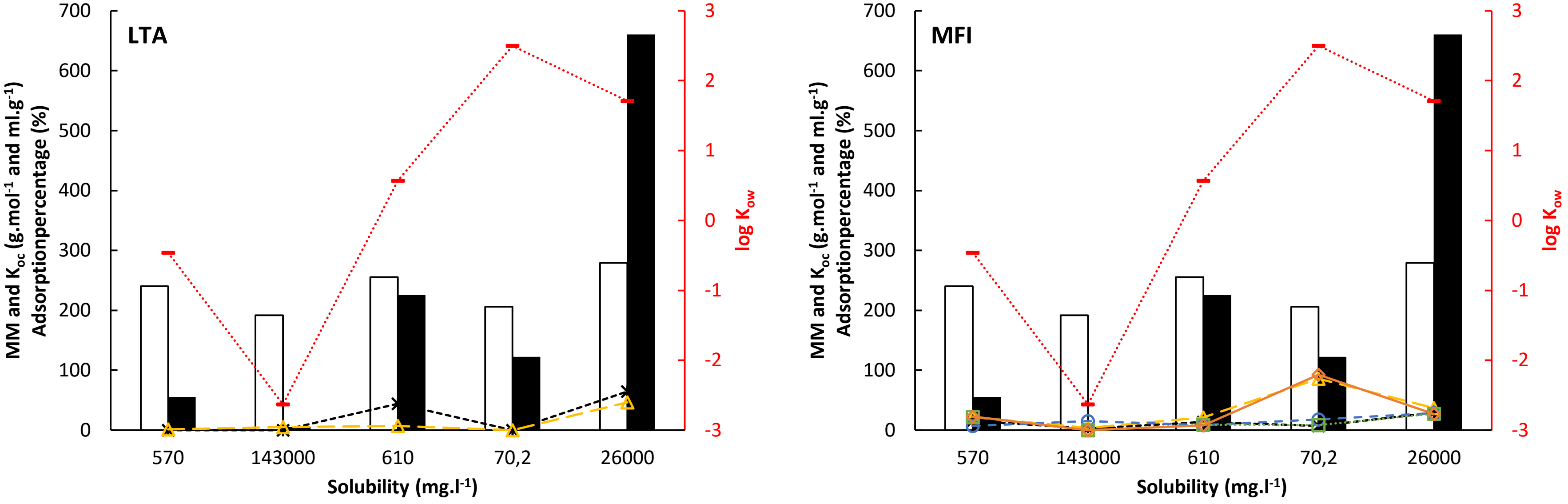


Figure 2-10. Comparison between the adsorption percentage (lines), the molar mass MM (☐), the adsorption coefficient K_{oc} (■), the octanol-water coefficient log K_{ow} (–) and the solubility (x-axis) of the different plant protection products (bentazon, clopyralid, imidacloprid, isoproturon and metalaxyl-M, from left to right, respectively) onto the different zeolite types BEA, CHA, FAU, HEU, LTA, MFI and MOR (**Table 2-8**).



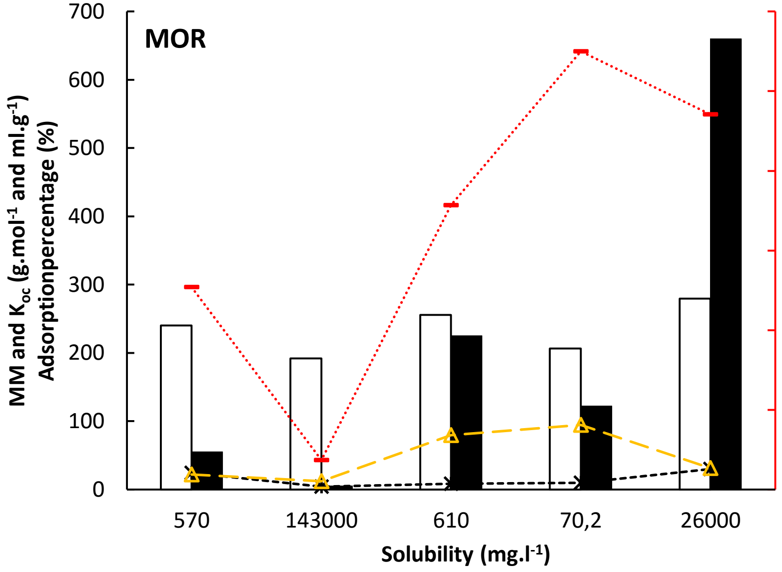


Figure 2-10. Comparison between the adsorption percentage (lines), the molar mass MM (☐), the adsorption coefficient K_{oc} (■), the octanol-water coefficient log K_{ow} (–) and the solubility (x-axis) of the different plant protection products (bentazon, clopyralid, imidacloprid, isoproturon and metalaxyl-M, from left to right, respectively) onto the different zeolite types BEA, CHA, FAU, HEU, LTA, MFI and MOR (**Table 2-8**) (continued).

An adsorption trend can be established between the different zeolites. Zeolite beta and zeolite Y had the best adsorption capacity, compared with other zeolites. These results are consistent with the higher values of the surface area (porosity) and PLD for these zeolites. Some nitrogen adsorption and desorption isotherms of the different zeolites (**Appendix C**, **Figure C-1**) are type II isotherms, indicating a non-porous powder, which suggests that the pore openings are too small for nitrogen (0.364 nm) to enter at 77 K (de Lange et al., 1995; Jagiello and Thommes, 2004; Shakarova et al., 2014; Valtchev et al., 2005). This means that the measured BET surface areas in **Table 2-8** for most zeolites corresponded with their external surface area. Rezaei and Wembley (2009) have reported that the external surface area per unit volume is one of the important parameters in this regard and determines the mass transfer in an adsorbent. The higher the external surface area, the better the adsorption of molecules. The large-pore zeolites BEA and FAU, which were able to measure the BET surface area, showed better adsorption results. According to literature sources, the external surface areas of these zeolites are as follows: H-BEA-25, 130 m^2.g^{-1}; H-BEA-35, 41 m^2.g^{-1}; CP 811C-300, not available; CBV 720, 93 m^2.g^{-1}; and CBV 780, 77 m^2.g^{-1} (Kobayashi et al., 2015; Wagholika et al., 2004). A comparison between the adsorption percentages and the obtained surface areas is shown in **Figure 2-11**.

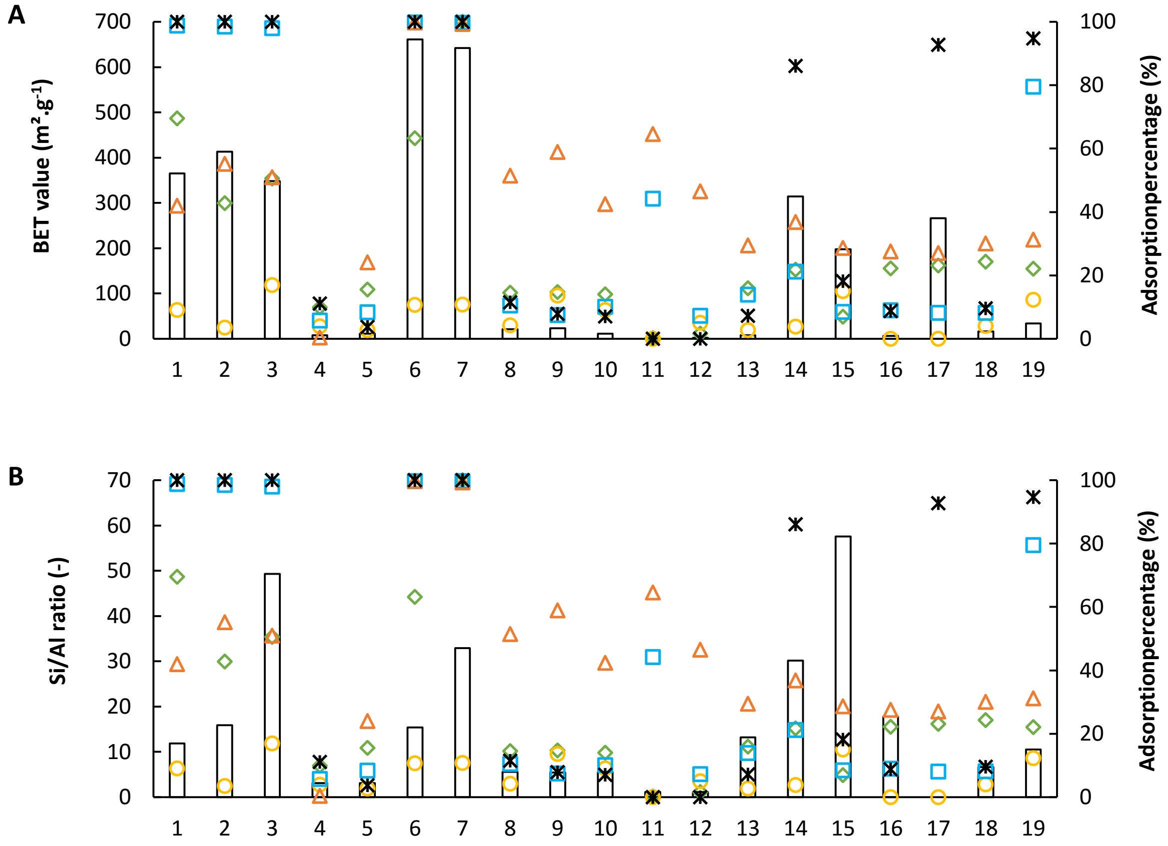


Figure 2-11. Comparison between the adsorption percentage (points), the specific BET-surface area (□; A) and the Si/Al-ratio (□; B) of the different zeolites (1-19, **Table 2-8**) for the different plant protection products, i.e. bentazon (◇), clopyralid (○), imidacloprid (□), isoproturon (⋇) and metalaxyl-M (△).

An additional explanation can be given by the hydrophobic properties, depending on the Si/Al ratio, of the zeolites. The zeolitic hydrophobic property decreases as the aluminium content in the zeolite framework increases and vice versa. These Si/Al values, summarized in **Table 2-8**, show that zeolites BEA and FAU are hydrophobic zeolites, and therefore, preferentially adsorb the intermediate and non-polar PPPs (**Figure 2-11**). This hydrophobic/hydrophilic nature of zeolites also appears to depend on their framework structure, which explains why the MFI zeolites did not adsorb the PPPs well (Cekova et al., 2006). Based on these outcomes, zeolites beta and Y, having the highest adsorption percentages, were selected to determine the kinetic and isotherm adsorption characteristics.

Finally, the sorption behaviour is also strongly affected by the chemical characteristics of the sorbate. The adsorption mechanism depends on the nature of the functional groups of the adsorbate, the type of adsorbent, and the acidity of the system (Sitea, 2001).

Both bentazon and clopyralid are acid herbicides and are often present in their anionic form in aqueous solutions. Electrostatic repulsion between negatively charged adsorbents and anionic herbicides is expected to result in low sorption (Shang and Arshad, 1998). This is in line with the observed results. The amount of anionic PPPs adsorbed onto the negatively charged zeolites was less compared to the other non-ionic PPPs, due to electrostatic repulsion (Dubus et al., 2001).

Despite the fact that bentazon and clopyralid are both ionic herbicides, the observed sorption capacity of bentazon on zeolites was obviously higher. This result is related to the pKa values and the molecular structure of the two organic materials. The pKa values indicate that clopyralid was more dissociated in water compared to bentazon. Unlike clopyralid, however, bentazon has both ionic charge and non-polar moiety and is a hydrophobic ionizable organic compound, which leads to a better adsorption onto the non-polar and especially the hydrophobic zeolites (Liu et al., 2011).

The non-ionic PPPs imidacloprid, isoproturon and metalaxyl-M showed better adsorption behaviour onto the zeolites due to the absence of electrostatic repulsions that prevent them from adsorbing. However, it is expected that the guanidine and phenylurea group of imidacloprid and isoproturon, respectively, will form stronger hydrogen bondings with water, resulting in more cationic properties of these PPPs (Chefetza et al., 2004; Hoepfner et al., 2012). However, based on the obtained results, it can be derived that the better adsorption behaviour of these PPPs were caused due to the hydrophobic properties instead of possible cation exchange properties.

B.3.1.2 ADSORPTION KINETICS

The adsorption kinetics of the PPPs on zeolite types BEA and FAU are presented in **Figure 2-12**. An initial steep increase in the adsorbed PPP concentration was observed in all cases with 72-100% of the equilibrium concentration adsorbed after 1 hour. (The equilibrium concentrations adsorbed, derived from **Figure 2-12**, were different for each zeolite and each PPP**.**) Isoproturon was adsorbed quickly by all zeolites during the first hour of solid-solution contact time, followed by a quick progress towards an apparent equilibrium after 15 and 30 minutes on zeolites BEA and FAU, respectively. Metalaxyl-M was adsorbed best on zeolite FAU, for which the equilibrium was achieved after 15 minutes. Bentazon was less quickly adsorbed on the adsorbents, followed by imidacloprid.

To shed more light on the kinetic process, two kinetic models, which are commonly used to study the kinetics of adsorption, namely, the pseudo-first-order and the pseudo-second-order models, were applied to the experimental data obtained in **Figure 2-12**. The higher values of the determination coefficient (R^2) and the accuracy to predict $q_{e,calc}$ were used as criteria to define the most suitable model to describe the adsorption kinetics. The R^2 values for the pseudo-first-order model were low for all PPPs, ranging from 0.0029 to 0.6284. Moreover, the $q_{e,calc}$ values did not agree with the $q_{e, exp}$ values. Hence, this model was not applicable to fit the obtained experimental data.

The pseudo-second-order model correlated much better with the experimental data (R^2 = 0.9991-1.0000), and the calculated adsorption capacity was also much closer to the experimental value. **Table 2-13** summarizes the kinetic parameters of the PPPs adsorbed at equilibrium using the pseudo-second-order equation. The pseudo-second-order constant k_2 ($g.mg^{-1}.h^{-1}$) gives an indication of the adsorption rate. The bigger the k_2 value, the faster the equilibrium has been reached. It was noted that the k_2 values for isoproturon (BEA and FAU) and metalaxyl-M (FAU) were higher, which is due to the very fast adsorption at 15 and 30 minutes after their initial addition to reach equilibrium. Both isoproturon and metalaxyl-M had a stronger affinity for the zeolites based on their polarity (Gevao and Jones, 2002).

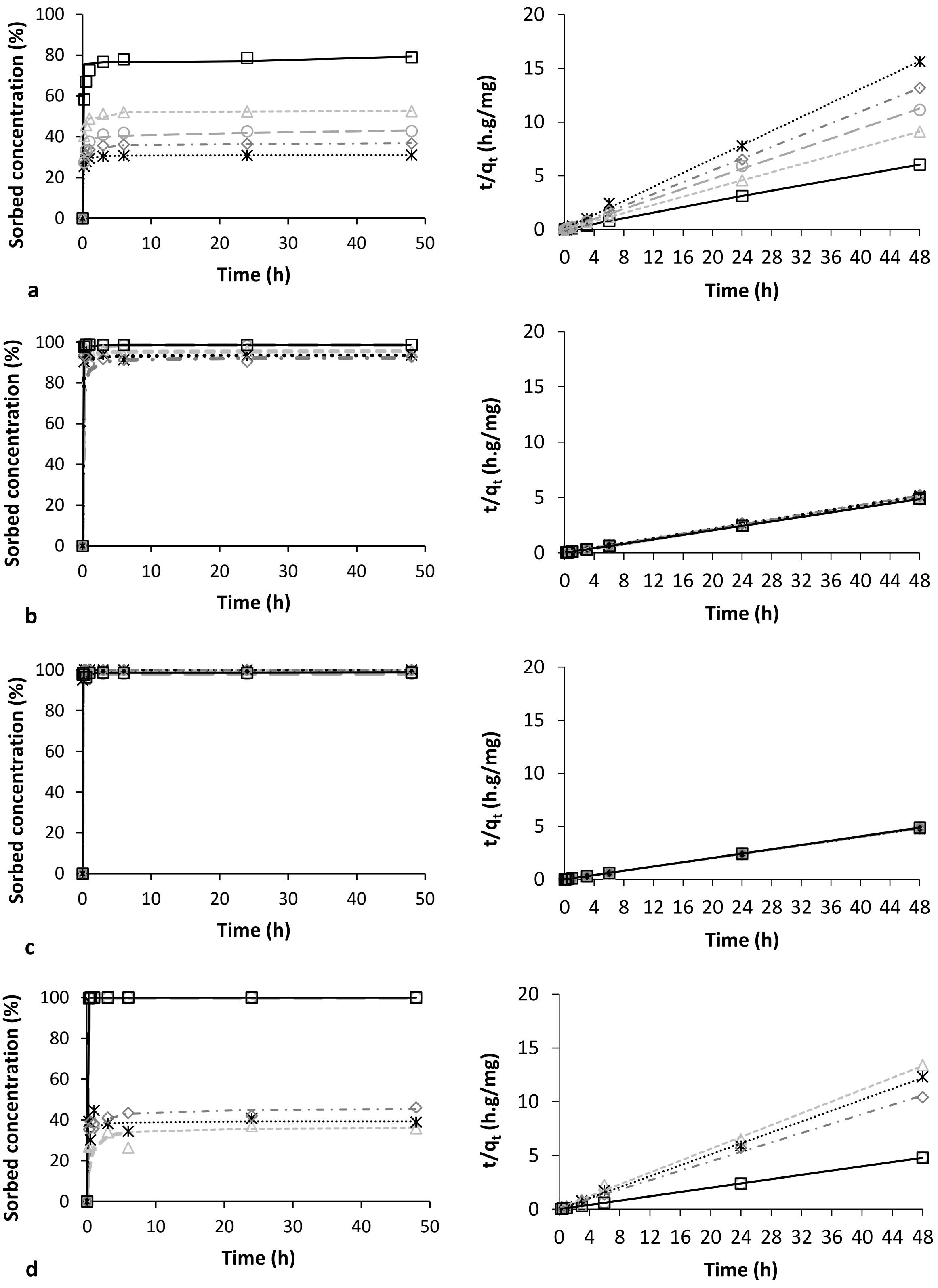


Figure 2-12. Experimental (indicated with the geometric symbols) and calculated (indicated with the lines) adsorption kinetics of (a) bentazon, (b) imidacloprid, (c) isoproturon and (d) metalaxyl-M for the different zeolites. Zeolite 1 (△), zeolite 2 (⚹), zeolite 3 (◇) (all BEA types, **Table 2-8**); zeolite 6 (○) and zeolite 7 (□) (all FAU types, **Table 2-8**). Initial plant protection product concentration, 10 mg.l^{-1} (n=3).

Table 2-13. Kinetic parameters of zeolite types BEA and FAU (**Table 2-8**) based on the pseudo-second order kinetic equations.

Zeolite		Parameter		Bentazon	Imidacloprid	Isoproturon	Metalaxy-M
BEA	1	$q_{e,exp}$	(mg g^{-1})	5.26	9.68	10.00	3.93
		$q_{e,calc}$	(mg g^{-1})	5.28	9.55	10.00	3.63
		k_2	(g mg^{-1} h^{-1})	1.13	6.45	500.00	0.70
		R^2		0.9998	1.000	1.000	0.9977
	2	$q_{e,exp}$	(mg g^{-1})	3.10	9.48	10.00	4.47
		$q_{e,calc}$	(mg g^{-1})	3.08	9.35	10.00	3.93
		k_2	(g mg^{-1} h^{-1})	1.50	4.57	333.33	2.88
		R^2		0.9991	1.000	1.000	0.9992
	3	$q_{e,exp}$	(mg g^{-1})	3.68	9.37	10.00	4.53
		$q_{e,calc}$	(mg g^{-1})	3.65	9.24	10.00	4.56
		k_2	(g mg^{-1} h^{-1})	4.06	1.46	1666.67	0.59
		R^2		0.9999	0.9999	1.000	0.9987
FAU	6	$q_{e,exp}$	(mg g^{-1})	4.31	9.89	9.82	10.00
		$q_{e,calc}$	(mg g^{-1})	4.27	9.87	9.81	10.00
		k_2	(g mg^{-1} h^{-1})	1.41	4.89	25.96	20000.00
		R^2		0.9992	1.000	1.000	1.000
	7	$q_{e,exp}$	(mg g^{-1})	7.94	9.88	9.87	10.00
		$q_{e,calc}$	(mg g^{-1})	7.89	9.87	9.86	10.00
		k_2	(g mg^{-1} h^{-1})	1.22	4.89	51.41	500.00
		R^2		0.9997	1.000	1.000	1.000

B.3.1.3 ADSORPTION ISOTHERMS

Adsorption isotherms are generally important to describe how adsorbates will interact with zeolites and are critical in optimizing the use of zeolites as an adsorber (Juang et al., 1996; Teng and Hsieh, 1998). Although the adsorption mechanism in aqueous solutions is complicated in nature, the correlation of equilibrium data with a theoretical equation giving a satisfactory description of adsorption often offers a clue to the key mechanistic steps involved in the overall adsorption process (Hsieh and Teng, 2000). The different adsorption isotherms obtained in this study are shown in **Figure 2-13**. According to IUPAC classification (1985), these isotherms can be divided into six different types (see **Figure 2-8**). Based on the results, types I, II and III were observed (**Table 2-14**). Although these isotherms shed no light on the mechanism of adsorption, they are useful for comparing results from different sources on a quantitative basis, providing information about the adsorption potential of a material with easily interpretable constants (Dawodu et al., 2012).

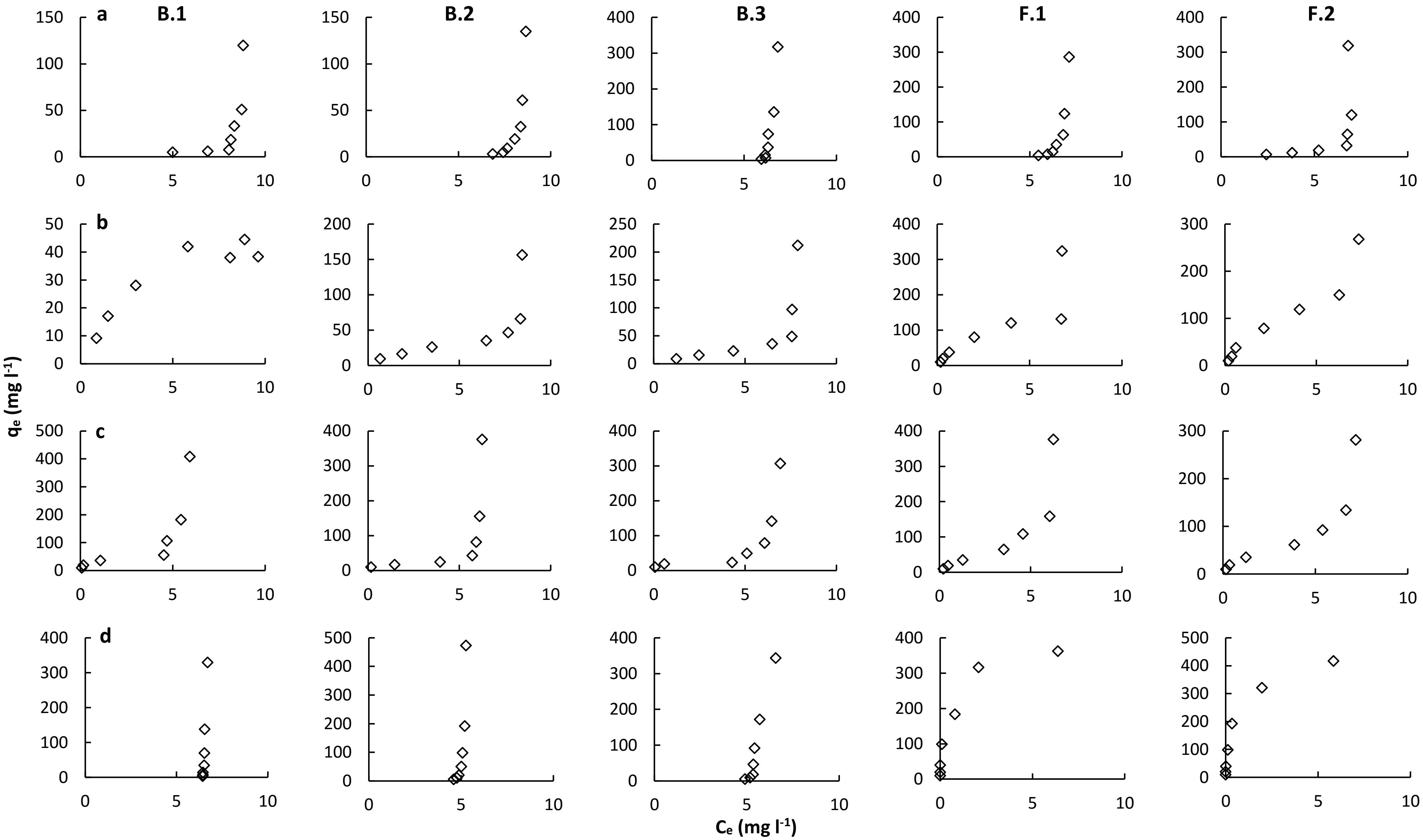


Figure 2-13. Adsorption isotherms of (a) bentazon, (b) imidacloprid, (c) isoproturon and (d) metalaxyl-M for the different zeolites (**Table 2-8**), BEA: (B.1) = zeolite 1, (B.2) = zeolite 2, (B.3) = zeolite 3 and FAU: (F.1) = zeolite 6 and (F.2) = zeolite 7 (n=3).

Table 2-14. Type of adsorption isotherm.

Zeolite		**Bentazon**	**Imidacloprid**	**Isoproturon**	**Metalaxyl-M**
BEA	1	III	I	III	III
	2	III	III	III	III
	3	III	III	III	III
FAU	6	III	II	III	I
	7	III	II	II/III	I

Langmuir adsorption isotherms

The Langmuir model is probably the best known and most widely applied adsorption isotherm (Ho et al., 2002). The Langmuir equation quantitatively describes the formation of an adsorbate monolayer on the outer surface of a microporous adsorbent containing a finite number of identical binding sites (Dawodu et al., 2012; Yousef et al., 2011). This type of behaviour is typical for chemisorption. The determination coefficients suggest that the applied Langmuir isotherm appears to produce a reasonable model of the adsorption system and the separation values indicate that the equilibrium adsorption was favourable for imidacloprid and isoproturon onto all zeolites. The negative values in **Table 2-15** observed for K_L and q_m are improbable (Monkiedje and Spiteller, 2002). These values, together with a small number of type I isotherms, indicate that the Langmuir model is not suitable to describe the adsorption of the PPPs on the zeolites (Carberry, 2001).

Freundlich adsorption isotherms

In this case, type III isotherms were the most frequently obtained. This isotherm is seen in systems in which the adsorbate-adsorbent interaction is small compared with the adsorbate-adsorbate reaction. The uptake of the adsorbate is initially slow until surface coverage is sufficient when the interactions between adsorbed and free molecules start to dominate the process (IUPAC, 1985).

The Freundlich model, which describes this type of isotherm, was found to give a good fit for the adsorption of the PPPs (**Table 2-15**). The Freundlich isotherm is the earliest known adsorption isotherm equation and is commonly used to describe the adsorption characteristics for the heterogeneous zeolite surface and considers multilayer adsorption (Boivon et al., 2005; Ho et al., 2002; Romero et al., 2006).

Table 2-15. Isotherm parameters of zeolite types BEA and FAU (**Table 2-8**) based on the Langmuir and Freundlich equations.

Zeolite		Parameter		Bentazon	Imidacloprid	Isoproturon	Metalaxyl-M
				Langmuir isotherm			
BEA	1	**K_L**	(l.g^{-1})	-0.076	0.130	3.275	-0.153
		q_m	(mg.g^{-1})	-7.651	94.340	59.880	-0.079
		R_L	(-)	0.808	0.303	0.029	2.133
		R^2	(-)	0.6689	0.9801	0.9773	0.6201
	2	**K_L**	(l.g^{-1})	-0.119	0.350	2.418	-0.198
		q_m	(mg.g^{-1})	-0.745	48.780	33.898	-0.584
		R_L	(-)	1.230	0.182	0.038	42.553
		R^2	(-)	0.9649	0.9699	0.8070	0.9603
	3	**K_L**	(l.g^{-1})	-0.158	0.066	5.820	-0.183
		q_m	(mg.g^{-1})	-0.286	113.636	34.364	-0.567
		R_L	(-)	2.385	0.375	0.017	5.850
		R^2	(-)	0.9055	0.9854	0.8322	0.9800
FAU	1	**K_L**	(l.g^{-1})	-0.147	0.0805	0.414	376.667
		q_m	(mg.g^{-1})	-1.122	714.286	114.943	88.496
		R_L	(-)	1.901	0.357	0.163	3 E-04
		R^2	(-)	0.9672	0.9779	0.9946	0.9062
	2	**K_L**	(l.g^{-1})	-0.080	0.100	0.927	380.000
		q_m	(mg.g^{-1})	-30.864	555.556	78.740	87.719
		R_L	(-)	0.832	0.333	0.089	3 E-04
		R^2	(-)	0.9694	0.9915	0.9923	0.8993
				Freundlich isotherm			
BEA	1	**K_F**	(mg.g^{-1})	0.053	11.858	38.098	3 E-134
		n	(-)	0.369	1.530	2.232	0.006
		R^2	(-)	0.5323	0.9296	0.9260	0.8681
	2	**K_F**	(mg.g^{-1})	2 E-10	11.741	19.436	1 E-19
		n	(-)	0.083	1.586	2.121	0.034
		R^2	(-)	0.9621	0.9872	0.7520	0.9702
	3	**K_F**	(mg.g^{-1})	2 E-33	6.827	24.626	5 E-18
		n	(-)	0.023	1.109	2.735	0.039
		R^2	(-)	0.8458	0.9764	0.7590	0.7938
FAU	1	**K_F**	(mg.g^{-1})	2 E-9	45.092	30.297	202.96
		n	(-)	0.080	1.277	1.360	3.080
		R^2	(-)	0.9475	0.9785	0.9831	0.9991
	2	**K_F**	(mg.g^{-1})	1.355	43.611	31.550	247.172
		n	(-)	0.564	1.274	1.717	2.823
		R^2	(-)	0.858	0.9730	0.9869	0.9845

One important characteristic of the Freundlich isotherm is its ability to give an appropriate description of equilibrium data over a restricted range of concentration. According to Kadirvelu and Namasivayam (2000), the value of n between 1 and 10 represents a beneficial adsorption process. The value of n obtained for all PPPs and zeolites lies within this range, which implies that the zeolites have a high affinity for the PPPs in solution. This high affinity is more or less in accordance with their reported K_{oc} values.

In general, non-ionic PPPs are relatively less mobile than ionic PPPs, and consequently their affinity for zeolites is higher (De Wilde et al., 2009). This effect was also observed in the obtained results. The anionic PPP bentazon showed the lowest adsorption intensity, compared to the other PPPs. In contrast, the non-ionic PPP obtained higher adsorption intensities. However, the affinity of the adsorbent for some types of substances can be much greater than the affinity for others. Imidacloprid and isoproturon, both to be expected to result in more cationic PPPs in water, obtained a higher affinity for zeolite BEA compared to metalaxyl-M. Nevertheless, it was noticed that the adsorption capacity of the zeolites for the PPPs was highest for the FAU zeolites. This can be explained by their higher specific surface area. The obtained results are in line with the second-order rate constants.

As mentioned earlier, the interpretation of different shapes of isotherms is not an easy task, especially for type III. There is no simple theory that predicts the exact meaning of isotherms, but different assumptions can be made to explain these isotherms. First, according to literature, type III isotherms may be the result of very large adsorbate molecules. If these molecules are too large and/or access to the micropores is somehow hindered, adsorption virtually takes place in extra-crystalline pores with surface adsorption being the main adsorption mechanism (Elaiopoulos, 2012). As already described for the type II isotherms, this description seems to be applicable by looking at the molecular size of the adsorbates.

Another explanation, given by Zhao et al. (2013), is that a type III isotherm is commonly observed in the adsorption of H_2O molecules in crystalline materials. In this case, the adsorption of the PPPs, which were dissolved in water, could be the result of the adsorption of water. This statement can be verified by testing the hygroscopicity of the zeolites, which was carried out in Section B.3.2, in order to investigate the water holding capacity of zeolites.

Finally, a type III isotherm can be the result of a situation in which previously adsorbed molecules lead to a modification of the adsorbent, which favours further adsorption. Such effects have been reported in studies involving anionic or cationic surfactants as adsorbates (Schwarzenbach et al., 2003). However, non-ionic products forming hydrogen bonds with the hydroxyl groups on the solid surface can also cause these modifications. Because the hydrogen bonding is weaker than the electrostatic interaction, the adsorption of the non-ionic product to most solids is less than that of ionic products (Zhang and Somasundaran, 2006). To examine whether this type of isotherm is the result of a modification in the crystal structure of the zeolites, XRD patterns of the different zeolites before and after adsorption were compared.

Based on the decrease or increase of the relative intensity of the strongest diffraction peak, the zeolite was found to be less crystalline. In other words, this change of intensity is associated with the presence of some defects in the structure (Shanjiao et al., 2007). In particular, it will be shown that different types of defects may lead either to the occurrence of transport resistance on a crystal surface or to an enhancement of the adsorption rate due to the existence of cracks on the surface. For molecules with characteristic diameters comparable with the size of the pore openings, a small change in the latter could easily cause an order-of-magnitude change in the mass transfer rate (Kortunov et al., 2004). However, in case of these large PPP molecules, the cracks will not be large enough to make a big difference in our results. According to the XRD patterns (**Figure 2-14**), a change in the intensities can be observed. These little changes are plausible after the adsorption of PPPs, and thus, do not indicate a modification of the structures.

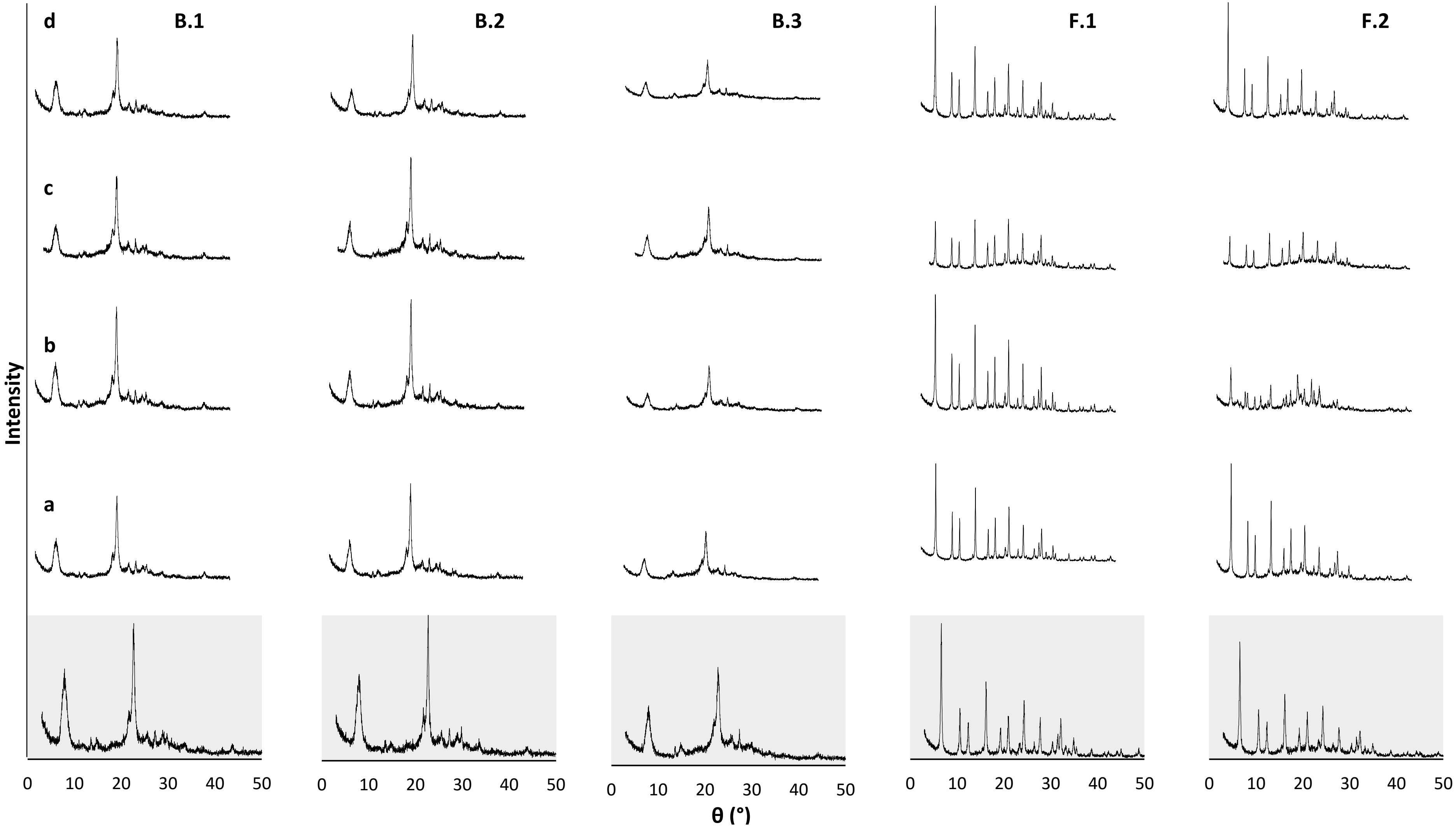


Figure 2-14. XRD patterns of the different zeolites (**Table 2-8**), BEA: (B.1) = zeolite 1; (B.2) = zeolite 2, (B.3) = zeolite 3 and FAU: (F.1) = zeolite 6 and (F.2) = zeolite 7 before and after adsorption of (a) bentazon, (b) imidacloprid, (c) isoproturon and (d) metalaxyl-M.

B.3.2 HYGROSCOPICITY EXPERIMENTS

Hygroscopicity is the tendency of a material to adsorb humidity, and thus, a hygroscopic product will have a large water adsorptive capacity. These products may have a porous nature and preferentially adsorb large amounts of water vapour onto specific sites with high binding energy; thus, depressing a_w dramatically for relatively large moisture contents (Decagon Devices, 2006).

The WHC is one of the main and most important properties of zeolites (Tzia and Zorpas, 2012). The obtained WHCs, after drying and wetting all zeolites, are shown in **Table 2-16**. These results follow a similar trend as the PPP adsorption percentages presented in **Table 2-12**. Zeolites BEA and FAU were also able to adsorb more water compared with the other zeolites (**Figure 2-15**). This is in contrast to the hydrophobic properties based on the measured Si/Al ratio. According to Corma (2003), all zeolites containing charges are normally more hydrophilic materials which, depending on the number of charges (extra-framework cations and framework Si/Al ratio), can be more or less selective adsorbents for polar or non-polar molecules. However, pure silica zeolites with no positive charges are highly hydrophobic materials. This was confirmed by a WHC of 0.52% for the pure silica zeolite 17.

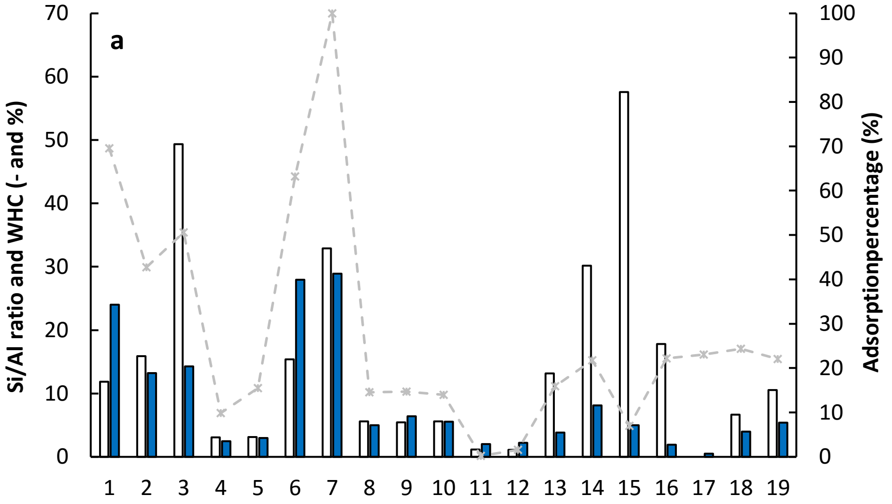


Figure 2-15. Comparison between the adsorption percentage (✳), the Si/Al-ratio (□) and the water holding capacity WHC (■) of the different zeolites (1-19, **Table 2-8**) for the different plant protection products: (a) bentazon, (b) clopyralid, (c) imidacloprid, (d) isoproturon and (e) metalaxyl-M.

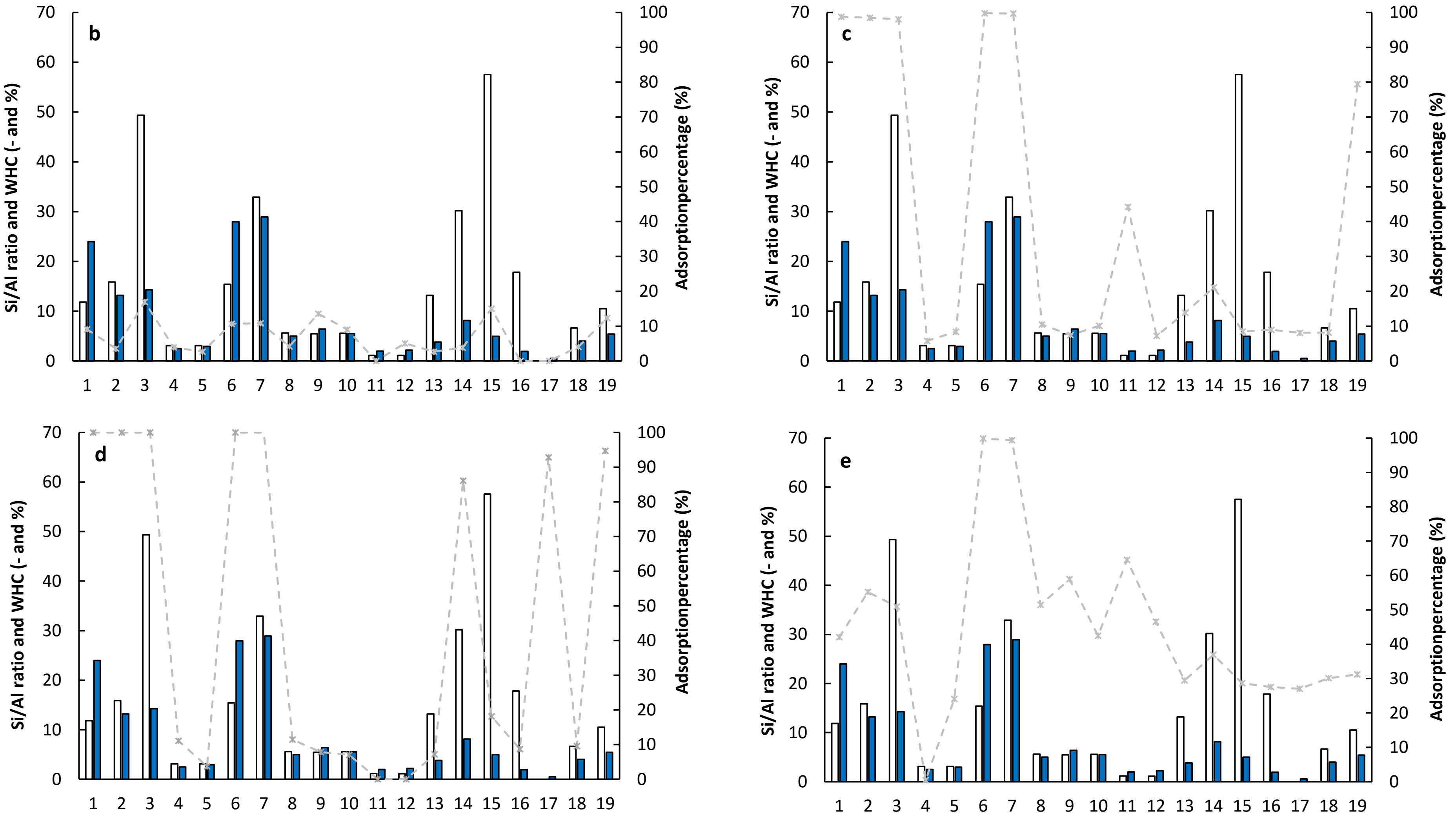


Figure 2-15. Comparison between the adsorption percentage (×), the Si/Al-ratio (□) and the water holding capacity WHC (■) of the different zeolites (1-19, **Table 2-8**) for the different plant protection products: (a) bentazon, (b) clopyralid, (c) imidacloprid, (d) isoproturon and (e) metalaxyl-M (continued).

Table 2-16. Hygroscopic effect of the zeolites (**Table 2-8**) based on their water holding capacity (WHC) percentage.

Zeolite		**% Initial moisture**	**% weight change**	**% weight change**	**% WHC**
			$a_{w,\,max}$	$a_{w,\,min}$	
BEA	1	5.34	24.52	0.52	**24**
	2	4.58	16.58	3.37	**13.21**
	3	8.62	11.66	-2.62	**14.28**
CHA	4	3.66	1.44	-1.04	**2.48**
	5	3.90	1.66	-1.30	**2.96**
FAU	6	10.29	21.74	-6.21	**27.95**
	7	12.63	18.82	-6.21	**28.91**
HEU	8	3.35	3.52	-1.47	**4.99**
	9	5.11	4.22	-2.18	**6.40**
	10	3.60	3.95	-1.58	**5.53**
LTA	11	2.19	1.72	-0.27	**1.99**
	12	6.80	0.99	-1.22	**2.21**
MFI	13	3.58	2.32	-1.50	**3.82**
	14	2.57	6.39	-1.75	**8.14**
	15	6.99	2.56	-2.42	**4.98**
	16	15.38	1.49	-0.44	**1.93**
	17	1.42	0.56	0.04	**0.52**
MOR	18	6.47	2.59	-1.40	**3.99**
	19	5.81	4.22	-1.19	**5.41**

The obtained water adsorption isotherms represented type II instead of type III isotherms, which can be explained in the same way as explained in the "BET adsorption isotherms" section. These results were also observed by Halasz et al. (2002), who found that less hydrophobic zeolites give rise to a type II isotherm, indicating that water adsorption does not take place in its microchannels. This means that the higher water adsorption of zeolites BEA and FAU can be the result of higher external surface areas. As was also observed by others, the surface area increased with increasing Si/Al ratios of the zeolites (**Figure 2-16**; Ali et al., 2003; Shanjiao et al., 2007). The external surface of zeolites consists of bridging hydroxyls and silanol groups (SiOH), which can form hydrogen bonds with water, making the zeolite hydrophilic (Kühl, 1999). These last two statements in turn explain why the hydrophobic properties based on the Si/Al ratio are not applicable here.

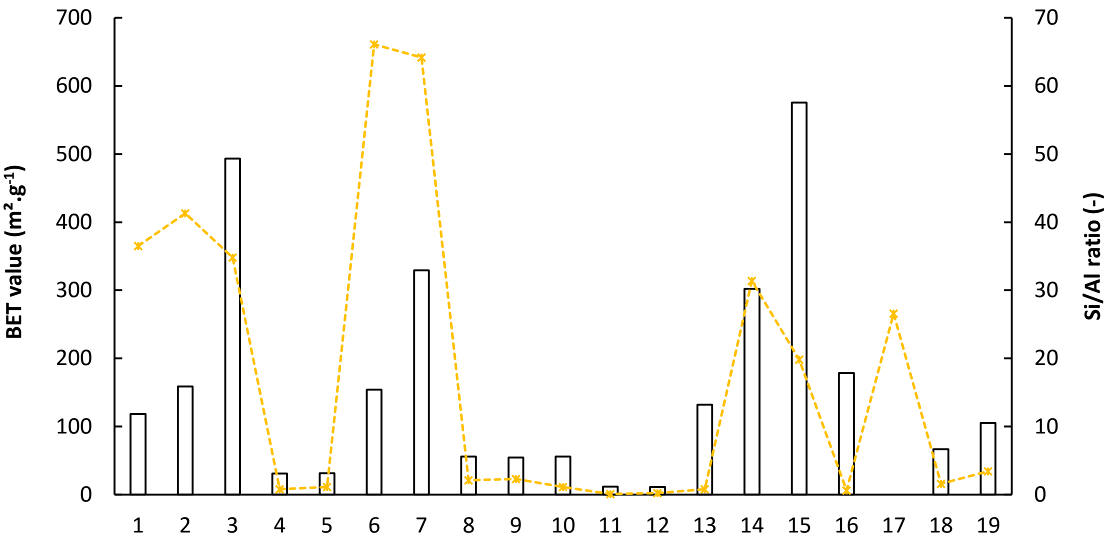


Figure 2-16. Comparison between the specific surface area (✳) and the Si/Al-ratio (□) of the different zeolites (1-19; **Table 2-8**).

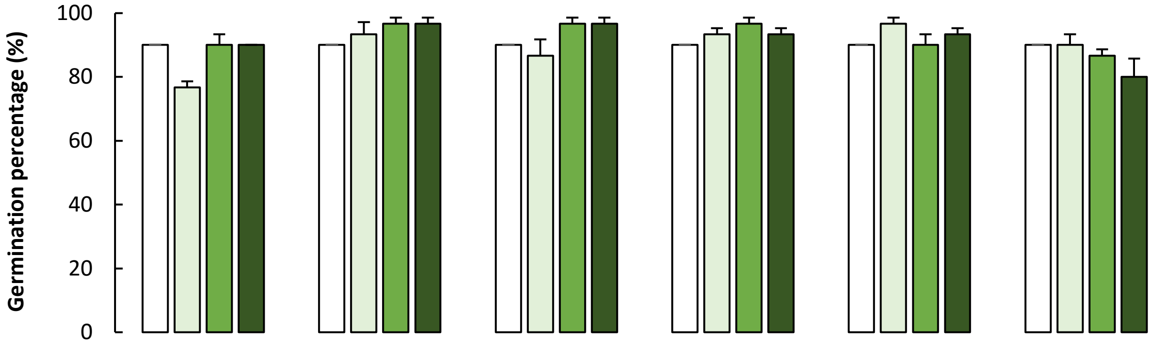
B.3.3 PHYTOTOXICITY EXPERIMENTS

As concluded in Sections B.3.1 and B.3.2, zeolites BEA (B.1, B.2, B.3) and FAU (F.1, F.2) showed the best PPP adsorption and water holding capacities. The phytotoxic effects of these zeolites were determined by measurement of seed germination and root growth of three plant species (Microbiotests Inc, 2008). The obtained results were also compared with the phytotoxic effect of the natural product kaolin, which is already commercially used in agriculture.

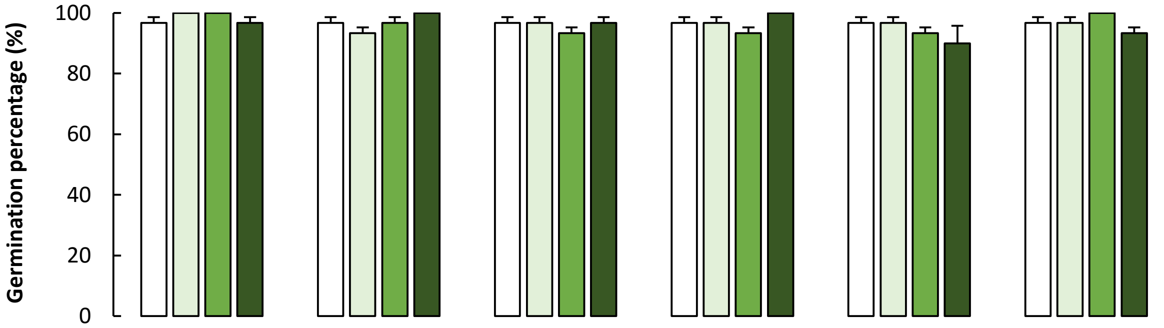
The germination percentage of the three plant species was higher than 76% for all groups (30 seeds per group), even reaching 100% for some. **Figure 2-17** shows no significant differences between germination percentages with and without the zeolites. Subsequently, the degree of inhibition was not in function of the zeolite concentrations. These values were used to calculate the inhibition of seed germination. The IG values of the test plants ranged from -7.41% to 14.81% (**Table 2-18**), which indicates low to no influence of the zeolites on germination percentage.

The rooth length results of the test samples, expressed relative to the control, are summarized in **Table 2-17.** It was noticed that the zeolite solutions had a greater influence on root growth inhibition than on germination inhibition for all the tests species. Generally, a descending trend in function of the zeolite concentrations was determined. Nevertheless, two species of dicotyledonous plants (*S. alba*, *L. sativum*) and one species of monocotyledonous plants (*S. saccharatum*) showed a diverse reaction to the test products, which is in agreement with works by Czerniawska-Kusza et al. (2006); Czerniawska-Kusza and Kusza (2011) and Baran and Tarnawski (2013). In all studies, *S. saccharatum* was the most sensitive species compared to *S. alba* and *L. sativum*. This trend was also illustrated by the calculated values of root growth inhibition (IR), listed in **Table 2-18**. Only positive values were observed, ranging from 11.02% to 46.54%.

a: ***Sorghum saccharatum***



b: ***Sinapis alba***



c: ***Lepidium sativum***

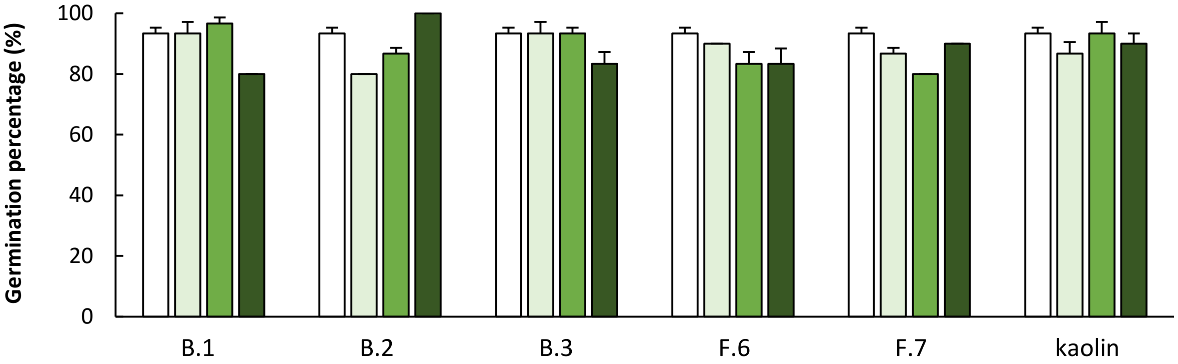


Figure 2-17. Germination percentages (± SE) of the three plant species (a) *Sorghum saccharatum*, (b) *Sinapsis alba* and (c) *Lepidium sativum* treated with different zeolites (**Table 2-8**), i.e. BEA: (B.1) = zeolite 1; (B.2) = zeolite 2, (B.3) = zeolite 3, FAU: (F.1) = zeolite 6, (F.2) = zeolite 7 and kaolin. All products were tested at a concentration of 0 (☐), 100 (☐), 1000 (🟩) and 10000 (■) mg.l^{-1} (n=3).

Table 2-17. Relative root length percentage (± SE) of the three plant species treated with different concentrations of zeolite types BEA and FAU (**Table 2-8**) and kaolin (n=3).

Concentration	BEA 1	BEA 2	BEA 3	FAU 6	FAU 7	Kaolin
			Sorghum saccharatum			
0	100.00 ± 1.94	100.00 ± 1.94	100.00 ± 1.94	100.00 ± 1.94	100.00 ± 1.94	100.00 ± 1.94
100	57.69 ± 1.85 ^{a*}	67.14 ± 1.40 ^{ab*}	75.43 ± 1.90 ^{ab*}	74.78 ± 1.76 ^{ab*}	80.47 ± 1.64 ^{b}	79.13 ± 1.85 ^{b}
1000	78.36 ± 1.63 ^{a}	58.84 ± 1.31 ^{a*}	86.15 ± 1.90 ^{a}	88.98 ± 1.72 ^{a}	75.73 ± 1.63 ^{a}	79.52 ± 1.71 ^{a}
10000	71.62 ± 1.74 ^{a}	53.46 ± 1.27 ^{a*}	78.85 ± 1.53 ^{a}	72.71 ± 1.34 ^{a}	66.21 ± 1.45 ^{a*}	74.57 ± 1.64 ^{a}
			Sinapis alba			
0	100.00 ± 1.74	100.00 ± 1.74	100.00 ± 1.74	100.00 ± 1.74	100.00 ± 1.74	100.00 ± 1.74
100	106.08 ± 1.96 ^{a}	111.12 ± 1.77 ^{a}	89.18 ± 2.05 ^{ab}	106.46 ± 1.91 ^{a}	68.26 ± 1.24 ^{b*}	148.17 ± 2.02 ^{c*}
1000	102.35 ± 2.15 ^{abc}	112.65 ± 1.96 ^{ac}	90.50 ± 2.07 ^{abc}	92.71 ± 2.08 ^{abc}	72.44 ± 1.30 ^{b*}	99.60 ± 1.71 ^{ac}
10000	107.52 ± 2.04 ^{a}	102.26 ± 1.60 ^{a}	71.43 ± 1.51 ^{b}	99.02 ± 1.79 ^{ab}	91.77 ± 1.61 ^{ab}	74.42 ± 1.84 ^{ab}
			Lepidium sativum			
0	100.00 ± 1.27	100.00 ± 1.27	100.00 ± 1.27	100.00 ± 1.27	100.00 ± 1.27	100.00 ± 1.27
100	87.24 ± 1.53 ^{a}	100.58 ± 1.76 ^{a}	98.02 ± 1.87 ^{a}	137.58 ± 1.87 ^{b*}	81.96 ± 1.81 ^{a}	107.82 ± 1.57 ^{ab}
1000	84.78 ± 1.67 ^{ab}	79.02 ± 1.66 ^{a}	113.27 ± 1.27 ^{b}	99.88 ± 1.78 ^{ab}	155.56 ± 2.49 ^{c*}	100.85 ± 1.58 ^{ab}
10000	82.36 ± 2.06 ^{ab}	66.97 ± 1.15 ^{a*}	92.74 ± 2.04 ^{ab}	94.83 ± 1.89 ^{b}	101.42 ± 1.68 ^{ab}	104.02 ± 1.36 ^{b}

* Asterisks indicate significant differences ($P < 0.05$) in relative root length percentage between sample and control treatments.

$^{a, b, c}$ Significant differences amongst the tested products within a certain concentration are indicated with a different letter.

Table 2-18. Toxicity classification of zeolite types BEA and FAU (**Table 2-8**) and kaolin, based on the inhibition of seed germination (IG) and root growth (IR) and the germination index (GI)

		Inhibition of seed germination (IG)			Inhibition of root growth (IR)			Germination index (GI)[a]		
Concentration (mg.l^{-1})		100	1000	10000	100	1000	10000	100	1000	10000
					Sorghum saccharatum					
BEA	1	14.81	0.00	0.00	42.31	21.64	28.38	49.15	78.36	71.62
	2	-3.70	-7.41	-7.41	32.86	41.16	46.54	69.63	63.20	57.42
	3	3.70	-7.41	-7.41	24.57	13.85	21.15	72.63	92.54	84.69
FAU	6	-3.70	-7.41	-3.70	25.22	11.02	27.29	77.55	95.57	75.41
	7	-7.41	0.00	-3.70	19.53	24.27	33.79	86.43	75.73	68.66
Kaolin		0.00	3.70	11.11	20.87	20.48	25.43	79.13	76.58	66.28
					Sinapis alba					
BEA	1	-3.45	-3.45	0.00	-6.08	-2.35	-7.52	109.73	105.88	107.52
	2	3.45	0.00	-3.45	-11.12	-12.65	-2.26	107.29	112.65	105.79
	3	0.00	3.45	0.00	10.82	9.50	28.57	89.18	87.38	71.43
FAU	6	0.00	3.45	-3.45	-6.46	7.29	0.98	106.46	89.52	102.43
	7	0.00	3.45	6.90	31.74	27.56	8.23	68.26	69.95	85.44
Kaolin		0.00	-3.45	3.45	-48.17	0.40	25.58	148.17	103.04	71.86
					Lepidium sativum					
BEA	1	0.00	-3.57	14.29	12.76	15.22	17.64	87.24	87.81	70.59
	2	14.29	7.14	-7.14	-0.58	20.98	33.03	86.21	73.38	71.75
	3	0.00	0.00	10.71	1.98	-13.27	7.26	98.02	113.27	82.81
FAU	6	3.57	10.71	10.71	-37.58	0.12	5.17	132.66	89.18	84.67
	7	7.14	14.29	3.57	18.04	-55.56	-1.42	76.10	133.34	97.80
Kaolin		7.14	0.00	-4.02	-7.82	-0.85	-4.02	100.12	100.85	100.30

[a] The colours indicate (1) inhibtition: GI < 90 (🟨), (2) no effect: 90<GI<110 (🟪) and (3) stimulation: >110 (🟦).

In general, no significant differences were observed within and between the zeolite types tested on *S. saccharatum*. However, for *S. alba*, significant differences were noticed for the root length within the BEA and FAU zeolites at 10000 mg.l^{-1} and 100 mg.l^{-1}, respectively. Significant differences between the zeolite types were also noticed. Similar results were obtained for *L. sativum*. Significant differences within the zeolites were observed at 100 mg.l^{-1} for both BEA and FAU zeolites and at 1000 mg.l^{-1} for the FAU zeolites. Significant differences between the zeolite types were also noticed. The IR values of these test plants ranged from -48.17% to 31.74% and from -55.56% to 33.03%, respectively (**Table 2-18**). Since most inhibition values were lower than 20%, the observed effects were assumed to be negligible.

Based on various toxicity indices that focus on germination and the early rot growth, germination index (GI) appears to be a good method for assessing the toxicity (Beltrami et al., 1999; Emino and warman, 2004; Devesa-Rey et al., 2008; Czerniawska-Kusza and Kusza, 2011). Growth inhibition was caused by 89% of the examined zeolites in the most sensitive plant, *S. saccharatum* (GI < 90%). The other 11% caused neither inhibition nor stimulation (90 < GI < 110). *Sinapis alba* was the least sensitive to zeolite substances. Not toxic effect was noticed in 50% of the examined samples and 11% had a stimulating effect on its growth (GI ≥ 110). *Leptidium sativum* had the intermediate position. Germination index of *L. sativum* assumed values from 90 to 110% (no effect) in 28% of the zeolite samples. In 56% of the samples GI was lower than 90% (growth inhibition) and in 17% the GI was more than 110% (growth stimulation). Despite a better overview given by the GI values in **Table 2-18**, no clear differences were observed between the various zeolites. A general assumption can be made that zeolite B.3 (BEA) and F.6 (FAU) exhibited the lowest phytotoxic effects.

Nevertheless, phytotoxicity does not only affect germination and root growth of plants. In addition, phytotoxic effects may be observed on the crop at emergence or during its growth or may be expressed at harvest. They may be temporary or lasting. The symptoms may affect the whole plant or any particular part of the plant (roots, shoots, leaves, flowers or fruits). Hence, besides inhibition of seed germination and root growth, other phytotoxic symptoms occur, i.e. modifications in the development cycle, thinning, modifications in colour, necrosis, deformations and effects on quantity and quality of the yield (EPPO, 2014). However, tests to determine these phytotoxic effects are very time-consuming, since a lot of time is needed to breed fully grown plants.

B.4 CONCLUSION

In this work, it was found that zeolites showed the applicability as adsorbent for treating waters contaminated with PPPs. The adsorption of bentazon, clopyralid, imidacloprid, isoproturon and metalaxyl-M on different zeolites was evaluated in this study. The adsorption of clopyralid was too low onto all zeolites. Subsequently, the adsorption processes of the selected PPPs and zeolites BEA and FAU were found to follow the pseudo-second-order kinetics. The FAU zeolites were able to adsorb the PPPs faster. The mobility of the PPPs also has an influence of the adsorption rate.

The equilibrium data were analysed based on the obtained type of isotherms. In this study, three different types were observed. The type I and II isotherms occurred less frequently compared with the type III isotherms. The Freundlich model, which describes this type of isotherm, was found to give a good fit for the adsorption of the PPPs. Different assumptions could be made to describe the meaning of type III isotherms. First, this isotherm can be the result of the presence of very large adsorbate molecules, which seems applicable taking into account the molecular size of the adsorbents. Subsequently, these isotherms can also be the result of water adsorption. In this case, the adsorption of PPPs, which were dissolved in water, could be the result of the adsorption of water. However, hygroscopic experiments, indicating type II isotherms for the adsorption of water, presented different results in contrast to this statement. This means that the obtained water adsorption was also the result of the higher external surface area of the BEA and FAU zeolites. Finally, adsorbed molecules can lead to modifications of the adsorbent. The XRD patterns of the different zeolites before and after adsorption demonstrate that the observed isotherms are most likely not the result of modifications in the crystal structure of the zeolites.

Based on the phytotoxicity experiment, it was not possible to come to clear conclusions. Nevertheless, a greater influence was observed on root growth inhibition compared to germination inhibition. Looking at the germination index for assessing the toxicity, zeolites B.3 (BEA) and F.6 (FAU) exhibited the least toxicity of each zeolite type.

Finally, one zeolite of each type had to be selected for further measurements. An overview of the zeolites with the best adsorption, water holding capacity and the least phytotoxic properties is given in **Table 2-19**. For zeolite type BEA, a selection had to be made between zeolites B.1 and B.3. Since no significant phytotoxicity was observed for both zeolites and no significant differences were noticed between the zeolites, the preference was given to the hygroscopicity results, which may have a bigger impact on the effect of zeolites on diseases, pests and plants. A higher water holding capacity was observed for zeolite B.1. For zeolite type FAU zeolite F.6 was selected, based on its test results.

Table 2-19. A final overview of the zeolites (**Table 2-8**) that obtained the best adsorption and water holding capacity and the least phytotic properties.

Selected zeolites		**Adsorption**	**Hygroscopicity**	**phytotoxicity**
BEA	1	x	x	
	2	x		
	3	x		x
FAU	6	x	x	x
	7	x	x	

CASE STUDY:

COMPARISON OF DIFFERENT SOLID ADSORBENTS FOR THE REMOVAL OF MOBILE PLANT PROTECTION PRODUCTS FROM AQUEOUS SOLUTIONS

In this part, the results of a case-study on the adsorption capacity of different adsorbents for the removal of mobile PPPs are presented. The main purpose of this study was to investigate another possible application of zeolites, based on the results of Part B. This work looked at different sorption materials, of which zeolites was one of them, to be used in wastewater treatements.

C.1 INTRODUCTION

Environmental problems associated with the use of PPPs, particularly the highly mobile ones, are a matter of concern because of the increasing presence of these agrochemicals in ground and surface waters (Kalkhoff et al., 1998; Kolpin et al., 1998). In this work, the herbicides bentazon, clopyralid and isoproturon were used as an example of mobile PPPs. The ability of different adsorbents to delay the release of the herbicide into water and to reduce herbicide leaching in soil or to remove this compound from contaminated water by adsorption, was investigated.

Techniques generally applied to reduce PPPs from effluents, include adsorption, photocatalytic degradation, electrochemical degradation, oxidation, membrane filtration, nanofiltration (Srivastava et al., 2009). Adsorption processes are recognized as the most efficient and promising fundamental approaches in the wastewater treatment processes (Foo and Hameed, 2010). The use of various solid-phase sorbents, such as activated carbon, polymeric resins, alkylsilane-modified silica, organoclays, zeolites, agricultural products/by-products and industrial products/by-products have also been explored for the removal of PPPs from water (Masque et al., 1998a, 1998b; Bagheri and Mohammadi, 2003; Groisman et al., 2004).

The majority of sorbents are from one of the three general classes of materials: activated carbons, zeolites, and metal/metalloid oxides including silica gel and activated alumina. A fourth category is relatively new and consists of crystalline metal-organic frameworks (MOFs). MOFs are a class of hybrid materials that exist as infinite crystal lattices with inorganic vertices and molecular-scale organic connectors (O'Keeffe et al., 2008; Ferey, 2009). They are essentially coordination polymers formed by connecting metal ions with polytopic organic linkers. They combine the well-defined structural characteristics of zeolites with surface areas exceeding those of the best activated carbons. The pores in MOFs have a very uniform distribution unlike in heterogeneous carbon materials in which a broad pore size distribution is observed (Fletcher et al., 2005). Furthermore, incorporation of functionality (e.g., halogen, nitrogen, sulphur, carboxy, cyano, nitro) on the organic linker, as well as the ability to select different metals, allows the electronic nature of the pore surface to be tuned, a feature very difficult to achieve in zeolites and activated carbons (Fletcher et al., 2005; Cychosz et al., 2010; Farha and Hupp, 2010). One of the most attractive features of MOFs is the simplicity of their synthesis. Typically they are obtained via one-pot solvothermal preparations (Farha and Hupp, 2010).

Some of the many applications of MOFs are: gas storage (Murray et al., 2009; Hu and Zhang, 2010), molecular separations (Bae et al., 2009; Britt et al., 2009; Finsy et al., 2009; An et al., 2010), chemical catalysis (Lee et al., 2009; Ma et al., 2009; Leus et al., 2010), chemical sensing (Allendorf et al., 2009), ion exchange (Min and Suh, 2000) and drug delivery (Horcajada et al., 2006; An et al., 2009; Taylor-Pashow et al., 2009). Gas adsorption by MOFs has extensively been studied. This in contrast to the aqueous sorption of chemical compounds by MOFs. Adsorption from liquid solution is more complex compared to gas phase adsorption. A number of additional factors, such as polarity and composition of host and guest, solubility of adsorbate in the solvent, temperature, adsorptive concentration, as well as competitive solvent adsorption should be taken into account. Beside adsorbent-adsorbate interaction, the solvent dramatically influence the resulting adsorption capacity (Henschel et al., 2011). Liquid phase separations by MOFs have been studied and were reviewed by Cychosz et al. (2010), however most of these studies treated sorption by MOFs from an organic solvent. Water stability is an important property for any sorbent to be used in waste water treatment plants. The labile nature of many metal-oxygen bonds can lead to hydrolysis of the network and thus irreversibly destroying the structure (Leus et al., 2016). There are only limited examples of sorption from water using MOFs (Bai et al., 2006; Jhung et al., 2007; Qin et al., 2009). However, water stable MOFs do exist and these structures have great potential for the treatment of wastewater (Cychosz and Matzger, 2010).

In present study the efficiency of the four classes of adsorbent materials was compared on sorption potential in order to remove mobile PPPs from an aqueous solution to understand adsorption behaviour in terms of equilibrium isotherms and adsorption kinetics.

C.2 EXPERIMENTAL ANALYSIS

C.2.1 PLANT PROTECTION PRODUCT PROPERTIES (ADSORBATES)

Based on their polarity (Log K_{ow}) and mobility (Log K_{oc}) PPPs can be classified into four categories. The K_{ow} value is the octanol-water partition coefficient and the K_{oc} value is the organic carbon partition coefficient. Plant protection products are classified as polar when the Log K_{ow} value is lower than 3.0 and as mobile when the Log K_{oc} is lower than 2.5 (Wang and Liu, 2007; De Wilde et al., 2008). Bentazon (Sigma Aldrich), clopyralid (Sigma Aldrich), and isoproturon (Sigma Aldrich) for this study are all selected polar and mobile PPPs (**Figure 2-18**). The chemical structure and the chemical property of each PPP are shown in **Table 2-6** in Part B of this chapter. The test solutions were prepared by diluting stock solutions to the test concentration of 10 mg.l^{-1}.

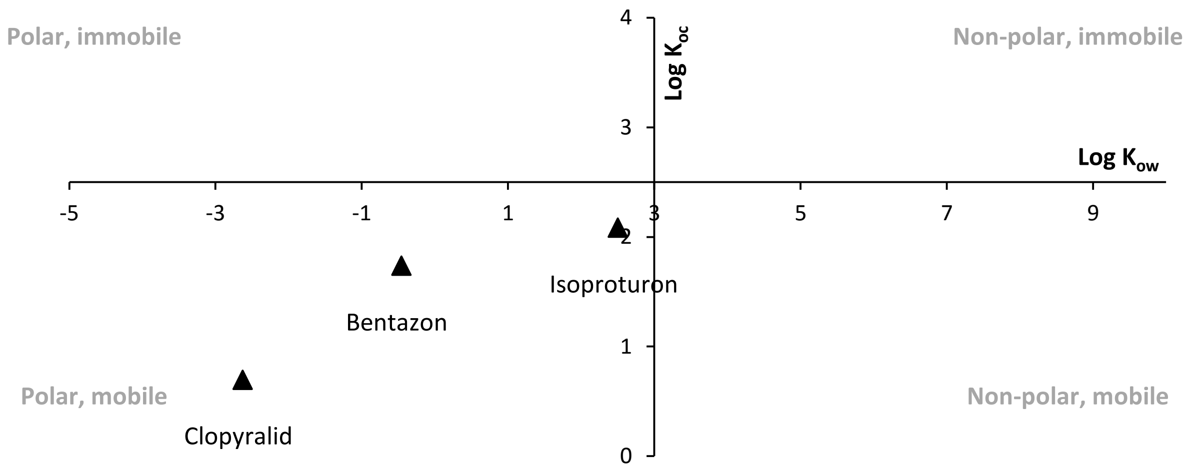


Figure 2-18. Selected plant protection products presented as a function of polarity (Log K_{ow}) and mobility (Log K_{oc}).

C.2.2 ADSORBENT PROPERTIES

Four classes of adsorbents were tested. The adsorbents were selected from the activated carbons, the resins, the zeolites and the MOFs. The first three types of materials were commercially available. However, most of the MOFs were synthesized following the recipes in the references given in **Table 2-20**. The selection of the different adsorbents was based on their adsorption properties, water stability and toxicity.

The Langmuir specific surface area (**Table 2-20**) of the adsorbents was determined using nitrogen adsorption/desorption measurements (**Appendix C**, **Figure C-2**). The isotherms were recorded on a Belsorp Mini II equipment (Bel Japan Inc, Osaka, Japan) at -196°C. The samples were pretreated at 150°C under vacuum.

X-ray diffraction measurements (**Figure 2-19**) of the synthesized MOFs were performed on an ARL X'tra X-ray diffractometer of Thermo Scientific (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a Cu KR1 tube and a Peltier cooled lithium drifted silicon solid stage detector.

Table 2-20. Different types of adsorbents.

Metal organic frameworks (MOFs)

N°	**Name**	**Metal**	**Linker**	**Langmuir-value ($m^2.g^{-1}$)**	***Reference***
M-1	Basolite® F300	Fe^{3+}	benzenetricarboxylate	326	*Sigma-aldrich, 2012a*
M-2	Basolite® C300	Cu^{2+}	benzenetricarboxylate	1871	*Sigma-aldrich, 2012b*
M-3	MIL-53	Al^{3+}	terephthalate	1354	*Loiseau et al., 2004*[a]
M-4	DUT-5	Al^{3+}	biphenyldicarboxylate	2378	*Senkovska et al., 2009*[a]
M-5	MIL-53-$(OH)_2$	Al^{3+}	dihydroxyterephthalate	non porous	*Biswas et al., 2011*[a]
M-6	MIL-53-NH_2	Al^{3+}	aminoterephthalate	212	*Savonnet et al., 2011*[a]
M-7	CAU-1-NH_2	Al^{3+}	aminoterephthalate	1192	*Savonnet et al., 2011*[a]
M-8	CAU-1-$(OH)_2$	Al^{3+}	dihydroxyterephthalate	1320	*Ahnfeldt et al., 2011*[a]
M-9	MIL-125	Ti^{4+}	terephthalate	1186	*Hardi et al., 2009*[a]
M-10	MOF-235	Fe^{3+}	terephthalate	non porous	*Haque et al., 2011*[a]

Resins

N°	**Name**	**Type**	**Langmuir-value ($m^2.g^{-1}$)**	***Reference***
R-1	Lewatit AF 5	carbon	1324	*Lanxess, 2011a*
R-2	Lewatit VP OC 1064	polystyrene	953	*Lanxess, 2011b*

Activated carbons

N°	**Name**	**Type**	**Langmuir-value ($m^2.g^{-1}$)**	***Reference***
A-1	GCN-1240	granular	1245	*Norit, 2011a*
A-2	GAC-1240	granular	1024	*Norit, 2011b*
A-3	C-GRAN	granular	1388	*Norit, 2011c*
A-4	DARCO-KB-WJ	powder	1800[b]	*Norit, 2009a*
A-5	DARCO-KB-G	powder	2588	*Norit, 2009b*
A-6	ORGANOSORB 10	granular	1075	*Desotec, 2011*

Zeolites

N°	**Name**	**Type**	**Langmuir-value ($m^2.g^{-1}$)**	***Reference***
Z-1	H-BEA-25	BEA	424.52	*Clariant,2010*
Z-2	H-BEA-35	BEA	482.57	*Clariant,2010*
Z-3	CP 811C-300	BEA	391.51	*Clariant,2010*
Z-4	CBV 720	FAU	757.57	*Zeolyst, 2011*
Z-5	CBV 780	FAU	757.25	*Zeolyst, 2011*

[a] These MOFs are synthesized following the procedures from these references.

[b] BET-value ($m^2.g^{-1}$)

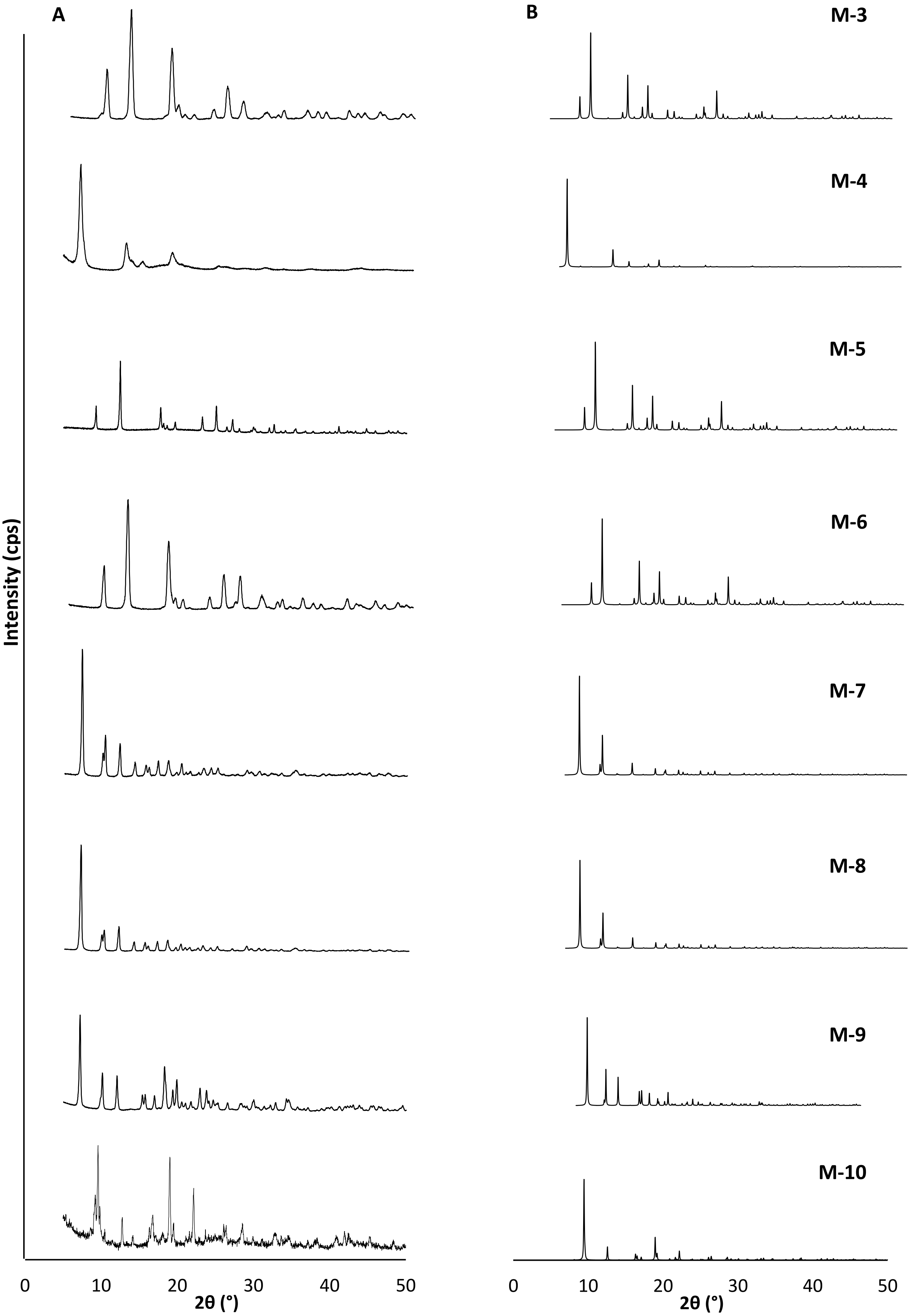


Figure 2-19. Experimental (A) and theoretical (B) powder X-ray diffraction patterns of the synthesized MOFs M-3 until M-10 (**Table 2-20**).

C.2.3 ADSORPTION EXPERIMENTS

Adsorption experiments, performed in a batch reactor system, were conducted in three ways, dependent on sorption (percentage), time (kinetic) and concentration (isotherm). The suspensions (sorbent and PPP aqueous solution) were shaken on an orbital shaker at 150 rpm at room temperature. The supernatants were filtered with a syringe filter containing a PVDF membrane with a pore size of 0.22 µm (Carl Roth, Karlsruhe-Rheinhafen, Germany). The aliquots with bentazon, clopyralid and isoproturon were injected into the HPLC-DAD. The amounts adsorbed were determined from the initial and final concentration of the contaminant solution. All the tests were carried out in triplicate and control runs containing the PPP but without the addition of an adsorbent were also included.

The protocols for the adsorption experiments were previously reported in Part B of this chapter (Section B.2.4). Additionally to the Freundlich and Langmuir equations, two other adsorption isotherm equations were used to describe the performance of the adsorbents, i.e. the Temkin and Dubinin-Radushkevich equations (**Table 2-21**).

Table 2-21. Temkin equation and Dubinin-Radushkevich equation.

	Non-linear form	Linear form
Temkin	$q_e = B_T \ln (A_T C_e)$	$q_e = B_T \ln A_T + B_T \ln C_e$
Dubinin-Radushkevich	$q_e = q_D \exp (-B_D \varepsilon^2)$	$\ln q_e = \ln q_D - B_D \varepsilon^2$

where q_e (mg.g^{-1}) is the amount of PPP sorbed at equilibrium concentration C_e (mg.l^{-1}). $B_T = RT.b_T^{-1}$ (J.mol^{-1}) corresponds to the heat of adsorption, with R as the ideal gas constant (J.K^{-1}.mol^{-1}) and T (K) as the absolute temperature. A_T (l.g^{-1}) and b_T are Temkin constants representing the Temkin isotherm equilibrium binding constant and the Temkin isotherm constant. Finally, q_D (mg.g^{-1}) is the maximum sorption capacity and B_D (mol^2.J^{-2}) and $\varepsilon = RT.\ln(1+1.C_e^{-1})$ are the Dubinin-Radushkevich isotherm constants.

C.2.4 $FeCl_4^-$-LEACHING

The presence of coordinated iron cations and chloride anions was confirmed by XRF analysis. This analysis is based on the emission of X-rays by the material when an electron drops down to a vacant level and releases energy. For this test 1.0 g of the adsorbent together with 100 ml distilled water or PPP aqueous solution were shaken on an orbital shaker at 150 rpm at room temperature. After 48 h the supernatants were filtered with a syringe filter containing a PVDF membrane with a pore size of 0.22 µm. The aliquots with water, bentazon, clopyralid and isoproturon were injected into the XRF. The amounts of iron and chloride were compared with the results obtained for a 1000 mg.l^{-1} $FeCl_3$ standard solution. The measurements were performed on a NEX CG from Rigaku using a Mo-X-ray source.

C.3 RESULTS AND DISCUSSION

C.3.1 SORPTION PERCENTAGE

A first selection of the different adsorbents was made based on their PPP adsorption capacity. The observed sorption percentages are presented in **Table 2-22**. The analysis of the obtained data demonstrated that activated carbon showed the best results for all PPPs. The three alternative materials (MOFs, resins and zeolites) gave various results.

The adsorption of the PPPs on zeolites was previously discussed in Part B of this Chapter (Section B.3.1.1). The adsorptions were found to take place through a number of different mechanisms such as electrostatic interactions, hydrophobic interactions and hydrogen bonding. Zeolites Z-1 and Z-5 were selected for further experiments.

Table 2-22. Sorption percentage (± SD) of the different adsorbents (**Table 2-20**) determined at an initial concentration of 10 mg.l^{-1}.

Adsorption %	Bentazon	Clopyralid	Isoproturon
		Metal organic frameworks	
M-1	30.35 ± 4.36	45.69 ± 0.45	44.91 ± 6.79
M-2	10.20 ± 2.67	17.18 ± 6.30	33.55 ± 1.94
M-3	52.45 ± 1.05	4.50 ± 6.37	99.40 ± 0.24
M-4	68.19 ± 2.08	67.63 ± 0.98	88.65 ± 6.54
M-5[a]	0.00 ± 0.00	54.32 ± 2.83	72.35 ± 6.71
M-6	31.30 ± 2.18	15.96 ± 0.20	95.15 ± 0.15
M-7	85.99 ± 1.47	72.68 ± 5.09	96.15 ± 0.68
M-8	89.24 ± 0.53	4.10 ± 1.30	100.00 ± 0.00
M-9	0.00 ± 0.00	0.00 ± 0.00	51.81 ± 0.47
M-10[a]	100.00 ± 0.00	100.00 ± 0.00	89.68 ± 0.21
		Resins	
R-1[a]	100.00 ± 0.00	100.00 ± 0.00	99.73 ± 0.50
R-2[a]	71.44 ± 3.17	5.34 ± 0.51	99.52 ± 0.03
		Activated carbons	
A-1	100.00 ± 0.00	98.88 ± 0.57	99.95 ± 0.07
A-2[a]	100.00 ± 0.00	100.00 ± 0.00	99.94 ± 0.08
A-3	100.00 ± 0.00	98.75 ± 0.16	99.68 ± 0.00
A-4	100.00 ± 0.00	99.77 ± 0.03	100.00 ± 0.00
A-5	100.00 ± 0.00	99.47 ± 0.05	99.87 ± 0.03
A-6[a]	100.00 ± 0.00	100.00 ± 0.00	99.80 ± 0.13
		Zeolites	
Z-1[a]	69.56 ± 2.46	9.12 ± 1.20	100.00 ± 0.00
Z-2	42.77 ± 2.26	3.55 ± 0.49	100.00 ± 0.00
Z-3	50.58 ± 2.17	16.94 ± 1.75	100.00 ± 0.00
Z-4	63.22 ± 1.63	10.67 ± 1.76	100.00 ± 0.00
Z-5[a]	100.00 ± 0.00	10.81 ± 1.53	100.00 ± 0.00

[a] Selected sorbents for further testing.

Given the similarity to zeolites, MOFs have the advantage to control the pore sizes. They can overcome the limitation in pore sizes due to their flexibility as compared to the relatively rigid zeolites (Peralta et al., 2012; Opanasenko et al., 2013; Qi-Long and Qiang, 2014). The thicker framework walls of zeolites, compared to MOFs, provide a smaller surface area (US-EPA, 1999; Farrusseng et al., 2009; Cychosz et al., 2010; Denayer et al., 2011). However, this benefit was not noticed in the adsorption results of the MOFs.

The different adsorption mechanisms of the PPPs onto the MOFs also depend on the surface chemistry of this latter. Based on the functional groups of the PPPs and the organic linker molecules of the MOFs, it could be derived that electrostatic interactions, hydrophobic interactions, hydrogen bonding and π-π interactions occurred. MOFs M-7 and M-10 showed the best adsorption results. MOF 7 has an aminoterephtalate as organic linker, which in particular may induce hydrogen bonding. In contrast, the high adsorption of PPPs on MOF 10 can be explained through electrostatic interactions. Haque et al. also showed that an iron terephthalate MOF (MOF-235) can successfully remove both anionic (MO) and cationic (MB) dyes from contaminated water through adsorption (Hasan and Jhung, 2015).

The variation in the results of zeolites and MOFs could be explained by their pore sizes. In contrast to these materials, activated carbons have a wide range of pore size distributions from micro- to macropores. Because of these micropores, activated carbon has a larger internal surface area compared to the other adsorbents (Inagaki and Feiyu, 2006). However, it is not only the pore size distribution that leads to a good adsorption. On an atomic level, activated carbon is considered to consist of graphene layers, i.e. layers of interlocking aromatic rings, also referred to as basal planes (Brennan et al., 2001).

These layers contain carbon atoms that are bonded together with three sigma bonds and one pi bond having sp^2 hybridization. It is also possible for sp^3 hybridization (tetrahedron) to occur, which may result in cross-linking among the graphite layers (Coughlin and Ezra 1968). It is asserted by Coughlin and Ezra (1968) that the basal face of the benzene ring can weakly adsorb through π interactions, while at edge sites adsorption is much stronger. Each PPP also consist of an aromatic ring, resulting in π-π interactions with the activated carbon. These interactions lead to an overall high adsorption capacity for all PPPs.

Polymeric adsorbents have also a range of pore sizes, but usually lack the very small micropores. The smallest pores are usually larger than the micropores of the activated carbon. Resin R-1 obtained better results compared to R-2, with adsorption percentages equal to those of activated carbon. This might be explained by the fact that this resin is a microporous carbonaceous bead type material, with a unique adsorptive surface with excellent selectivity for small polar molecules from polar solvents (Lanxess, 2011a, 2011b). The term "carbonaceous material" is used for materials with high content of elemental carbon, high porosity and large specific surface area (Wolowicz and Hubicki, 2016). These adsorber resins adsorb through mechanisms similar to activated carbon. They can be regarded as a kind of "fully synthetic" activated carbon (Lanxess, 2009).

Not only the adsorbate, but also the adsorbent can have an influence on the adsorption results. Isoproturon adsorbed the best, followed by bentazon and clopyralid. The results correlate with their Koc value. Especially the MOFs and zeolites were selective sorbents. Both carbon and polymer sorbents were not considered as highly selective (US-EPA, 1999). Despite the fact that zeolites are the precursors of MOFs, the completely different chemical nature (organic vs. inorganic) is expected to result in a different adsorption behaviour. The absence of extra-framework cations will lead to less strong interactions. On the other hand, organic linkers containing aromatic groups might also result in favorable interactions. A weaker but still selective interaction can even be beneficial for the design of an adsorptive separation process, since desorption requires less energy (Denayer et al., 2011).

In the following, two materials of each sorbent material with the best adsorption were selected for further testing. An overview is presented in Table 2-22. Because of the low adsorption results for clopyralid on R-2, Z-1 and Z-5, these materials were not further tested for this PPP.

C.3.2 SORPTION KINETICS

Sorption kinetics of bentazon, clopyralid and isoproturon on the selected adsorbents are presented in **Figure 2-20**. As mentioned in Part B of this chapter (Section B.2.4.2), two kinetic models were used to study the kinetics of sorption processes, i.e. the pseudo-first order and the pseudo-second-order models. Based on the R^2 values for the pseudo-first-order and pseudo-second-order model, ranging from 0.699 to 0.998 and from 0.9960 to 1.0000, respectively, this last model correlated much better with the experimental data. **Table 2-23** summarizes the kinetic parameters of the PPPs adsorbed at equilibrium. The pseudo-second-order constant k_2 ($g.mg^{-1}.h^{-1}$) gives an indication of the adsorption rate. The bigger the k_2 value, the faster equilibrium has been reached. This can also be derived from **Figure 2-20**.

Table 2-23. Kinetic parameters of the different adsorbents (**Table 2-20**) based on the pseudo-second order kinetic equations.

	Parameter		Bentazon	Clopyralid	Isoproturon
Metal organic frameworks					
M-7	$q_{e,exp}$	$(mg.g^{-1})$	9.56	8.23	9.37
	$q_{e,calc}$	$(mg.g^{-1})$	9.61	8.18	9.40
	k_2	$(g.mg^{-1}.h^{-1})$	0.53	0.48	1.09
	R^2		1.0000	0.9992	1.0000
M-10	$q_{e,exp}$	$(mg.g^{-1})$	10.00	9.76	7.15
	$q_{e,calc}$	$(mg.g^{-1})$	10.00	9.81	9.81
	k_2	$(g.mg^{-1}.h^{-1})$	0.27	0.51	0.51
	R^2		0.9997	0.9999	0.9999
Resins					
R-1	$q_{e,exp}$	$(mg.g^{-1})$	10.00	10.00	10.00
	$q_{e,calc}$	$(mg.g^{-1})$	10.09	10.12	10.06
	k_2	$(g.mg^{-1}.h^{-1})$	0.30	0.21	0.44
	R^2		0.9999	0.9999	1.0000
R-2	$q_{e,exp}$	$(mg.g^{-1})$	7.19	/	9.80
	$q_{e,calc}$	$(mg.g^{-1})$	7.22	/	9.86
	k_2	$(g.mg^{-1}.h^{-1})$	0.11	/	0.45
	R^2		0.9960	/	1.0000
Activated carbons					
A-2	$q_{e,exp}$	$(mg.g^{-1})$	10.00	10.00	9.99
	$q_{e,calc}$	$(mg.g^{-1})$	10.05	10.04	10.05
	k_2	$(g.mg^{-1}.h^{-1})$	0.49	0.62	0.49
	R^2		0.9999	1.0000	1.0000
A-6	$q_{e,exp}$	$(mg.g^{-1})$	10.00	10.00	9.99
	$q_{e,calc}$	$(mg.g^{-1})$	10.14	10.14	10.04
	k_2	$(g.mg^{-1}.h^{-1})$	0.19	0.19	0.47
	R^2		0.9997	0.9999	1.0000
Zeolites					
Z-1	$q_{e,exp}$	$(mg.g^{-1})$	5.26	/	10.00
	$q_{e,calc}$	$(mg.g^{-1})$	5.28	/	10.00
	k_2	$(g.mg^{-1}.h^{-1})$	1.13	/	500.00
	R^2		0.9998	/	1.000
Z-5	$q_{e,exp}$	$(mg.g^{-1})$	7.94	/	9.87
	$q_{e,calc}$	$(mg.g^{-1})$	7.89	/	9.86
	k_2	$(g.mg^{-1}.h^{-1})$	1.22	/	51.41
	R^2		0.9997	/	1.0000

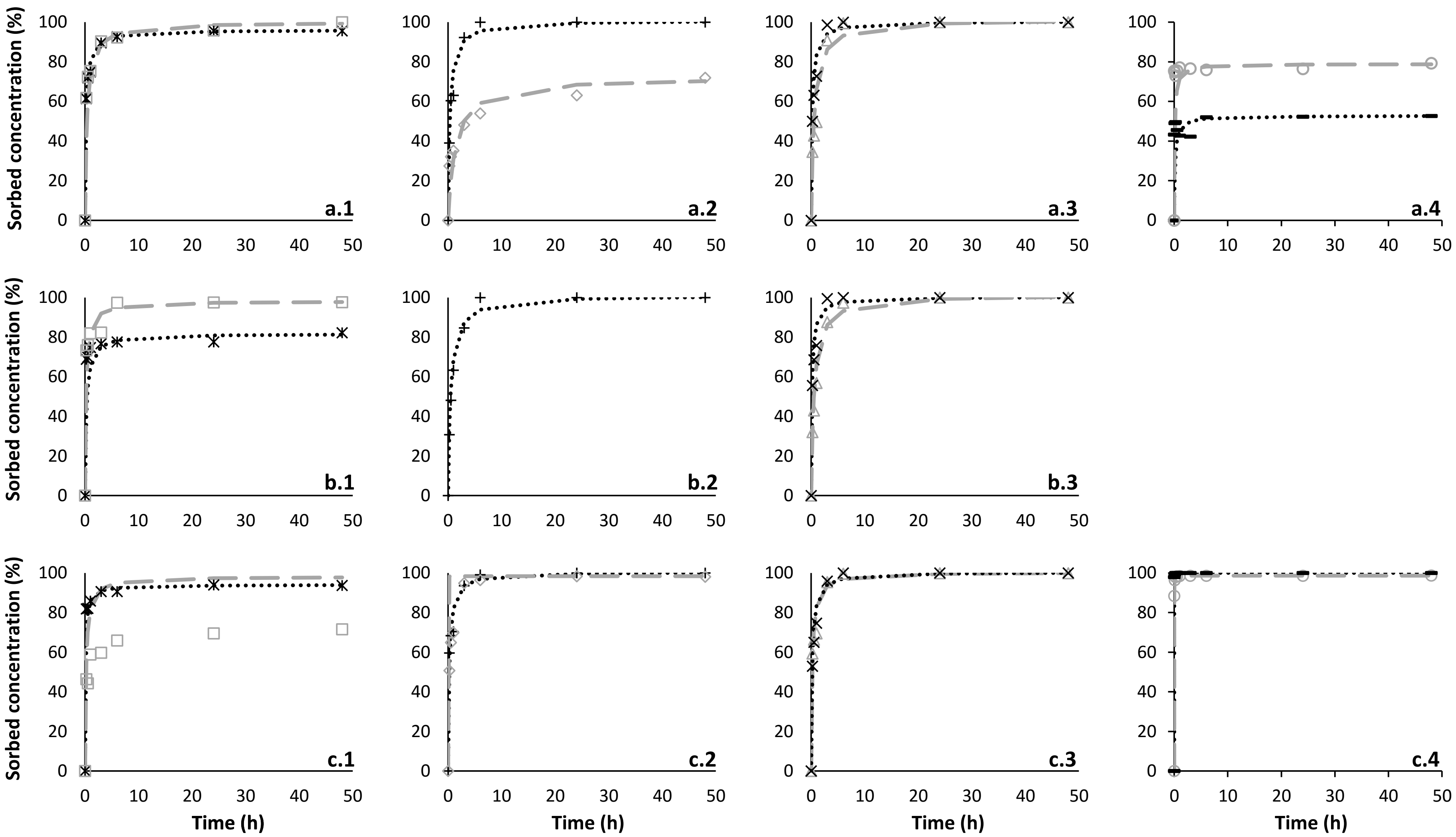


Figure 2-20. Experimental (indicated with the geometric symbols) and calculated (indicated with the lines) sorption kinetics of (a) bentazon, (b) clopyralid and (c) isoproturon for the different adsorbents (**Table 2-20**), 1: metal organic frameworks (M-7, ⁎; M-10, □), 2: resins (R-1, + ; R-2, ◇), 3: activated carbons (A-2, x ; A-6, △) and 4: zeolites (Z-1, ━ ; Z-4, ○) at 10 mg.l^{-1} as initial plant protection product concentration.

Generally seen, isoproturon was adsorbed the fastest by all adsorbents. This is in line with the K_{oc} values of the PPPs. When comparing the adsorbents, zeolites, followed by MOFs, were able to adsorb the PPPs much more rapidly in comparison with the other adsorbents. Activated carbon, MOFs, resins and zeolites showed respectively 66-86%, 74-91%, 44-100% and 86-100% of the equilibrium concentration being adsorbed after 1 h. This is explained by the particle size of the different adsorbents. As the particle size influences the flow characteristics and also adsorption kinetics (Torrado and Valiente, 2004; Marsh and Reinoso, 2006). The rate of adsorption is inverse to the particle size, in other words small particles have the fastest rate of adsorption (Marsh and Reinoso, 2006). Two resistances hinder the progression of the compound: the crossing of the laminar boundary layer surrounding the particle and the diffusion within the particle. The latter can be a diffusion in the liquid phase into the pores or a diffusion of the molecules in the adsorbed state at the pore's surface. This surface diffusion depends highly on the nature and structure of the adsorbent (Baup et al., 2000). The tested activated carbons were both granular types that had a relatively larger particle size compared to the powder types. Powder activated carbon (PAC) usually has an average diameter between 1 and 150 µm, while granular activated carbon (GAC) usually has a diameter between 0.5 and 5 mm (Verliefde et al., 2011). The resins had also smaller particle sizes compared to the zeolites and MOFs. These fine sizes provide a relatively high external surface area-volume ratio and reduces mass transfer resistance (Torrado and Valiente, 2004).

C.3.3 SORPTION ISOTHERMS

Adsorption isotherms are generally important to describe how adsorbates will interact with the adsorbents (Juang et al., 1996; Teng and Hsieh, 1998). More information about the different isotherms was described in Part B of this chapter (Section B.2.4.3). The experimental data showed a reasonable good fit to the equations for most adsorbents. The determination coefficients for the Langmuir sorption ranged from 0.4645 to 0.9954, for the Freundlich equation from 0.5323 to 0.9831, for the Temkin equation from 0.3804 to 0.9778 and for the Dubinin-Radushkevich (D-R) from 0.4643 to 0.9550.

Despite the good fit for most adsorbents to the Langmuir model and separation values between 0 and 1, the Langmuir model is not suitable to describe the sorption of the PPPs. Negative values were calculated for K_L and q_m which is improbable (Monkiedje and Spiteller, 2002). Additional data are given in **Table 2-24.**

The Freundlich model was found to give a better fit in the adsorption of the PPPs, especially for isoproturon (**Table 2-25**). This better fit of equilibrium data suggest multilayer adsorption (Boivon et al., 2005; Romero et al., 2006). The values of n obtained for all PPPs and adsorbents lies within the range of 1-10, which implies that the adsorbents had a high affinity for the PPPs in solution (Kadirvelu and Namasivayam, 2000).

Table 2-24. Isotherm parameters of the different adsorbents (**Table 2-20**) based on the Langmuir equations.

	Parameter		Bentazon	Clopyralid	Isoproturon
Metal organic frameworks					
M-7	K_L	(l.g^{-1})	0.888	-0.055	0.001
	q_m	(mg.g^{-1})	52.632	-22.272	14285.71
	R_L	(-)	0.092	0.692	0.497
	R^2	(-)	0.9528	0.9935	0.9775
M-10	K_L	(l.g^{-1})	8.558	0.104	0.009
	q_m	(mg.g^{-1})	22.472	52.910	119.048
	R_L	(-)	0.011	0.329	0.478
	R^2	(-)	0.9313	0.8513	0.8104
Resins					
R-1	K_L	(l.g^{-1})	26.308	16.789	5.857
	q_m	(mg.g^{-1})	29.240	31.348	243.902
	R_L	(-)	0.004	0.006	0.017
	R^2	(-)	0.7911	0.8116	0.9058
R-2	K_L	(l.g^{-1})	-0.089	/	0.777
	q_m	(mg.g^{-1})	-12.579	/	71.942
	R_L	(-)	0.904	/	0.102
	R^2	(-)	0.9905	/	0.9888
Activated carbons					
A-2	K_L	(l.g^{-1})	3.267	3.275	10.348
	q_m	(mg.g^{-1})	51.020	59.880	42.017
	R_L	(-)	0.029	0.029	0.009
	R^2	(-)	0.4645	0.7237	0.8651
A-6	K_L	(l.g^{-1})	0.902	3.275	7.941
	q_m	(mg.g^{-1})	120.482	59.880	74.074
	R_L	(-)	0.091	0.029	0.0122
	R^2	(-)	0.9954	0.9756	0.7265
Zeolites					
Z-1	K_L	(l.g^{-1})	-0.076	/	3.275
	q_m	(mg.g^{-1})	-7.651	/	59.880
	R_L	(-)	0.808	/	0.029
	R^2	(-)	0.6689	/	0.9773
Z-5	K_L	(l.g^{-1})	-0.080	/	0.927
	q_m	(mg.g^{-1})	-30.864	/	78.740
	R_L	(-)	0.832	/	0.089
	R^2	(-)	0.9694	/	0.9923

Table 2-25. Isotherm parameters of the different adsorbents (**Table 2-20**) based on the Freundlich equations.

Parameter			Bentazon	Clopyralid	Isoproturon
			Metal organic frameworks		
M-7	**K_F**	(mg.g^{-1})	20.855	0.686	16.361
	n	(-)	1.682	0.634	1.098
	R^2	(-)	0.9726	0.9699	0.9379
M-10	**K_F**	(mg.g^{-1})	16.696	4.774	0.924
	n	(-)	4.608	1.181	0.947
	R^2	(-)	0.9447	0.7797	0.8210
			Resins		
R-1	**K_F**	(mg.g^{-1})	23.491	24.734	161.176
	n	(-)	4.003	3.388	1.982
	R^2	(-)	0.8043	0.8288	0.9363
R-2	**K_F**	(mg.g^{-1})	0.305	/	26.662
	n	(-)	0.450	/	1.675
	R^2	(-)	0.9299	/	0.9717
			Activated carbons		
A-2	**K_F**	(mg.g^{-1})	40.179	38.098	37.471
	n	(-)	2.006	2.232	2.369
	R^2	(-)	0.8690	0.8835	0.9454
A-6	**K_F**	(mg.g^{-1})	43.162	38.098	59.869
	n	(-)	1.852	2.232	2.592
	R^2	(-)	0.9463	0.9469	0.9173
			Zeolites		
Z-1	**K_F**	(mg.g^{-1})	0.053	/	38.098
	n	(-)	0.369	/	2.232
	R^2	(-)	0.5323	/	0.9260
Z-5	**K_F**	(mg.g^{-1})	1.355	/	31.550
	n	(-)	0.564	/	1.717
	R^2	(-)	0.858	/	0.9869

The affinity depends on the type of adsorbent and on the adsorbate. Activated carbon, together with the carbonaceous resin R-1, showed a high affinity for all PPPs. This can be explained by the π-π interactions between the PPPs and the adsorbents. Also the fact that both adsorbents are not highly selective, as mentioned before, can be a possible clarification of the obtained results. Carbon is neither fully hydrophobic nor hydrophilic and retains some adsorption area with affinity for both polar and non-polar molecules.

Polymers are usually hydrophobic unless modified with another affinity. There appeared to be some preference for isoproturon. In general, non-ionic PPPs are relatively less mobile than ionic PPPs, and consequently their affinity for adsorbents is higher (De Wilde et al., 2009). This seems to be an explanation why the non-ionic PPP isoproturon had a higher sorption intensity compared to the other PPPs. Striking was the high affinity of resin 1 for the anionic PPPs bentazon and clopyralid. This resin has a unique adsorption surface with excellent selectivity for small polar molecules from polar solvents, which explains the observed results (Lanxess, 2011a, 2011b).

Subsequently, the results obtained by the zeolites and MOFs are given in **Table 2-25**. All naturally occurring zeolite is hydrophilic, having an affinity for polar substances, such as water, but synthetic zeolites can be either hydrophilic or hydrophobic. The hydrophilicity and hydrophobicity of MOFs is adjustable by the introduction of various organic groups, but it is still not as flexible as adjusting the charge density of zeolites (US-EPA, 1999; Bu and Feng, 2003). Based on the results, a higher affinity for isoproturon was also observed on these materials. Striking here was the high affinity of M-10 for bentazon. The preference for the anionic PPP bentazon by this MOF can be explained by its structure. Electrostatic interactions may occur between the adsorbate and adsorbent. More information about this is shown in Section C.3.4 ($FeCl_4^-$-leaching).

Furthermore, adsorbents based on activated carbon have generally been found to exhibit higher adsorption capacities than those based on zeolites, and thus also on MOFs (Acton, 2013). This statement is in line with the obtained results. After considering the limitation of the Langmuir adsorption isotherm, the Temkin model takes into account the indirect adsorbate interactions. The model assumes that the fall in the heat of sorption (B_T) decreases linearly with coverage, rather than logarithmic as supposed by the Freundlich equation (Abasi et al., 2011). Examination of the data showed a good fit of the Temkin isotherm for the activated carbon, and a reasonable fit for the other adsorbents (**Table 2-26**). Generally, the values for B_T obtained in the present study indicate somewhat weak ionic interactions (physisorption). This result is in line with the multilayer sorption observed by the Freundlich equation. As shown by the Freundlich model, the potential of adsorption A_T also suggests that the affinity of the PPPs for activated carbon and the carbonaceous resin seemed to be higher than for zeolites and MOFs.

Finally, the D-R isotherm is reported to be more general than the Langmuir and Freundlich isotherms (Abasi et al., 2011). This model helps to estimate the characteristic porosity in addition to the apparent free energy (E_D) of adsorption. Based on the determination coefficient values, the D-R isotherm provided a poor fit for all adsorbents compared to the other energy parameter model Temkin (**Table 2-27**). The values of E_D in this work were lower than 8 $kJ.mol^{-1}$ and pointed to a physisorption-dominated process for the sorption of PPPs on to the adsorbents. Just as obtained by the Temkin equation, the higher values obtained by activated carbon and the carbonaceous resin sticked out, indicating a stronger connection between the adsorbate and the adsorbent.

Table 2-26. Isotherm parameters of the different adsorbents (**Table 2-20**) based on the Temkin equations.

	Parameter		**Bentazon**	**Clopyralid**	**Isoproturon**
Metal organic frameworks					
M-7	**A_T**	(l.g^{-1})	4.624	0.322	1.856
	B_T	(J.mol^{-1})	17.600	19.750	31.487
	b_T	(-)	140.771	125.447	78.686
	R^2	(-)	0.8293	0.8692	0.7933
M-10	**A_T**	(l.g^{-1})	171.870	0.866	0.262
	B_T	(J.mol^{-1})	3.489	14.494	14.871
	b_T	(-)	710.069	170.938	166.604
	R^2	(-)	0.8979	0.6225	0.4957
Resins					
R-1	**A_T**	(l.g^{-1})	136.398	59.348	141.480
	B_T	(J.mol^{-1})	5.727	7.505	29.787
	b_T	(-)	432.605	330.145	83.176
	R^2	(-)	0.6294	0.5853	0.9159
R-2	**A_T**	(l.g^{-1})	0.285	/	5.866
	B_T	(J.mol^{-1})	37.359	/	20.162
	b_T	(-)	66.318	/	122.883
	R^2	(-)	0.7484	/	0.8314
Activated carbons					
A-2	**A_T**	(l.g^{-1})	14.306	175.712	28.404
	B_T	(J.mol^{-1})	19.850	8.230	14.651
	b_T	(-)	124.815	301.060	169.106
	R^2	(-)	0.8926	0.8790	0.7352
A-6	**A_T**	(l.g^{-1})	13.412	99.275	80.546
	B_T	(J.mol^{-1})	21.566	8.524	16.187
	b_T	(-)	114.883	290.662	153.059
	R^2	(-)	0.9600	0.9463	0.9778
Zeolites					
Z-1	**A_T**	(l.g^{-1})	0.212	/	21.133
	B_T	(J.mol^{-1})	34.424	/	14.486
	b_T	(-)	71.972	/	150.283
	R^2	(-)	0.3804	/	0.7203
Z-5	**A_T**	(l.g^{-1})	0.420	/	7.653
	B_T	(J.mol^{-1})	40.751	/	20.746
	b_T	(-)	60.798	/	119.424
	R^2	(-)	0.5997	/	0.8926

Table 2-27. Isotherm parameters of the different adsorbents (**Table 2-20**) based on the Dubinin-Radushkevich equations.

	Parameter		**Bentazon**	**Clopyralid**	**Isoproturon**
			Metal organic frameworks		
M-7	q_D	(mg.g^{-1})	44.372	26.552	63.434
	B_D	(mol^2.J^{-2})	1 E-07	5 E-06	4 E-07
	E_D	(kJ.mol^{-1})	2.236	0.316	1.118
	R^2	(-)	0.7422	0.8817	0.8866
M-10	q_D	(mg.g^{-1})	22.227	25.290	2.821
	B_D	(mol^2.J^{-2})	2 E-08	9 E-07	6 E-06
	E_D	(kJ.mol^{-1})	5.000	0.745	0.289
	R^2	(-)	0.8596	0.6237	0.5425
			Resins		
R-1	q_D	(mg.g^{-1})	32.034	34.716	146.804
	B_D	(mol^2.J^{-2})	1 E-08	2 E-08	2 E-08
	E_D	(kJ.mol^{-1})	7.071	5.000	5.000
	R^2	(-)	0.5977	0.5907	0.9550
R-2	q_D	(mg.g^{-1})	45.146	/	54.473
	B_D	(mol^2.J^{-2})	6 E-06	/	9 E-08
	E_D	(kJ.mol^{-1})	0.289	/	2.357
	R^2	(-)	0.8345	/	0.8304
			Activated carbons		
A-2	q_D	(mg.g^{-1})	63.612	50.608	50.174
	B_D	(mol^2.J^{-2})	5 E-08	2 E-08	2 E-08
	E_D	(kJ.mol^{-1})	3.162	5.000	5.000
	R^2	(-)	0.6762	0.8071	0.6673
A-6	q_D	(mg.g^{-1})	76.792	49.112	80.214
	B_D	(mol^2.J^{-2})	6 E-08	2 E-08	2 E-08
	E_D	(kJ.mol^{-1})	2.887	5.000	5.000
	R^2	(-)	0.9500	0.9275	0.8793
			Zeolites		
Z-1	q_D	(mg.g^{-1})	34.426	/	59.842
	B_D	(mol^2.J^{-2})	1 E-05	/	4 E-08
	E_D	(kJ.mol^{-1})	3 E-04	/	3.536
	R^2	(-)	0.4643	/	0.8272
Z-5	q_D	(mg.g^{-1})	46.740	/	60.087
	B_D	(mol^2.J^{-2})	3 E-06	/	8 E-08
	E_D	(kJ.mol^{-1})	0.408	/	2.500
	R^2	(-)	0.7237	/	0.8664

C.3.4 $FeCl_4^-$-LEACHING

The MOF-235 framework, $[Fe_3O(C_8H_4O_4)_3(C_3H_7NO)_3]^+.[FeCl_4]^-$, has a positive charge, which is balanced with the negative $[FeCl_4]^-$-ion (Haque et al., 2011). Based on this information it seems possible that the negative ion is able to dissociate from the positive ion. In this case, more anionic PPPs can adsorb on the MOF-235. In order to prove this statement, the XRD pattern of the MOF-235 before adsorption was compared with the XRD pattern after adsorption, demonstrated in **Figure 2-21**. Additional information about the water stability of the other MOFs is given in **Appendix D** (**Table D-1**).

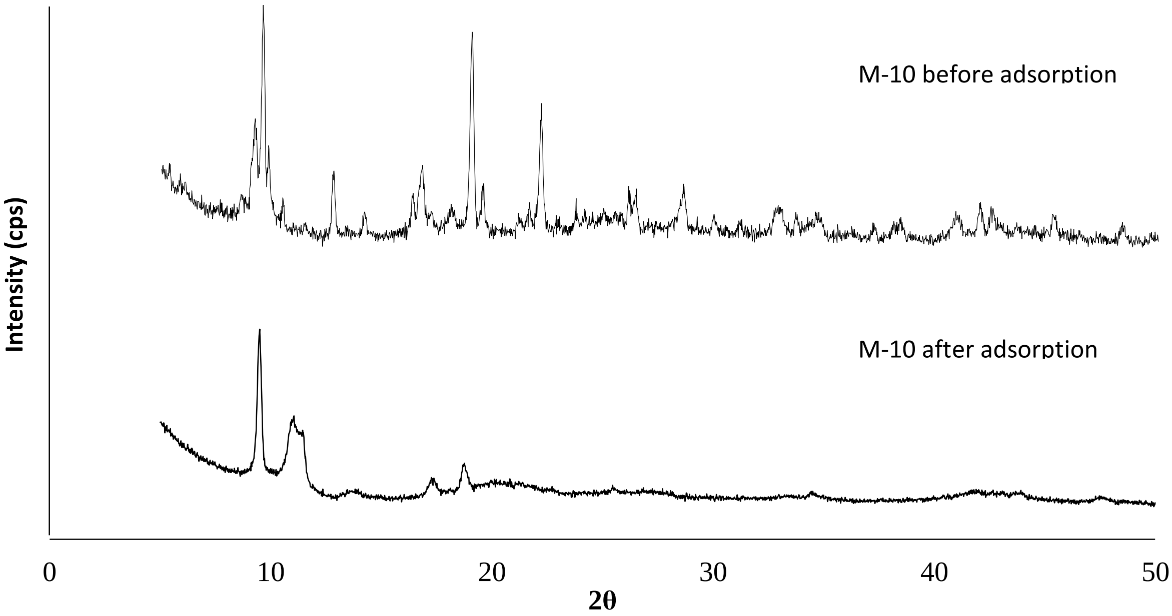


Figure 2-21. The X-ray diffraction pattern of MOF-235 before and after adsorption.

Based on the obtained results, it can be deduced that the structure of MOF-235 did not remain stable after the adsorption process. In order to demonstrate that this was the result of $[FeCl_4]^-$-leaching, the presence of these ions in the PPP solutions was examined by XRF. Therefore 1 g adsorbent was added to 100 ml solution. The results are summarized in **Table 2-28**.

Table 2-28. The Fe^{3+} and Cl^- intensities in the solutions, observed by XRF analyses.

Solution	Fe^{3+} (cps)	Cl^- (cps)
Standard (1 g l^{-1} $FeCl_3$)	49644	130110
Distilled water	42534	147805
Bentazon	43650	144989
Clopyralid	44791	142139
Isoproturon	42399	148149

Figure 2-22 demonstrates the theoretically amount of 0.344 g.l^{-1} Fe^{3+} and 0.656 g.l^{-1} Cl^{-} in a solution of 1 g.l^{-1} FeCl$_3$. Based on these values and the values in **Table 2-28**, it can be calculated that the solution with distilled water contained 0.294 g.l^{-1} Fe^{3} and 0.747 g.l^{-1} Cl^{-}. The molar ratio Fe^{3+}/Cl^{-} derived from these values was equal to 0.25. This indicated that the iron and chlorine in the solution were present as FeCl$_4^-$-ions. In liquid this negative ion dissociates from the adsorbent. This dissociation resulted here in a positive charged adsorbent, which indicates that the MOF-235 structure is unstable. Similar observations were noted for the PPP solutions. The result gives an explanation for the higher adsorption percentage and affinity of the PPPs for this MOF.

Figure 2-22. Theoretically amount of iron and chlorine present in a solution of 1 g.l^{-1} FeCl$_3$.

Fe	**+**	**3 Cl**	**→**	**FeCl$_3$**
				1 g.l^{-1}
55.845 g.mol^{-1}		35.453 g.mol^{-1}		162.204 g.mol^{-1}
0.006 mol.l^{-1}		**0.018 mol.l^{-1}**		**0.006 mol.l^{-1}**
0.344 g.l^{-1}		0.656 g.l^{-1}		

C.4 CONCLUSION

Sorption of PPPs on four types of adsorbent materials were studied in an aqueous environment. Despite the fact that zeolites and MOFs were able to adsorb the PPPs more rapidly in comparison with the other adsorbents, activated carbon and the carbonaceous resin showed generally seen the best adsorption results for all PPPs. This slower rate of adsorption might be explained by the bigger particle size of these adsorbents. The equilibrium data were analysed using four different models, Langmuir, Freundlich, Temkin and D-R. The Freundlich isotherm was the best model to describe sorption of the PPPs. The activated carbon and carbonaceous resin have generally been found to exhibit higher adsorption capacities than those based on zeolites and MOFs. Their disadvantage was their high affinity for the PPPs, which makes it difficult to regenerate these materials. Therefore, zeolites and MOFs are attractive materials when it comes to regeneration. Nevertheless, the Temkin equation, which showed a reasonable fit, predicted physisorption for all adsorbents. MOF-235 obtained its good results based on the FeCl$_4^-$-ion exchange. However, this led to an unstable structure, which makes regeneration of this material impossible.

“If a cluttered desk is a sign of a cluttered mind,
of what, then, is an empty desk a sign?”
-Albert Einstein-

CHAPTER 3


ZEOLYST
INTERNATIONAL
To:
BEA 950
BEA 950
CLARIANT
EZ 00178
GALENIKA-FITOFARMACIJA
R&D LABORATORY
ZEOLITE 850 WP
LOT:34/14
100 g

Chapter 3

FORMULATION OF ZEOLITES

Before zeolites can be used in agriculture, they need to be formulated. In order to select the most appropriate formulation type physicochemical tests need to be performed. In the following chapter, three different formulation types were investigated with respect to evaporation, surface tension, spreading, rainfastness and deposition.

1 INTRODUCTION

1.1 WHAT IS A FORMULATION?

A PPP is rarely used or applied in its pure form. The technical grade compound must first be formulated, whether it is an herbicide, insecticide, fungicide, or another classification, because they may not mix well with water, may be chemically unstable or may be difficult to handle and transport. Even though the active ingredient in the PPP formulation must ensure biological activity, other constituents, such as adjuvants, are needed to improve application effectiveness, safety, handling and storage (Fishel, 2013).

Generally, adjuvants are compounds that modify the activity of the PPP or the physical properties of the PPP mixture (Hazen, 2000). They improve the product by having an influence on the solubility of the active ingredient, physicochemical and spreading properties, biological activity and safety issues (Spanoghe, 2005).

Since a wide variety of PPPs is available on the market, many different types of formulations have been developed. The type of formulation mainly depends on the physicochemical properties of the active ingredients (Knowles, 2008). Due to the physicochemical properties of zeolites, different types of formulations are possible. In the current study, three formulation types were regarded as qualified in function of their application, i.e. an oil dispersion (OD), a suspension concentrate (SC) and a wettable powder (WP) formulation.

1.2 PHYSICOCHEMICAL PROPERTIES OF A FORMULATION

The surface of leaves, roots, insects, fungi and the composition of the soil vary a lot and largely determine the contact, plant uptake, persistence, availability and the final effect of PPPs. The formulation design has become a crucial step in optimizing PPPs. The formulator takes into account many aspects in which the physicochemical properties are very important (Spanoghe, 2014). Relevant physicochemical properties for selecting the most appropriate zeolite formulation in this research were evaporation, surface tension, spreading, deposition and rainfastness.

1.2.1 EVAPORATION

Evaporation of adjuvants is usually underestimated. When adjuvants intend to modify the leaf surface by stimulating the uptake of the active substance, it may be that volatile additives are no longer present at the right moment. A fast evaporation of the spray liquid results in smaller drops and less deposition on the leaf surfaces.

When water or solvent evaporates, the concentration of the surfactants used in the present solution increases. This can give rise to the formation of 'liquid crystals', which influence the biological effectiveness. One possible consequence is a limited diffusion of the PPPs. Evaporation of water can be prevented by adding adjuvants that move into the liquid-air interface (Spanoghe, 2005).

1.2.2 SURFACE TENSION

At liquid-air or solid-air interfaces, there is a force that makes the interface as small as possible. This force is called the 'surface tension', denoted by the Greek letter gamma (γ). The stronger the intermolecular interactions, the greater the surface tension. That is why most of the oil-like liquids have a small surface tension with a value around 25 $mN.m^{-1}$ at room temperature. Water has a much higher attraction, with a value around 73 $mN.m^{-1}$, because of its hydrogen bonds and dipoles. This surface tension can be reduced by using surfactants (Spanghe, 2005).

1.2.3 SPREADING

In order to have a good activity, the spray droplet of a PPP must be able to wet the foliage and spread out evenly. As described above, surfactants physically change the surface tension of a deposited spray droplet. They make the area of PPP coverage larger, which increases the pest's exposure to the chemical. Without proper wetting and spreading, spray droplets often run off or fail to provide good coverage of the leaf surfaces. Too much surfactant, however, can cause phytotoxicity or excessive runoff, which makes the plant protection product less effective.

The better the spreading, the smaller the contact angle. The contact angle (α) is the angle at which a liquid meets a solid. In case of partial wetting, the contact angle $\alpha > 0$, and in case of complete wetting, the contact angle $\alpha = 0$. The shape of the droplet is the result of the two opposing forces, i.e. surface tension tries to create a spherical droplet, while gravity wants to flatten it. The lower the surface tension of a liquid, the better its wettability, and the smaller the contact angle (Spanoghe, 2005).

1.2.4 RAINFASTNESS

A PPP’s rainfastness, or its ability to withstand rainfall, is an important factor affecting the efficacy of foliar-applied PPPs (Wells and Fishel, 2014). Rainfastness can be defined as the retention of activity of the active substance when the application of the PPP is shortly followed by a rainfall (Penner, 2000). Rainfall can adversely affect a plant protection product application by (1) directly washing the PPP away or physically removing it, (2) diluting the product to a less effective form, (3) redistributing the active ingredient, or (4) extracting the PPP from the plant tissue altogether. The overall rainfastness of a PPP depends on the time between the application and the rainfall event, the amount of rainfall, the formulation of the PPP, and the properties of the target surface (Wells and Fishel, 2014).

Sticking adjuvants enhance rainfastness and reduce wash-off. A sticker is an additive that increases the adhesion of solid particles to target surfaces. According to Leung and Webster (1994) and Steurbaut et al. (2001b), solutions with low surface tension and low contact angle may dry up rapidly on foliage, resulting in crystalline, rainfast deposits. The indirect effect of adjuvants on rainfastness is related to deposit characteristics and is discussed in the next paragraph.

1.2.5 DEPOSITION

Besides the active ingredients, spraying techniques can also have an important impact on the effect of PPPs in crop protection. The physical properties of the spray liquid can interact with nozzle design and affect spray quality (Butler Ellis and Tuck, 1999; Butler Ellis et al., 2001; Stainier et al., 2004). One of the most overlooked factors, that can dramatically influence the effectiveness of a given PPP, is the spray distribution. The uniformity of the spray distribution across the spray boom or within the spray swath affects the biological effect of the spray application treatment and, also, the dose required to achieve a specific effect (Larsolle et al., 2002).

There are a number of factors contributing to the distribution quality of a spray boom, i.e. nozzles (type, pressure, spacing, spray angle, flow rate, etc.), boom height, pressure losses, plugged filters, etc. (TeeJet, 2014). Several adjuvants also improve spray deposition on the surfaces (Balsari et al., 2001), due to their ability to reduce surface tension of PPP solutions.

2 MATERIALS AND METHODS

2.1 TEST ITEMS

Galenika Fitofarmacija (Belgrade, Serbia) developed three different types of formulations of zeolite LTA, i.e. oil dispersion, suspension concentrate and wettable powder (**Table 3-1)**.

Oil dispersions (OD) are multiphase formulations where the active ingredient is suspended in oil. An oil-based suspension concentrate is a stable suspension of one or more insoluble solid active ingredients in an organic fluid (mineral oils, vegetable oils) intended for dilution with water before use. Subsequently, suspension concentrates (SC) are multiphase formulations where the active ingredient is suspended in water. This formulation type is a stable suspension of one or more insoluble solid active ingredients in a fluid (water) intended for dilution with water before use. Finally, wettable powder (WP) formulations are solid powder formulations for dilution in water. This formulation type is a solid formulation of one or more liquid or solid active ingredients, which are blended and mixed with inert diluents (fine mineral clays). After dispersion in water, this formulation type is applied as a suspension (ECO-ZEO, 2016).

2.2 PHYSICOCHEMICAL EXPERIMENTS

2.2.1 EVAPORATION

A gravimetric method was used to compare the volatility behaviour of non-water soluble formulations (Sundaram, 1986). A circular polyurethane sponge (S; **Figure 3-1**) of 4.7 cm diameter and 1.5 cm thickness was placed inside the lid of a plastic petri dish (PD) of 4.8 cm diameter and 0.8 cm height, with four pins (P) fixed onto the sponge to form the corners of a square. The pointed ends of the pins provided the base for mounting a Whatman N_o. 1 filter paper of 4.25 cm diameter and 250 + 25 µm thickness. This system was placed on an analytical balance of sensitivity 0.0001 g and the weight was recorded. A 100 µl aliquot of the liquid to be studied was pipetted onto the filter paper, and the initial weight was immediately recorded. As the liquid continued to evaporate, the doors of the balance were left open to allow air circulation. Residual weights were recorded at 2, 4, 6, 10, 20, 30, 60, 120 and 180 minutes after the initial weighting. Results are presented in terms of the percentage of initial weight remaining at different intervals of time. The experiment was carried out in an environmental chamber maintained at 20 ± 1°C and 70-75% relative humidity (RH) for different concentrations (100 $mg.l^{-1}$, 1000 $mg.l^{-1}$ and 10000 $mg.l^{-1}$) of the different formulation types. This study was performed in triplicate.

Table 3-1. Properties of the developed oil dispersion (OD), suspension concentrate (SC) and wettable powder (WP) formulations.

Formulation types		**OD**	**SC**	**WP**
Properties	**Unit**			
pH	-	7.60	7.90	7.40
Relative density	g.cm^{-3}	1.11	1.48	-
Suspensibility/Dispersion	%	6.00	102.50	94.90
Persistent foam	cm³ (after 1 min)	0.00	0.00	0.00
Wet sieve test	% (75 µm)	0.05	0.21	0.41
Composition	**Function**			
Active ingredient		Zeolite 4A (LTA)	Zeolite 4A (LTA)	Zeolite 4A (LTA)
Adjuvants	Dispersing agent	Tensiofix NTM	Morwet D425, Reax 88B	Soprophor FL
	Structuring agent	Tixosil 365	-	PVP K-30
	Thickening agent	Tensiofix 869	Rhodopol 23	-
	Wetting agent	-	Supragil WP	-
	Antifoam agent	-	SAG 1572	-
	Biocide	-	Amebact C	-
Medium/carrier		Soya bean oil	Water	Ultrasil VN 3

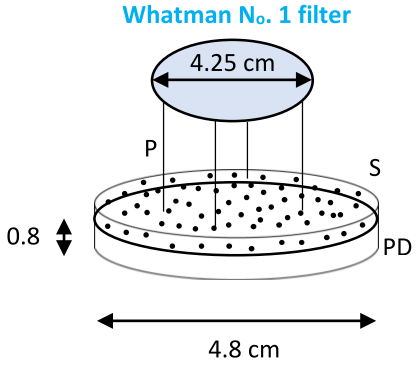


Figure 3-1. Experimental set-up for the gravimetric method (based on Sundaram, 1986).

2.2.2 SURFACE TENSION

The Wilhelmy plate method is used to measure the surface tension of liquids. This method utilizes the interaction between a thin platinum plate and the surface of the liquid. The plate has to be thoroughly cleaned with sulfochromic acid and rinsed with distilled water in order to maintain good wetting of the plate by the test liquid. The plate was attached to a balance via a thin metal wire put in a fixed position relative to horizontal surface of the liquid. First, the balance was calibrated using distilled water having a surface tension of 72 mN.m^{-1} at 20°C. This surface tension corresponded to a value of 0.183 g on the balance. This was done by placing a 100 ml beaker filled with 20 ml distilled water under the platinum plate. After bringing the plate into contact with the liquid, the beaker containing the liquid was gradually lowered, using an adjustable platform, until the plate had made contact with the surface of the liquid. The force exerted on the plate due to wetting was measured via the microbalance at the point of detachment. These forces were recorded for different concentrations of the formulation solutions: 0, 10, 100, 1000, 10000 and 100000 mg.l^{-1}. The experiment was carried out in triplicate. An illustration of this method is shown in **Figure 3-2**.

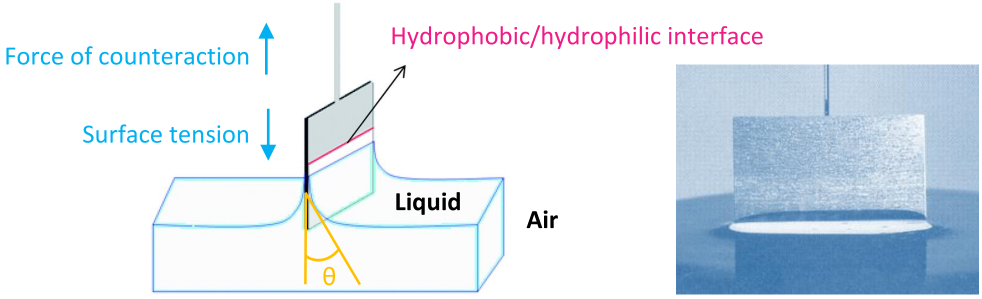


Figure 3-2. Illustration of the Wilhelmy plate method (Spanoghe, 2005; Sah, 2014).

The Wilhelmy equation is applied to determine the surface tension γ:

$$F = m \cdot g = W_{plate} + \gamma \cdot \cos \alpha \cdot perimeter_{plate}$$

where F is the measured force ($mN.m^{-1}$), m is the observed mass (g), g is the gravitational acceleration ($9.81\ m.s^{-2}$), W_{plate} is the mass of the plate (0.000072 kg), γ is the surface tension ($N.m^{-1}$), α is the contact angle (0°) and γ · cosα · perimeter is the interfacial strength (0.024 m).

The interfacial strength is a multiplication of the surface tension γ of the liquid with the perimeter of the plate (2L= perimeter of the plate, with L as the length of the plate). As a prerequisite, the plate has to be completely wetted. The value of F, W_{plate} and L can be measured accurately. Since the plate is manufactured of platinum, complete wetting (α = 0, cosα = 1) is assured, whereby the term cos α can be neglected. By plotting the surface tension in function of the concentration, the critical micelle concentration (CMC = critical micelle concentration) can be determined.

2.2.3 CONTACT ANGLE

Contact angle measurements were performed with a Krüss Drop Shape Analysis System G10/DSA10-device. The measuring device is equipped with an automatic image processing camera and software to calculate the contact angle. Before measuring, the syringe and the attached needle were washed five times with distilled water and then rinsed with the test solution. A 3 µL drop of each formulation was added to the leaf surfaces at a flow rate of 200 $µl.min^{-1}$. The contact angle was quantified by fitting the suitable model to the curve of the droplet on the surface. The experiment was carried out in triplicate for the different formulations, concentrations (0, 10, 1000 and 100000 $mg.l^{-1}$) and leaves (apple and tomato).

There is no universally suitable model for the drop shape analysis. Different fitting procedures are used to determine the best shape of the drop and thus the correct contact angle, i.e. circle method, height-width approach, tangent 1 method, tangent 2 method and Young-Laplace method. In this experiment, the Young-Laplace model was used to fit the contact angle of the different formulations.

After measuring the contact angle, the spreading coefficient (S) can be determined by applying the droplet radius ratio of a spray liquid R_1 to distilled water R_2 on a leaf surface (Uniqema, 2000):

$$S = \frac{R_1}{R_2}$$

where R_1 is the radius of the diluted formulation and R_2 is the radius of distilled water. The radius can be determined based on of the following formula:

$$V = \pi \cdot R^3 \cdot [1 - \cos\alpha + \frac{1}{3} \cdot (\mathrm{Cos}^3\alpha - 1)]$$

where V is the volume of the droplet (in this case, 3 µl), R is the radius of the droplet and α is the contact angle of the droplet (determined by the software). Since the volume and the contact angle of the droplet are given, or can be calculated, it is possible to determine the radius R the transformation of the formula:

$$R^3 = \frac{V}{\pi .\ [1 - \cos\alpha + \frac{1}{3} .\ (\mathrm{Cos}^3\alpha - 1)}$$

2.2.4 RAINFASTNESS

The zeolites were quantified based on the aluminium concentration in the formulation using XRF analysis. Each element has electronic orbitals of characteristic energy. Therefore, when materials are exposed to X-rays, ionization of their component atoms takes place. The removal of an electron makes the structure of the atom unstable, whereby the gap is filled up with another electron from a higher orbital. Hence, energy is released in the form of a photon (radiation), which has the energy characteristic of the atoms present. In this indicative test, the direct method was used to quantify rainfastness of the different formulations.

In order to determine the impact of the zeolite formulations on rainfastness, equal slices of leaves were made with the aid of a punch press. Subsequently, five 10 µl dots of a 30 $g.l^{-1}$ solution were placed on these leaf discs. Rainfastness was simulated after 0 seconds and 4 hours by immersing the leaves three times for 1 second in 10 ml distilled water. The amount of formulated zeolite washed off the leaves was determined using XRF analysis. The Al^{3+} and Si^{4+} concentration in the solution were calcultated based on the Al^{3+} and Si^{4+} calibration curves. The measurements were carried out in triplicate and were performed on a NEX CG from Rigaku using a Mo-X-ray source.

Rainfastness was then calculated based on the following formula:

$$\text{Rainfastness} = \frac{\text{Residual deposit on the leave after 4 hours}}{\text{Initial deposit on the leave after 0 seconds}} \cdot 100\%$$

2.2.5 SPREAD PATTERN

The different types of formulations have an influence on the spray deposition. To facilitate the determination of the droplet spreading patterns and droplet size of the different formulations, water sensitive papers (7.6 x 2.6 cm², TeeJet) were used. For each treatement, six of these water sensitive papers were sprayed and dried. Further analysis was done using image analysis software written in Halcon 8.0 (MVTec Software GmbH, Munich, Germany) (Foqué and Nuyttens, 2011).

An automated spray boom described by Foqué and Nuyttens (2011) was used in an adapted form (**Figure 3-3**). A greenhouse sprayer (Delvano NV, Hulste, Belgium) equipped with a membrane pump (AR 813, Annovi Reverberi , Modena, Italy) with a maximum flow rate of 79.9 l.min^{-1}, was connected to the boom. The boom was mounted on a track and pulled at a pre-set speed over the track by an electric motor. The following parameters were set for this automated spray boom, i.e. speed: 2.2 m.s^{-1} (= 8 km.h^{-1}), pressure: 3 and 6 bar, and boom height: 0.50 m above target. An area of 24 m² (8 m x 3 m) and 5 nozzles at a standard distance of 0.50 m from each other was used.

Four different spray nozzles were tested, i.e. a standard flat fan nozzle (Teejet ISO XR 110 03), an air induction nozzle (Teejet ISO AI 110 02 and 03), a wide angle nozzle (Teejet ISO TT 110 03) and a hollow cone nozzle (Teejet ISO TXA 80 02 and 03). A standard flat fan nozzle was selected because it is, by far, the most used nozzle in Belgian agriculture. The other selected nozzles aimed to cover a broad range of droplet sizes and spray patterns.

All other spraying parameters were kept constant, resulting in an application rate of 180 l.ha^{-1}. Furthermore, the ISO 02 and ISO 03 nozzles were used at a pressure of 3 and 6 bar, respectively. All three formulation types were used at a concentration of 1000 mg.l^{-1}. The tests were conducted in triplicate.

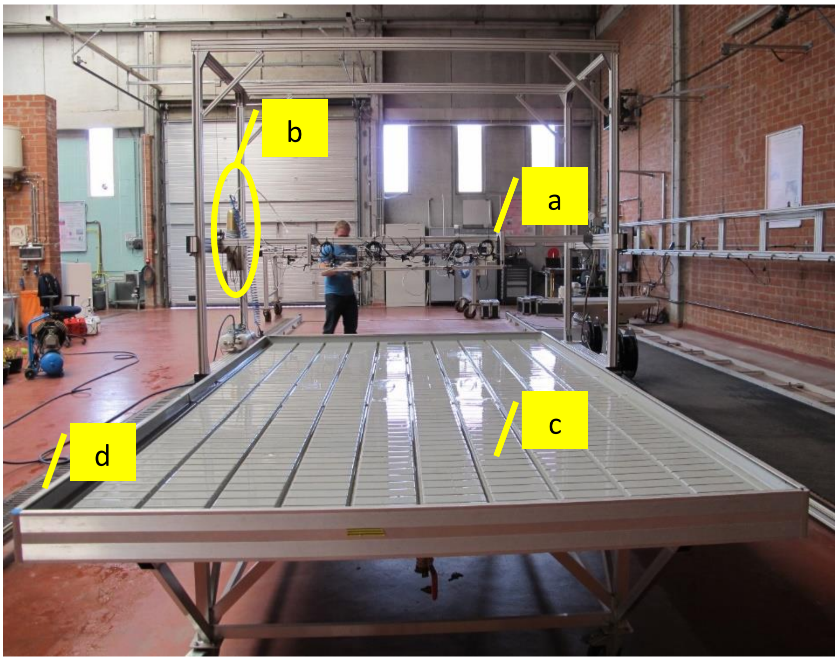


Figure 3-3. Automated spray boom, with (a) spray boom, (b) spray unit, (c) rolling bench and (d) spray track with fixed engine (not visible).

2.3 DATA ANALYSIS

Regression equations were calculated after measuring the surface tension of the different formulation types. A logistic model of the log concentration versus surface tension was fitted to the data using the following equation:

$$y = \frac{a}{1+e^{b.(x-c)}} + d$$

where a is the range of the mean surface tension, b is the slope coefficient, c is the concentration at the inflection point, d is the minimum mean surface tension, x is the log surfactant concentration and y is the mean surface tension.

3 RESULTS

3.1 PHYSICOCHEMICAL TESTS

3.1.1 EVAPORATION

The evaporation percentage of 100 µL of the different LTA zeolite formulation types is illustrated in **Table 3-2**. With the exception of the WP formulation, the evaporation percentage of the formulations decreased with increasing concentration of zeolites. It was noticed that the SC formulation evaporated faster than the other formulation types. Subsequently, the OD formulation obtained the lowest evaporation values.

Table 3-2. Evaporation percentage of water and different concentrations (100, 1000 and 10000 mg.l^{-1}) of the oil dispersion (OD), suspension concentrate (SC) and wettable powder (WP) formulations in function of time (min) (n=3).

Time	**0**	**2**	**4**	**6**	**10**	**20**	**30**	**60**	**120**	**180**
Concentration						**Water**				
	0.00	3.95	9.91	15.70	26.70	52.64	76.83	100.00	100.00	100.00
						OD				
100	0.00	5.11	8.01	12.76	22.31	53.71	66.58	88.67	88.97	90.15
1000	0.00	3.04	6.91	10.44	18.15	41.30	58.30	86.47	86.55	86.80
10000	0.00	2.98	6.29	9.93	17.87	37.63	54.51	83.37	83.79	83.87
						SC				
100	0.00	3.89	9.56	16.00	27.38	59.65	91.92	100.00	100.00	100.00
1000	0.00	2.57	8.02	14.48	20.90	49.23	77.57	96.44	100.00	100.00
10000	0.00	3.10	8.21	11.57	23.81	49.04	72.34	95.56	96.40	96.31
						WP				
100	0.00	1.84	7.63	13.24	23.89	49.87	76.45	94.30	95.14	95.81
1000	0.00	2.60	8.21	14.25	26.49	55.16	78.12	95.47	97.32	96.81
10000	0.00	5.87	10.98	17.27	29.67	57.67	80.39	94.13	94.72	95.64

3.1.2 SURFACE TENSION

The surface tension of the different formulations is illustrated in **Figure 3-4**. Theoretically, the relation of surfactant concentration and surface tension is a logistic distribution. Therefore, a logistic model was fitted for the different formulation types. For each formulation type, the estimated parameters in the logistic model and the CMC, which was calculated by the models, are presented in **Table 3-3**.

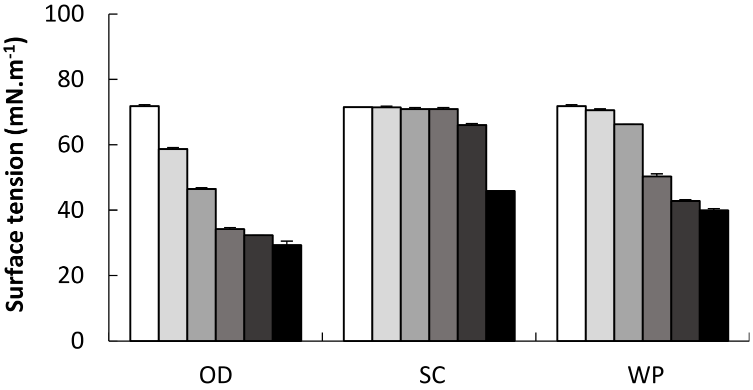


Figure 3-4. Surface tension (± SD) of different concentrations, i.e. 0 (□), 10 (□), 100 (■), 1000 (■), 10000 (■) and 100000 (■) mg.l^{-1}, of the oil dispersion (OD), suspension concentrate (SC) and wettable powder (WP) formulations (n=3).

Table 3-3. Critical micelle concentration (CMC) estimated from the regression equation obtained after measuring the surface tension of the oil dispersion (OD), suspension concentrate (SC) and wettable powder (WP) formulations.

Formulation type	**Equation parameters**				**Adjusted R²**	**CMC (mg.l^{-1})**
	a	b	c	d		
OD	28.07	2.00	3.00	26.14	0.7945	1010.05
SC	36.01	2.00	4.02	39.89	0.8372	10342.40
WP	32.58	2.00	3.59	33.86	0.8092	3917.05

Generally, the surface tension of all formulations also decreased with increasing zeolite concentration. However, the oil dispersion gave superior performance in lowering surface tension (CMC around 1010.05 mg.l^{-1}). Of the tested formulation types, the SC formulation reduced the surface tension less than the other two.

3.1.3 CONTACT ANGLE

Apple trees and tomato plants will be the central test species in the following chapters. Therefore, leaves of apple trees and tomato plants were used to measure the contact angle of the different zeolite formulations. Based on the results illustrated in **Figure 3-5**, smaller contact angles were found for the tomato leaves. Consequently, the formulations were spread more evenly over the surface of the tomato leaves. The OD formulation obtained the best spreading on both leaves, this in function of the tested concentration. In contrast, the SC formulation had the least impact in terms of spreading.

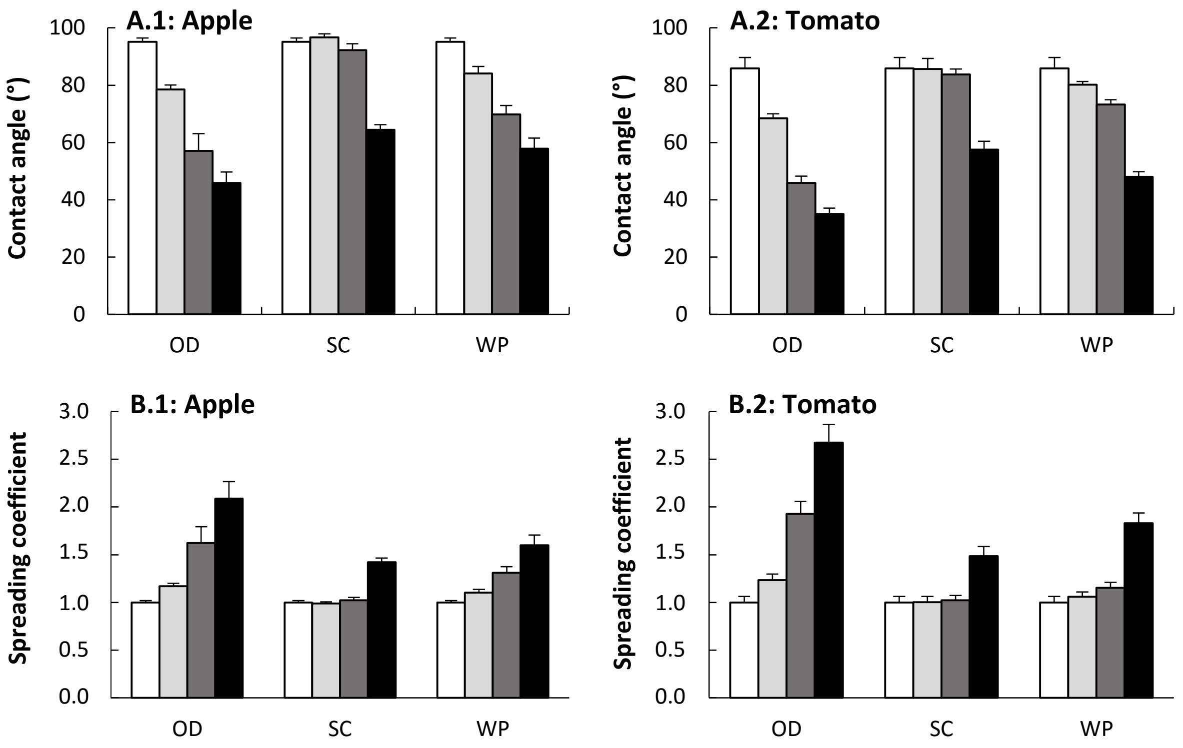


Figure 3-5. Contact angles (± SD; A) and spreading coefficients (± SD; B) of different concentrations, i.e. 0 (□), 10 (▨), 1000 (▩) and 100000 (■) mg.l^{-1} of the oil dispersion (OD), suspension concentrate (SC) and wettable powder (WP) formulations on apple (1) and tomato (2) leaves (n=3).

3.1.4 RAINFASTNESS

Calibration curves were plotted based on the concentration of aluminium in the formulations (**Figure 3-6**). The start concentration of the zeolite formulations used in this test was 150 mg.l^{-1}, since higher concentrations were no longer detectable with the calibration curves. A concentration of 200 mg.l^{-1} was the detection limit within the values giving a linear relationship.

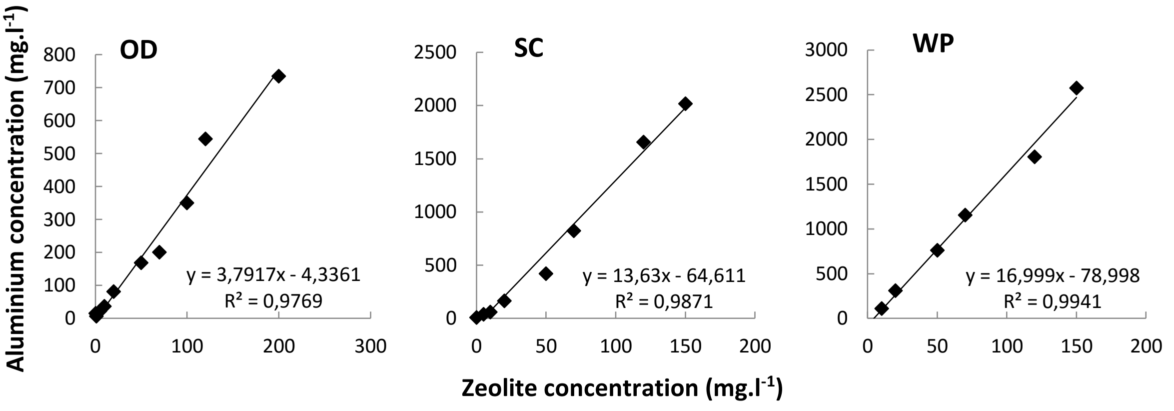


Figure 3-6. Calibration curves of the aluminium concentration present in the oil dispersion (OD), suspension concentrate (SC) and wettable powder (WP) formulations.

The results in **Table 3-4** indicate that all formulation types are more rainfast when applied on tomato leaves. However, the best results were obtained for the OD and WP formulations. In contrast, the SC formulation was not effective in terms of rainfastness.

Table 3-4. Rainfastness (± SD) of the oil dispersion (OD), suspension concentrate (SC) and wettable powder (WP) formulations (n=3).

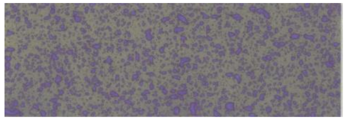
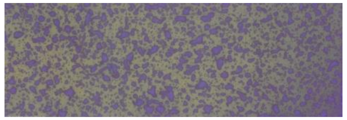
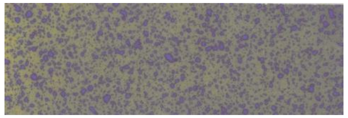
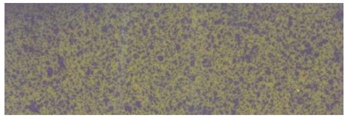
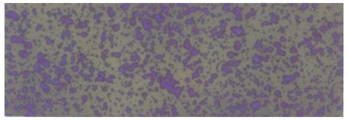
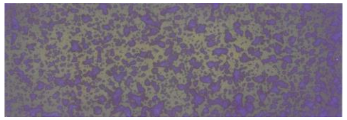
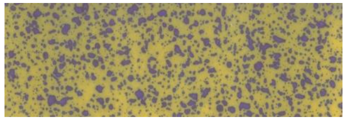
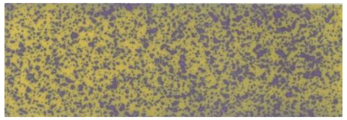
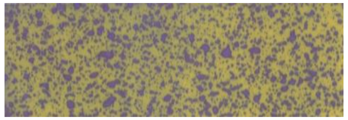
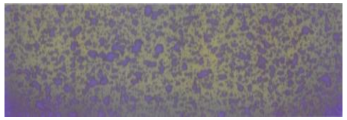
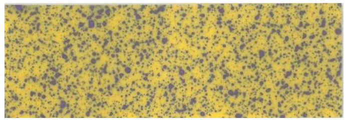
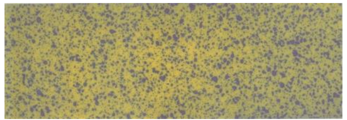
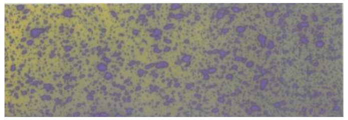
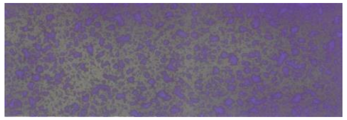
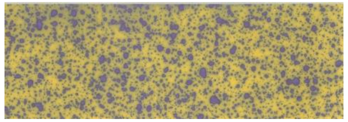
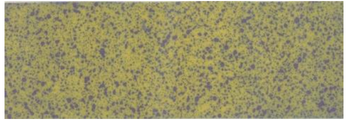
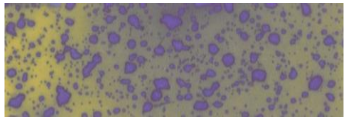
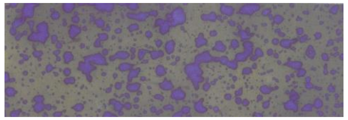
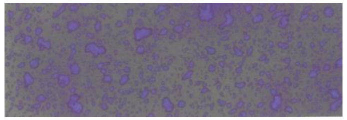
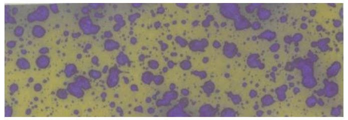
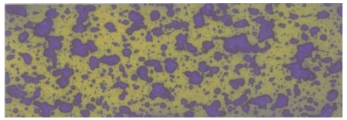
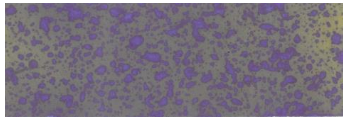
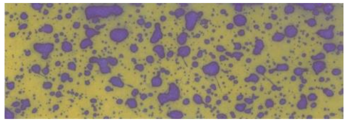
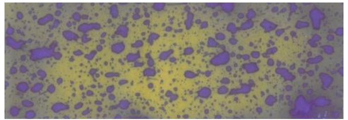
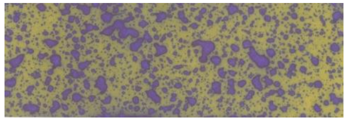
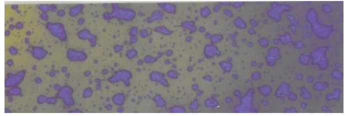
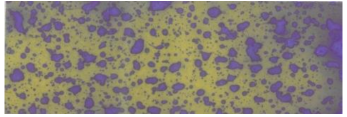
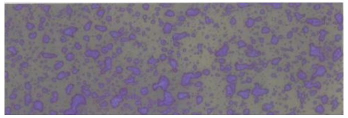
Rainfastness (%)	**OD**	**SC**	**WP**
Apple	28.00 ± 0.89	8.58 ± 4.52	22.54 ± 7.18
Tomato	40.32 ± 15.16	11.85 ± 5.13	34.88 ± 15.82

3.1.5 DEPOSITION

Water sensitive papers were used to detect the coverage by the different formulation droplets sprayed with different nozzles. All other spraying parameters were kept constant, resulting in an application rate of 180 $l.ha^{-1}$. Furthermore, the ISO 02 and ISO 03 nozzles were used at a pressure of 3 and 6 bar, respectively. Based on the pictures presented in **Table 3-5,** it becomes clear that the flat fan nozzle and the hollow cone nozzle resulted in an excellent spray distribution with very small droplets. This is confirmed in **Figure 3-7**, which shows the number of droplet particles. However, looking at the percentage of coverage in **Figure 3-7,** no clear differences are noticed at 3 bar. In contrast, at 6 bar these flat fan and hollow cone nozzles obtained a better coverage. But the number of droplets produced by the flat fan nozzle was lower compared to the air induction nozzle.

Looking at the different formulation types, the WP formulation obtained the best coverage compared to water. Significant differences were observed.

Table 3-5. Water sensitive papers covered with the oil dispersion (OD), suspension concentrate (SC) and wettable powder (WP) formulations using different types of spray nozzles.

	3 bar	6 bar	3 bar	6 bar
	Flat fan		**Hollow cone**	
Water				
OD				
SC				
WP				
	Air induction		**Wide angle**	
Water				
OD				
SC				
WP				

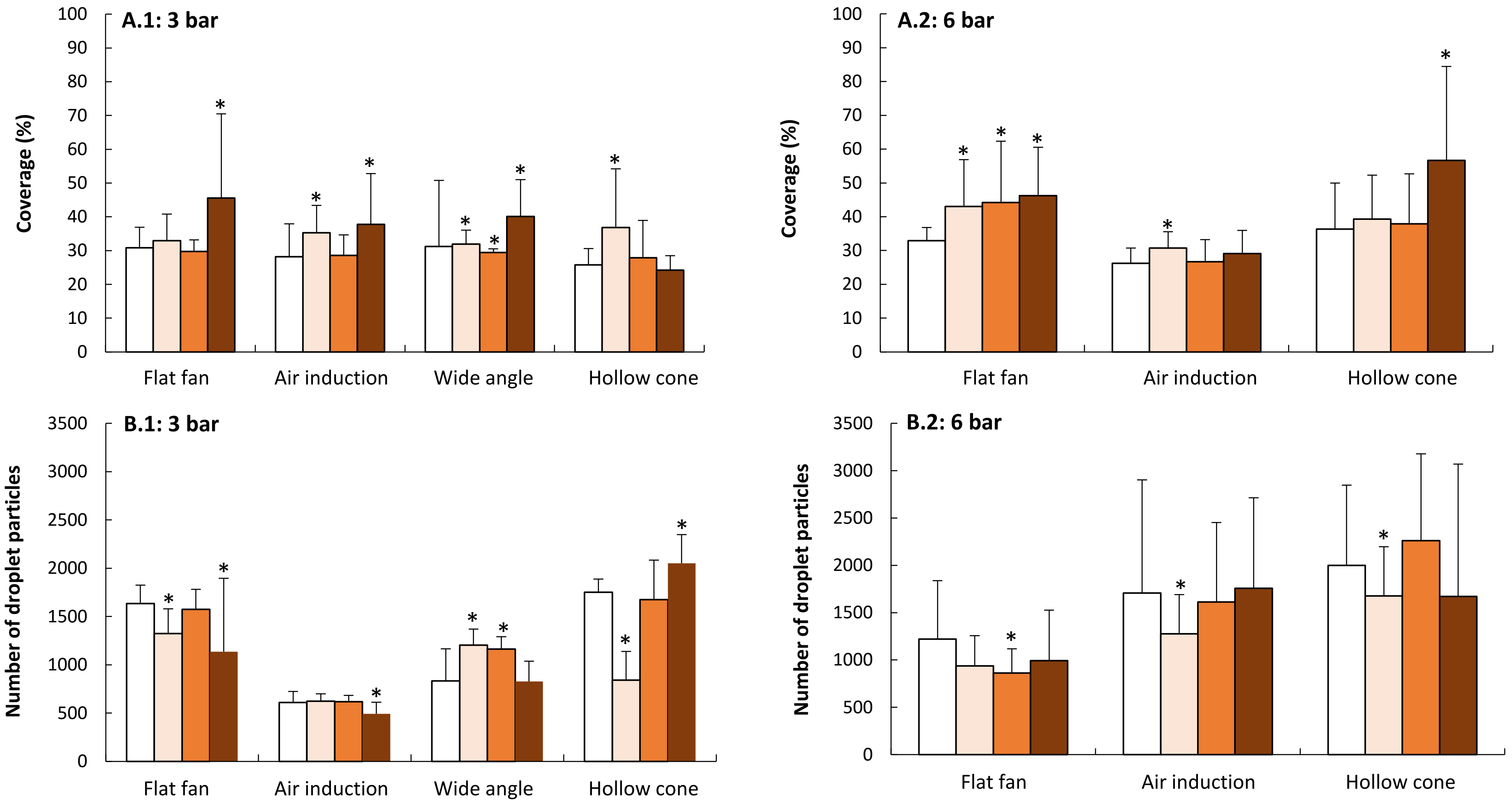


Figure 3-7. Coverage percentage (± SD; A) and number of droplet particles (± SD; B) of water (□) and the oil dispersion (□), suspension concentrate (🟧) and wettable powder (🟫) formulations, sprayed with a boom spray using 4 different types of nozzles (flat fan, air induction, wide angle and hollow cone) at 3 bar (1) and using 3 different types of nozzles (flat fan, air induction and hollow cone) at 6 bar (2). Asterisks indicate significant differences in coverage and number of droplet particles between the formulation types and water (n=3).

4 DISCUSSION AND CONCLUSION

Adjuvants are designed to perform specific functions, including wetting, spreading, sticking, reducing evaporation, reducing volatilization, buffering, emulsifying, dispersing, reducing spray drift and reducing foaming. No single adjuvant can perform all of these functions, but compatible adjuvants often can be combined to perform multiple functions simultaneously (Herzfeld and Sargent, 2011).

Physicochemical tests were carried out in function to score three different formulation types, i.e. oil dispersion, suspension concentrate and wettable powder. First of all, evaporation tests were carried out. It was found that the OD formulation obtained the lowest evaporation rate, followed by the WP and SC formulations, respectively. This can be explained by the fact that adjuvants may modify the evaporation of spray droplets (Somervaille et al., 2012). Evaporative loss of the carrier liquid (which is mostly water but can also be an oil or a liquid fertilizer) already begins during transport of the droplet to the target surface (Hall et al., 1993). After impaction, the droplet spreads along the water/leaf surface interface and evaporates via a mechanism of capillary flow (**Figure 3-8**) wherein the liquid phase is abandoned preferentially at the edge of the drying droplet (Faers, 2007). During evaporation, the active ingredient precipitates out as a residue at the contact area between the droplet and the leaf surface, resulting in an active ingredient deposition pattern which may be smaller, the same, or larger than the original droplet footprint (Bukovac et al., 2003).

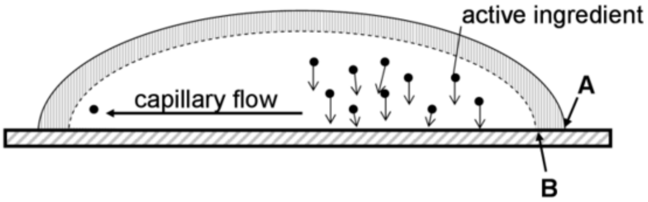


Figure 3-8. Schematic illustration of an evaporating droplet (modified after Deegan et al., 2000; Faers, 2007). During evaporation, the air/water interface moves from the solid line to the dashed line, and the contact line will move from A to B. The active ingredient precipitates out as a residue at the contact line between droplet and leaf surface.

Generally, oil evaporates more slowly than water. However, surfactants are necessary in a mixture of oil-water in order to obtain a steady emulsion. Consequently, these surfactants can reduce the evaporation effect of the oil. In contrast, some adjuvants can have a positive effect on evaporation. Thickeners increase the viscosity of spray mixtures. These adjuvants are used to control drift or slow evaporation after the spray has been deposited on the target area. (Herzfeld and Sargent, 2011). The OD and SC formulations of the zeolite both contained a thickening agent. However, the WP formulation obtained better results compared to the SC formulation in reduction of water volatilization. This is most likely due to the composition of the structuring agent of the WP formulation. PVP K-30, present in the WP formulation, is a polyvinyl polymer and, as indicated by Spanoghe (2005), some colloids, i.e. polyvinyl polymers, tend to absorb water and so reduce the rate of evaporation.

Secondly, the surface tension of all formulations was examined. Besides the fact that the surface tension influences the droplet size in a spray, it also influences the evaporation rate (Tu and Randall, 2001). As observed during the evaporation experiment, the oil dispersion gave superior performance in lowering surface tension, followed by the WP and SC formulations, respectively. Some adjuvants, i.e. surfactants, reduce surface tension by nature. Almost all surfactants reduce surface tension in the spray droplet, which ensures that the formulation spreads out and covers the plant with a thin film rather than beading up. Vegetable-derived oils (from soya bean, cotton seeds, etc.) also decrease surface tension, but they are not as effective as other surfactants at increasing spreading, sticking, or penetration (Miller and Westra, 1996). The WP formulation obtained better results compared to the SC formulation. This can most likely be explained due to the composition of the carrier of the WP formulation. Ultrasil VN 3, present in the WP formulation, is an organosilicon surfactant. It is known that organosilicones have the superior surface tension reducing ability. These products can give complete coverage on the target surface, resulting in better efficacy (Spanoghe, 2005).

Generally, the surface tension of all formulations decreased with increasing concentration. Increasing the concentration of a surfactant in a tank mix generally decreases the surface tension, which in turn increases the probability that a droplet will adhere to a leaf and spread onto its surface. Adding too much surfactant, however, sometimes negatively affects this wetting and spreading ability. For instance, too much surfactant can cause excessive runoff, resulting in less adhesion of the droplets to a leaf surface. Hence, it is best to decrease surface tension until the surfactant concentration reaches the CMC. At this point, addition of more surfactant does not decrease surface tension and does not necessarily translate into better control results (Tu and Randall, 2001).

Another important property related to the surface tension is the contact angle. This contact angle is the result of interaction between the droplet and the target surface. Generally, at normal use rates, the lower the surface tension is, the smaller the contact angle on target surfaces will be. This relationship was also observed in the current study. Zabkiewicz et al. (1985) reported that formulations with contact angle from 50 degrees or less are considered as having good wetting capability, while complete wetting is possible if the value is 20 degrees or less. Even this criterion seems to be somewhat arbitrary. The results of the contact angle measurements showed a good wetting capability for the oil formulation, starting at 1000 mg.l^{-1}.

The heterogenic composition of surfaces of plants may cause difficulty in covering the surface with the formulations. Surfactants are particularly important when applying a plant protection product to waxy or hairy leaves (Herzfeld and Sargent, 2011). Both leaf surfaces of the test species, apple and tomato, are hairy. However, apple leaves have a more smooth surface area compared to the hairy tomato leaves. Although surface roughness may aid in the deposition of dusts, it hinders the deposition of aqueous preparations. Roughness counters wettability because air entrapped in the cavities reduces the contact area between the surface and the liquid. A smooth leaf surface may be perfectly wettable by water, while a rough leaf surface repels water, which results in a contact angle above 100°. The composition of leaf surfaces changes with age, nutrition and weather during development. Hairs and cuticle are less developed on young leaves than on mature leaves. The cuticle is thinner on young and succulent leaves than on mature and hardy leaves and, thus, has greater hydrophilic properties. Leaves formed during cloudy weather may have less wax and smoother surfaces than those formed during sunny weather. Surfaces with little wax and smoothness are more easily wettable than those with a lot of wax and roughness (Lukens, 1971). Decreasing contact angles with increasing age are most probably caused by the accumulation of noncuticular material and its chemical constituents, although cuticular abrasion may also play a role (Hoad et al., 1992; Neinhuis and Barthlott, 1998; Bringe et al., 2006). Leaf surface particles influence plant-water relationships.

In this study, lower contact angles were observed on tomato leaves compared to the apple leaves, which can probably be explained due to the young tomato leaves that were used during the experiment. However, Yu et al. (2009) observed that adding a surfactant to an active ingredient increased the maximum coverage 4.5-10.1 times on the hairy leaves and 3.4-4.1 times on the waxy leaves. For the droplets with the same size, the opposite effect was noticed without the surfactant. On hairy leaves, droplets containing the surfactant continued to spread and demonstrated a different behaviour from similarly treated droplets on waxy leaves. Here, lipophilic compounds penetrate waxy, hydrophobic plant leaves more easily than hydrophilic compounds (Mashaya, 1993).

Subsequently, the effect of the different formulations on rainfastness was examined. A PPP's rainfastness, or its ability to withstand rainfall, is an important factor affecting the efficacy of foliar-applied PPPs. Generally, it is best to avoid PPP application when rainfall is likely. However, weather can be unpredictable, so it is best to choose a product with good rainfast characteristics. Based on the observed results, it can be said that the OD and WP formulations were more rainfast compared to the SC formulation. It is known that the oil in an OD formulation improves rain resistance (Tu and Randall, 2001). In contrast, WP formulations are more susceptible to wash-off. Dusts are finely ground mixtures of the active ingredient with clay, talc, or other such materials, and they usually contain a low percentage of active ingredients. This allows rain to easily wash off the active compound. Wettable powders are similar to dusts, but contain a wetting and dispersing agent. They also have a more concentrated active ingredient than dusts, but are still generally prone to wash-off. However, organosilicone surfactants are commonly used to improve rainfastness, to reduce surface tension, and to enhance spreading ability (Wells and Fishel, 2014). This surfactant, present in the WP formulation under the name of Ultrasil VN 3, is most likely responsible for a better rainfastness compared to the suspension concentrate formulation. Additionally, the formulations were more rainfast when applied onto hairy tomato leaves, compared to the smoother apple leaves.

Finally, spray deposition and coverage are the major components of spray performance. Water sensitive papers were used to detect the coverage by the different formulation droplets sprayed with different nozzles. All spraying parameters were kept constant, what resulted in a better reflection of the differences between the formulations and the applied nozzles. The flat fan and hollow cone nozzles were more efficient at depositing formulation droplets on the water sensitive papers. A good spray distribution was observed at both pressures, i.e. 3 and 6 bar. Flat fan nozzles usually operate between 1-4 bar, while hollow cone nozzles usually operate between 5-20 bar (Teejet, 2011). This means that the applied 6 bar was too high when using flat fan nozzles, and therefore explains the low amount of droplets observed. Because the high pressure, the fine droplets sprayed on the water sensitive papers, became one big droplet. As a result, the water sensitive papers coloured completely blue, making it difficult to distinguish the individual droplets by the applied software. Subsequently, the applied 3 bar was too low when using hollow cone nozzles. This explains the better distribution observed at 6 bar. However, in this experiment no specific disadvantage was observed. When using the air induction and wide angle nozzles, larger droplets were observed on the water sensitive papers. The recommended pressure range of both nozzles was between 2-8 bar and 1-6 bar, respectively.

Looking at the different formulation types, the WP formulation obtained the best coverage. This can be explained due to the smaller droplets of the WP formulation. As a rule, smaller droplets provide better coverage, but larger droplets are less likely to drift. The OD and SC formulations are more viscous compared to the WP formulation. This increase in viscosity will typically increase the drop size (Flint, 1993). Next, the better coverage of the WP formulation can also be explained by the physicochemical properties discussed above. In all tests, the OD and WP formulations were more efficient. However, oils often result in an increase in droplet size (Spanoghe, 2005), what may be an explanation of the observed results.

In conclusion, the OD and WP formulations yielded the best results related to contact formation and deposition. In order to select one formulation type, the advantages and disadvantages of these two formulations were taken into account (**Table 3-6**). Especially, the phytotoxicity property exerts a great influence on making the choice. In some cases, however, a powder formulation has advantage over the liquid formulations when oil-incompatible materials have been applied to the crop. For example, use of an oil-based formulation on a crop treated with elemental sulphur can cause severe phytotoxicity (Jackson et al., 2010).

Table 3-6. Advantages and disadvantages of the oil dispersion and wettable powder formulations.

	Advantages	Disadvantages
Oil dispersion	- Ideal for active ingredients not stable in water - Good sticking properties - Easy to handle - Little visible residues on treated surfaces	- Bulk storage difficult - Tank cleaning can be an issue - Physical instability on storage - May cause unwanted harm to plants - Difficult to dose - Easily absorbed through skin
Wettable powder	- Easy to storage - Easy to transport - Not expensive - No solvent - Less likely to cause unwanted harm to treated plants, animals and surfaces - Less odour	- Dust - Inhalation hazard while mixing - Require good and constant agitation - Often clog nozzles and screens - May be difficult to mix and measure - May leave white deposit on surfaces - Abrasive

This phytotoxicity was also confirmed by phytotoxicity studies conducted by CEMAS (Berkshire, UK) in the scope of the ECO-ZEO project. They observed that the OD formulated product at rate of 60 kg zeolite.ha^{-1} on apple and tomato showed extensive leaf tissue damage (white/brown necrotic lesions), leaf death, with chlorosis in the growing tips of tomatoes (**Figure 3-9**). Therefore, the WP formulation was selected for further research.

Figure 3-9. Apple (A) and tomato (B) leaves just after spraying (1) and 2 days after spraying (2) with the oil formulation.

5 DEVELOPED ZEOLITE FORMULATIONS

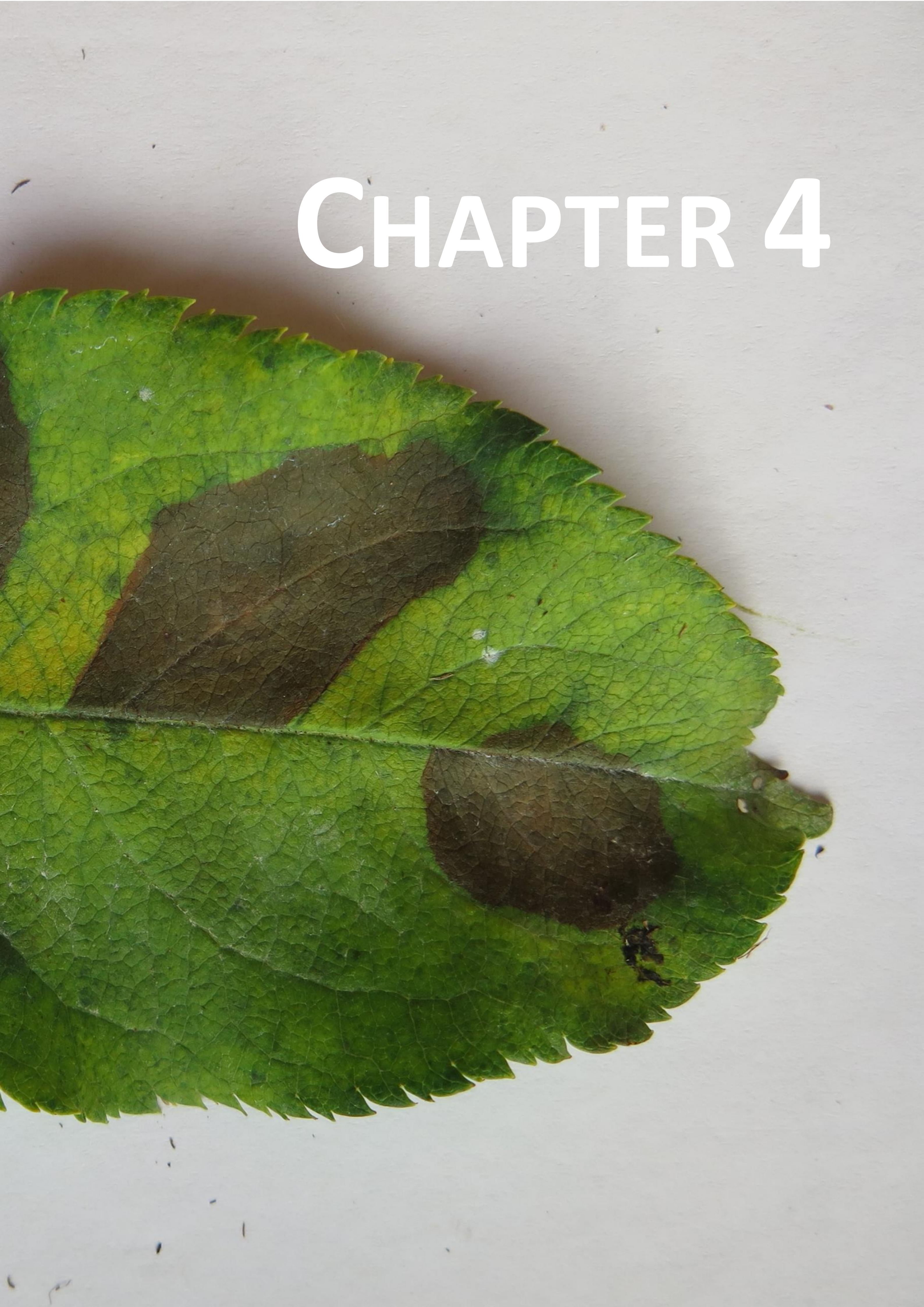
Galenika Fitofarmacija (Belgrade, Serbia) developed two different WP formulations for each of the two selected zeolites in Chapter 2 (**Table 3-7**). All tests were also carried out with zeolite type LTA, which was used in the ECO-ZEO project. Two LTA formulations were developed, i.e. 800 SC and 850 WP. The numbers in the formulation name refer to the zeolite concentration, in g.l^{-1}, present in the formulation.

Table 3-7. Zeolite formulations to be tested in the next chapters.

Material name	Framework type code	Product name	Supplier	Formulation
Beta-zeolite	BEA	H-BEA-25	Clariant, Germany	850 WP 950 WP
Zeolite Y	FAU	CBV 720	Zeolyst, The Netherlands	850 WP 920 WP
Zeolite A	LTA	Zeolite 4A	FMC, Spain	800 SC 850 WP

“The elevator to success is out of order.
You'll have to use the stairs... one step at a time.”
-Joe Girard-

CHAPTER 4



Chapter 4

FUNGICIDAL ACTIVITY OF ZEOLITES

As zeolites deposit a particle layer on the leaf surface, it may block liquid film formation, which eventually prevents disease propagule germination. Therefore, zeolites can be used as a potential fungicide. This chapter focuses on the fungicidal activity of the selected zeolites and its formulations against *Venturia inaequalis* and *Botrytis cinerea*. First, antifungal bioassays, together with incidence and severity assesments were carried out (Part A). Based on these results, the fungicidal activity of the adjuvants present in the formulations was examined (Part B).

1 INTRODUCTION

Plants cannot escape from pathogens or stress factors, such as droughts and floods. That is why they have developed complex mechanisms to defend themselves from attacks (adaptive response) (Dangl and Jones, 2001). In order to activate such defence mechanisms, plant hormones interact with each other in a synergetic or antagonistic way (cross talk), whereby a plant can regulate its immune system. Pathogens can abuse these interactions to disrupt the immune system of their host (Pieterse et al., 2009). Such pathogens can be divided into two broad classes, i.e. necrotrophic pathogens that directly kill host cells and use the cell contents, and biotrophic pathogens that do not kill the host cells directly but penetrate inside the apoplast and obtain nutrients through specialized structures such as haustoria. Many pathogens go through both life styles and are called hemibiotrophic (Kunkel and Brooks, 2002; Pieterse et al., 2009).

Fungicides are chemical compounds or biological organisms that destroy or inhibit the growth of these fungi or fungal spores. The use of fungicides for an effective control of plant diseases has become crucial in the last decades in agriculture since it is estimated that fungal infections cause yield reductions of almost 20% of crops worldwide (Gullino et al., 2000). Fungicides can be classified by their mode of action. They can have effects on membranes, nucleic acids and protein synthesis, signal transduction, respiration, mitosis and cell division. Hereby, a distinction can be made between site-specific and multi-site inhibition (Yang et al., 2011).

Between 1940 and 1970, organic antifungal compounds with broad-spectrum activity were developed by the newly emerging plant protection industry. Most of these fungicides are multi-site inhibitors that are active on thiol groups of glutathione and proteins (Bernard and Gordon, 2000; Hahn, 2014). Additionally, these inhibitors are contact fungicides that have a preventive action by killing or inhibiting fungi or fungal spores before the mycelia can grow and develop within the plant tissues. However, once the infection is established, this fungicide may not have any effect. Thus, this kind of fungicides can be used only as protectants (Dias, 2012).

A new generation of fungicides was developed in the 1960s. These fungicides have a specific mode of action towards a target protein in the fungal pathogens and are highly active. Most of these site-specific fungicides are systemic, i.e. they can penetrate the cuticle and are distributed within the plant, which increases their activity (Hahn, 2014). Additionally, these kind of inhibitors can kill the fungus after the mycelia has penetrated the parenchyma of the plant tissue, stopping the dispersal or infection within the plant (Dias, 2014). Based on these properties, this kind of fungicides have a protectant and curative activity.

Only few years after the introduction of site-specific fungicides, resistance development in the pathogen populations and the loss of fungicide activity were observed. *Botrytis cinerea* was one of the first fungi for which resistance was described. Since then, the awareness of the resistance problem has increased and it became a major focus of fungicide research (Hahn, 2014).

In order to overcome these resistances, alternative PPPs are needed. In this study fungicidal activity of zeolites on two important and wide spread diseases is studied.

1.1 *BOTRYTIS CINEREA*

1.1.1 THE DISEASE

Botrytis cinerea Persoon: Fries (teleomorph: *Botryotinia fuckeliana* (de Bary) Whetzel) is one of the first described genera of fungi and has been recognized as a genus since Micheli erected it in 1729. *B. cinerea* is probably the most common and most widely distributed plant pathogenic fungi of economically important crops including vegetables (e.g. tomato, cucumber, lettuce), ornamentals (e.g. rose, gerbera), bulbs (e.g. onion) and fruits (e.g. grapevine, strawberry, kiwifruit) (Elad et al., 2004; Zhang et al., 2007b). Based on its scientific and economic importance, *B. cinerea*, the causal agent of grey mould, has been recently ranked as number 2 among the top 10 fungal pathogens in molecular plant pathology (Dean et al., 2012).

The fungus (**Figure 4-1**) is most prevalent in temperate and subtropical areas where it commonly resides on the leaf surface (Leroux, 2007). It is a facultative parasite of a wide range of plants, which attacks many organs including leaves, stems and fruits as a nectrotroph, often with heavy losses after harvest. It is also a saprophyte on senescent and dead plant material (Elad et al., 2004).

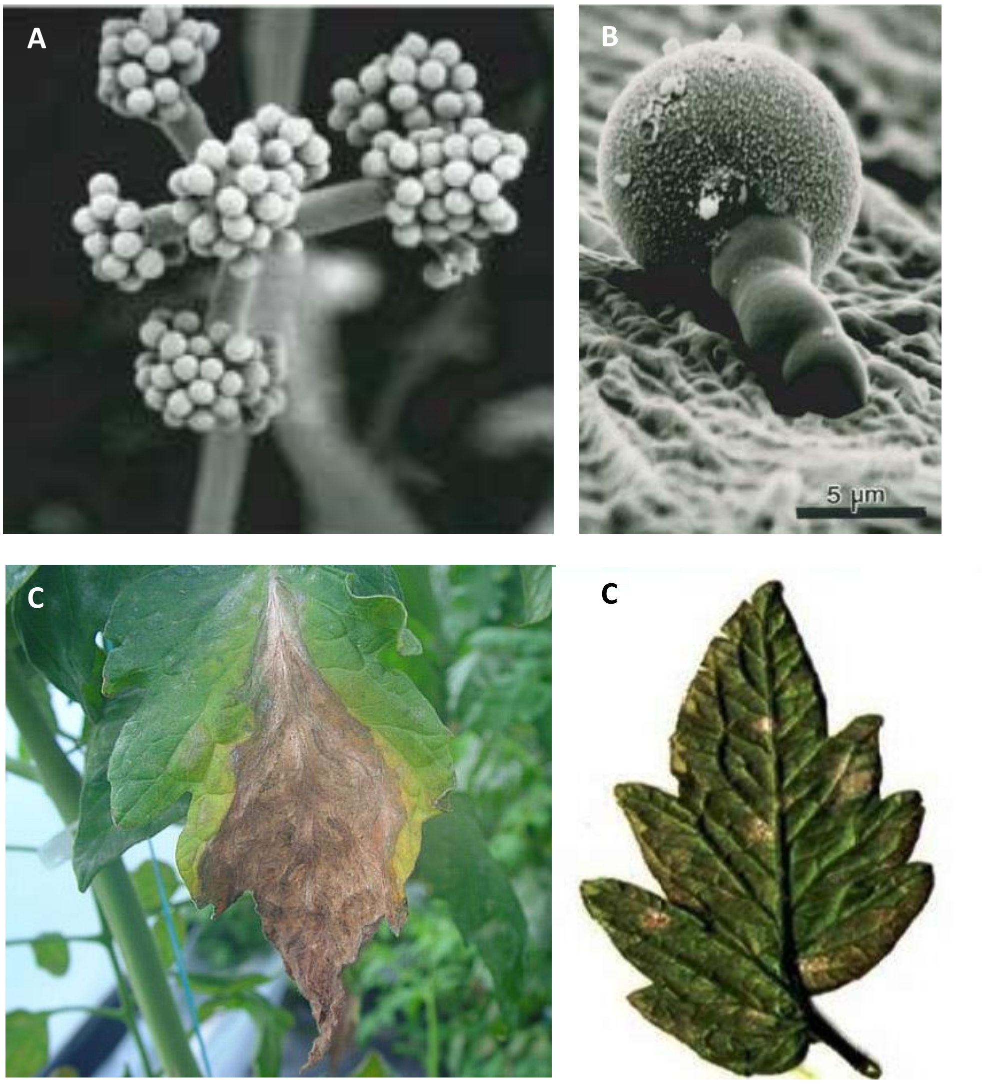


Figure 4-1. (A) *Botrytis cinerea* conidiophore with mature conidia in situ, (B) conidium germinating in absence of water droplet on abaxial surface of rose petal (C) *Botrytis cinerea* on tomato leaves (Ingram and Meister, 2006; Williamson et al., 2007; Chavera et al., 2013).

Greenhouse tomatoes are susceptible to a wide variety of diseases and pests. Among all diseases, Botrytis is the most common disease (Bardin et al., 2008; Nicot and Baille, 1996). Under humid conditions, the fungus produces a noticeable grey mould fruiting layer on the affected tissues that is characteristic of Botrytis diseases. Some of the most serious diseases caused by Botrytis include calyx end rot, blast and neck rot, blight or grey mould, bulb rot, corn rot, and others. Botrytis also causes secondary soft rots of fruits and vegetables during storage, transit, and marketing (Agrios, 2005). Stem lesions resulting mainly from infection of pruning by germinated spores of Botrytis are one of the most devastating symptoms in heated tomato greenhouses (Bardin et al., 2008; Decognet et al., 2010).

Conventional fungicides have been used to control *B. cinerea*. However, several investigations conducted all over the world have indicated that chemical control against grey mould of tomatoes was not effective, due to the development of resistant strains (Locke and Fletcher, 1988; Yourman and Jeffers, 1999; Rosslenbroich and Stuebler, 2000). Alternative methods for suppressing *B. cinerea* are therefore required.

1.1.2 TAXONOMY

The shortened taxonomic classification of *B. cinerea* is presented in **Table 4-1**.

Table 4-1. Short taxonomy of *Botrytis cinerea* (Williamson et al., 2007).

Kingdom	**Fungi**
Phylum	Ascomycota
Class	Leotiomycetes
Order	Helotiales
Family	Sclerotiniaceae
Genus	Botryotinia
Species	*Botrytis cinerea*
Preferred common name	Grey mould

1.1.3 LIFE CYCLE

Botrytis cinerea is a necrotrophic fungal pathogen that secretes toxic molecules and lytic enzymes that kill host cells, which leads to decomposition of the plant tissue and consumption by the pathogens for their own growth (Van Kan, 2006). The life cycle of *B. cinerea* is summarized in **Figure 4-2** (Agrios, 2005).

The fungus overwinters as mycelia or as sclerotia in/on plant debris and in soil. In spring, these sclerotia detach from the fungus and germinate. The organism grows and produces mycelium or conidia spores (Delcan and Melgarejo, 2002; Beever and Weeds, 2004). Sporulation is very important for the reproduction and spread of *B. cinerea*. It plays a key role in the development of epidemics, as the pathogen produces millions of spores within one week after inoculation, especially during favourable conditions. These spores are easily disseminated over large areas (Nicot et al., 1996).

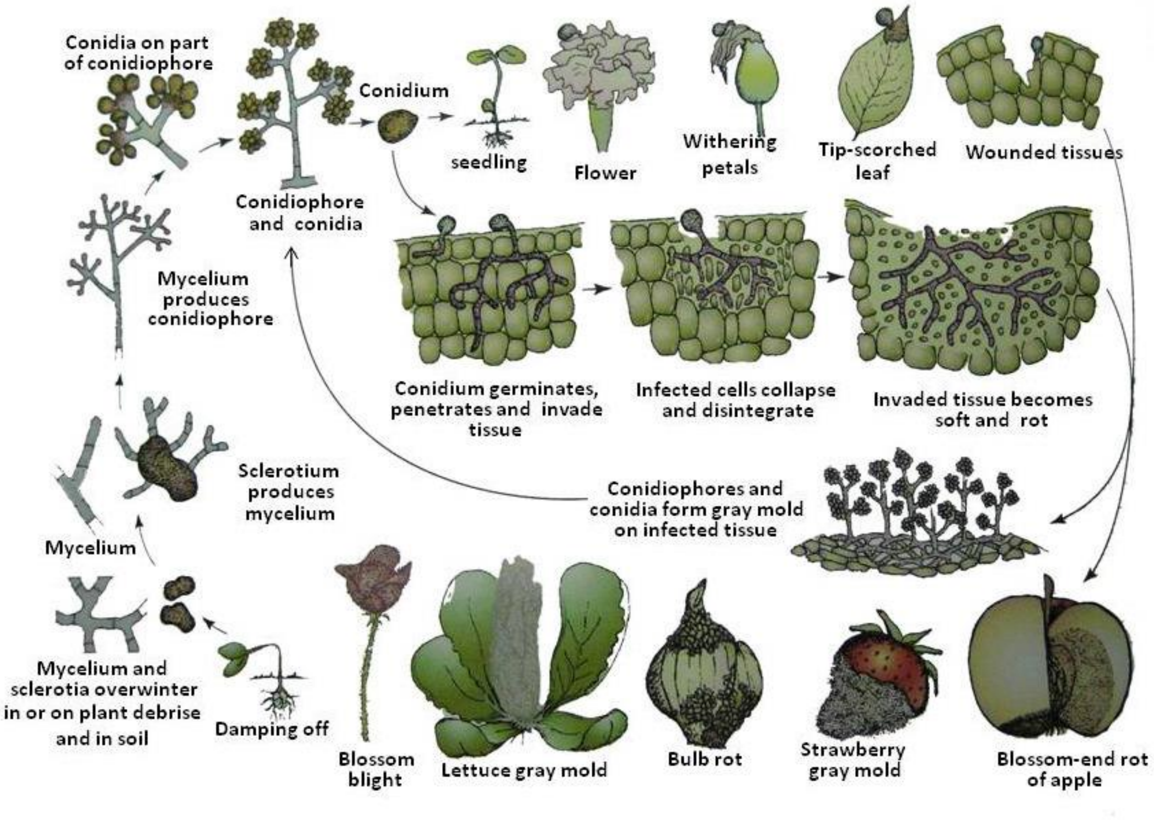


Figure 4-2. Life cycle of *Botrytis cinerea* (Agrios, 2005).

Botrytis cinerea exploits different infection modes of entry. Tubes of germinated conidia penetrate through injuries, natural openings like stomata, or intact, healthy plant tissues (Ernesto et al., 1998). Once the pathogen has entered the cells, haustoria are separated from the host cytoplasm by a host membrane that is contiguous with the plasma membrane. Hyphae consequently grow throughout the leaf, penetrating mesophyll cells where they form additional haustoria (Glazebrook, 2005). During the progress of infection, the fungus increases its biomass within the host tissue (Ernesto et al., 1998). After one week, conidiophores emerge from the stomata, and mature conidia are formed (Glazebrook, 2005). For some *Botrytis spp.*, sclerotia develop in dead plant tissue and form the fungal overwintering stage. Fungal mycelia can also last over winter in woody stem remains, where they form sclerotia that will germinate in the spring.

Mature conidia detach and are airborne and may also be carried on the surface of splashing rain drops (Williamson et al., 2007). In the field, spores landing on tomato plants germinate and produce an infection when free water from rain, dew, fog, or irrigation occurs on the plant surface. Dying flowers are a favourable site for infection, but infections can also result from direct contact with moist infested soil or plant debris. In the greenhouse, stem lesions develop either by direct colonization of wounds or through infected leaves. The presence of external nutrients, such as pollen grains in the infection droplet, increases infection (Elad et al., 2004).

This infection causes disintegration and collapse of the cells, which softens the tissue causing Botrytis blight or grey mould disease. With further growth, the mold spreads to produce new conida, and fresh spores are released. This process continues until the cold weather forces the organism into hibernation.

1.2 *VENTURIA INAEQUALIS*

1.2.1 THE DISEASE

Apple scab, caused by the fungus *Venturia inaequalis* Cooke (Wint.) (anamorph: *Spilocaea pomi (*Fr.) or *Fusicladium pomi* (Fr.) Lind), is the most important disease of apple worldwide. It occurs in almost every country where the cultivated apple (Malus x domestica) is grown (Carisse and Bernier, 2002).

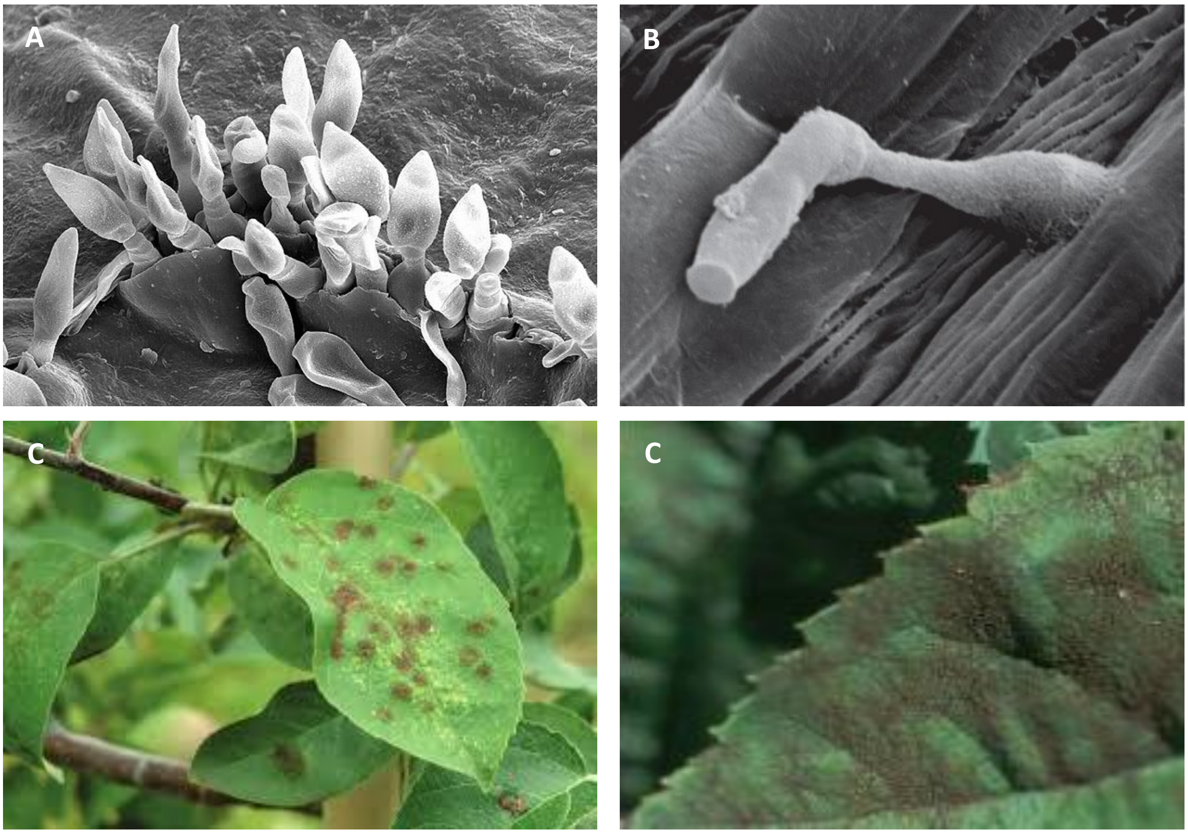


Figure 4-3. (A) *Venturia inaequalis* conidia erupting through the cuticle of a crabapple leaf, (B) conidium germinating and formation of an appressorium adhering to the leaf surface (C) *Venturia inaequalis* on apple leaves (Chevalier et al., 1991; Carisse and Jobin, 2006; Bowen et al., 2011; Walke, 2015).

Apple scab (**Figure 4-3**) is more severe in temperate regions with cool, moist climates during early spring, such as Belgium (MacHardy, 1996). It is not known when scab first appeared in orchards. The first report on scab was published by Fries in Sweden in 1819 (Fries, 1819), but the oldest evidence of the existence of scab dates from 1600, in a painting by Michelangelo Caravaggio ('The Supper at Emmaus'; **Figure 4-4**), held at the National Gallery in London (MacHardy et al., 2001).

Figure 4-4. 'The supper at Emmaus' by Michelangelo M. da Caravaggio (MacHardy et al., 2001).

The Apple Scab fungus itself does not kill the tree, but the fungal infection causes damages to leaves and fruits, which results in severe reductions in fruit quality and yield. In case of insufficient control of apple scab, the economic losses can increase up to 70% of the production value (Gupta, 1992). On the one hand, yield loss is caused by direct infection of fruits and pedicels. On the other hand, severe leaf damage can lead to a weakened tree with reduced flower bud formation (Verma and Sharma, 1999). Important losses occur also due to the development of scab in storage (Tomerlin and Jones, 1983).

1.2.2 TAXONOMY

The shortened taxonomic classification of *V. inaequalis* is presented in **Table 4-2**.

Table 4-2. Short taxonomy of *Venturia inaequalis* (Bowen et al., 2011).

Kingdom	**Fungi**
Phylum	Ascomycota
Class	Dothideomycetes
Order	Pleosporales
Family	Venturiaceae
Genus	Venturia
Species	*Venturia inaequalis*
Preferred common name	Scab

1.2.3 LIFE CYCLE

V. inaequalis is a hemibiotrophic fungus, which means that it does not only grow on/in living leaves, but also has a necrotrophic phase. The life cycle of *V. inaequalis* can be subdivided into two phases: a sexual or primary phase and an asexual or secondary phase (**Figure 4-4**). The primary phase mainly takes place in winter, the secondary in summer (MacHardy, 1996; Verma and Sharma, 1999).

Primary phase **-** *V. inaequalis* overwinters predominantly as pseudothecia (sexual fruiting bodies) in infected fallen leaves on the orchard floor. The primary infection is initiated in spring and mainly caused by ascospores (sexual spores). These ascospores are released by rainfall from the pseudothecium and are discharged over a period of five to nine weeks (Verma and Sharma, 1999). The optimal temperature for the formation and maturation of ascogonia is 8-12°C and 16-18°C, respectively (Turechek, 2004). When leaves on the orchard floor become wet from rain, the ascospores are released into the air. This release is favoured by sunlight and mainly takes place during the day (Rossi et al., 2001). These sexual spores have an inner and outer cell wall. The inner cell wall is thick and elastic and protects the ascospores from winter conditions, whilst the outer cell wall is thin and fragile (Jha et al., 2009).

The risk of infection is greatest early in the growing season, when leaves and fruit are young and most susceptible (Schwabe, 1979; Schwabe et al., 1984; Xu and Robinson, 2005). Air currents carry the ascospores into the trees and onto developing flowers, leaves and fruitlets, where they adhere quickly by contact and resist removal by more rain. Stronger wind can transport them throughout the orchard to neighboring or more distant orchards. The spores are spread up to 200 m from the source (Turechek, 2004). If the leaves and fruitlets remain wet, the ascospores germinate. The germ tubes penetrate through the cuticle, either directly or via an appressorium (Keitt and Jones, 1926; Smereka et al., 1987). They develop into multilayered, pseudoparenchymatous structures, termed stromata, which are presumed to obtain nutrients from the subcuticular space. The stromata, and the conidia that they produce, cause the characteristic leaf and fruit lesions that give the disease the name of scab or, in some countries, black spot.

Secondary phase **-** Conidia (asexual spores) are disseminated by wind and rain from lesions and allow secondary infection to occur within the orchard throughout the fruit development period. These spores are responsible for an increase in disease when suitable weather conditions occur during the growing season. Unlike the ascospores, conidia are released at any time of the day or night and stay in the air in both dry and wet weather. Wind carries them throughout the orchard. They spread by clothing, picking bags, insects and birds. Conidia are less widely spread than ascospores (less than 100 m from the source) and infect mostly the originating plant (Turechek, 2004). Once the conidia are deposited on leaves or fruits, they require free moisture and relative humidity above 95% to germinate. A few days later, new velvety scab lesions will appear, bearing more conidia. This cycle of secondary infections continues during summer, with the right weather conditions. Pseudothecia are formed on fallen infected leaves by the union of mycelium from compatible mating types. They mature during winter to build up an inoculum for the start of a new disease cycle in spring (Bowen et al., 2011).

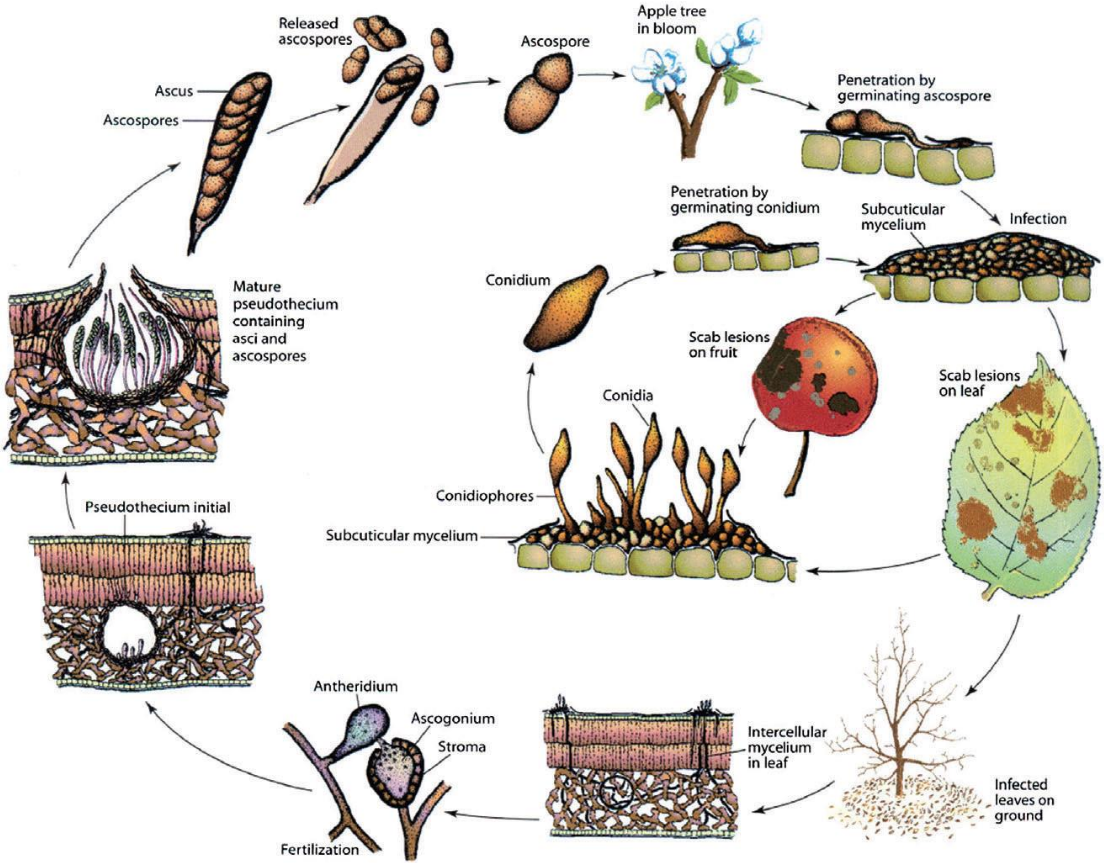


Figure 4-4. Life cycle of *Venturia inaequalis* (Bowen et al., 2011).

PART A: EFFECT OF ZEOLITES

This part aimed to verify the effect of different zeolites on the growth of *Botrytis cinerea* and *Venturia inaequalis*. First of all, antifungal activity bioassays were carried out. Subsequently, incidence and severity assesments were presented.

A.2 MATERIALS AND METHODS

A.2.1 TEST ITEMS

A.2.1.1 PLANT MATERIAL

The efficacy test was carried out on two different plant species. Measurements were conducted on cuttings of apple trees (*Malus domestica* vc. Golden Delicious) obtained from Schrama Nurseries, Biddinghuizen, Netherlands. The trees were approximately 26 cm high and their stem diameter at soil surface was approximately 2.3 mm. Subsequently, measurements were conducted on tomato plants (*Solanum lycopersicum* L. vc. Admiro) obtained from BPK, Duffel, Belgium. The plants were approximately 19 cm high and their stem diameter at soil surface was approximately 4.3 mm. All the plant materials were divided into twelve groups: 2 control and 10 treatment groups. Each group consisted of 5 plants.

A.2.1.2 MYCELIUM PRODUCTION

Mycelium of *Botrytis cinerea* (CBS 120092, Utrecht, The Netherlands) and *Venturia inaequalis* (CBS 121310, Utrecht, The Netherlands) was derived from fungal cultures grown on potato dextrose agar (PDA). The PDA was purchased from Becton Dickinson and Company, Le Pont de Clair, France. *Botrytis cinerea* plugs were transferred routinely on PDA at 22°C for 6 days in the dark. New subcultures of *B. cinerea* were made weekly. The mycelium growth of *V. inaequalis* is very slow and was grown for 4 weeks (2 cm colony diameter).

A.2.1.3 CONIDIAL PRODUCTION

Botrytis cinerea - A suspension of blended mycelium was used for conidial production. *Botrytis cinerea* was grown on PDA at 22°C under UV/dark (12h/12h) for 10 days. Spores were washed from the plates with distilled water containing 0.01 M glucose and 6.7 mM KH_2PO_4. Suspensions were filtered through cheesecloth and the conidia concentration was determined with a haemocytometer. The final spore concentration was adjusted to 5.10^5 spores.ml^{-1}. To synchronize conidia germination, the suspensions were kept for 2h at 22°C before being used.

Venturia inaequalis - *Venturia inaequalis* was isolated from infected leaves from unsprayed apple trees in PCFruit, Sint-Truiden, Belgium. Dry leaves were conserved at -20°C. The inoculum was prepared by blending the leaves with distilled water. Suspensions were filtered through cheesecloth and the conidia concentration was determined with a haemocytometer.

The final spore concentration was adjusted to 5.10^5 spores.ml^{-1}. To synchronize conidia germination, the suspensions were kept for 2h at 22°C before being used.

A.2.1.4 FUNGICIDE MATERIALS

The three zeolite types, BEA, FAU and LTA, together with two formulations of each type were compared with a commercial fungicide and a control treatment (**Table 4-3)**.

Table 4-3. Fungicide materials used in this study.

Treatment	Material name	Formulation type	Manufacturers
1	BEA	Technical product	Clariant
2	BEA 850[a]	WP	Fitofarmacia
3	BEA 950[b]	WP	Fitofarmacia
4	FAU	Technical product	Zeolyst
5	FAU 850[a]	WP	Fitofarmacia
6	FAU 920[b]	WP	Fitofarmacia
7	LTA	Technical product	FMC
8	LTA 800	SC	Fitofarmacia
9	LTA 850	WP	Fitofarmacia
10 (A)	Thiram (Hermosan 80)	WG	Taminco
10 (B)	Chlorothalonil (Daconil 50)	SC	Syngenta
11	Negative control	/	/
12	Positive control	/	/

[a, b] The formulations with a similar letter contain the same adjuvants.

A.2.2 MEASUREMENTS

A.2.2.1 ANTIFUNGAL ACTIVITY IN VITRO BIOASSAY

In vitro tests are usually performed as a first stage in the selection of active substances against the fungal pathogens. A mycelial plug from *Botrytis cinerea* or *Venturia inaequalis* was placed at the centre of a PDA plate amended with the substances to be tested (**Table 4-3**). The plates were incubated at 22°C for 3 days (*B. cinerea*) and 8 weeks (*V. inaequalis*) and examined for an inhibition zone.

Fungal colony diameters were determined as a measure of inhibition (I) of fungal growth and compared to those on control plates without active ingredients:

$$I = (1 - \frac{T_r}{C_r}) \, . \, 100$$

where T_r is the colony radius of the test plate (cm) and C_r is the colony radius of the control (mm).

The experiment was performed twice with 4 plates per treatment. Different concentrations (400 mg.l^{-1}, 4000 mg.l^{-1} and 20000 mg.l^{-1}) of the active ingredients were tested to estimate the biological activity.

A.2.2.2 DISEASE INCIDENCE AND SEVERITY ASSESSMENT

Inoculations with a *B. cinerea* or *V. inaequalis* suspension were carried out on apple trees and tomato plants under greenhouse conditions (at 26 $\pm$ 1°C, 70% RH and 16:8 (L:D)h). The conidial suspension was sprayed until runoff. Immediately after inoculation, the plants were incubated in a growth chamber at 90% RH for 48 h at 26°C to provide optimal infection conditions. Each treatment consisted of 5 plants and the experiment with the apple trees and tomato plants were carried out taken into account all leaves and 5 leaves per plant, respectively. Disease development was evaluated after 3 weeks. Incidence and severity were calculated visually.

Disease incidence (DI) was recorded as the proportion of diseased plants:

$$DI = \frac{N_l}{T_l} \, . \, 100$$

where N_l is the number of infected leaves and T_l the total number of leaves.

Disease severity (DS) was assessed based on the size and number of necrotic spots on a leaf surface (**Figure 4-5**). A 0-4 intensity scale was used, whereby: 0 = no symptoms observed; 1 = lesions covered less than 25% of leaf surface; 2 = lesions covered 25-50% of leaf surface; 3 = lesions covered 50-75% of leaf surface; 4 = lesions covered more than 75% of leaf surface or dead leaf.

The values obtained for each replication and each treatment were transformed to severity values (the percentage of apple scab or grey mould) with the following expression:

$$DS = \frac{\sum n_i \, . \, i}{T_l \, . \, v} \, . \, 100$$

where n_i is the number of leaves in class i, i is the class value; T_l is the total number of leaves and V is the highest class value.

A.2.3 DATA ANALYSIS

A.2.3.1 ANTIFUNGAL ACTIVITY IN VITRO BIOASSAYS

Statistical analysis of the antifungal activity bioassay was examined using a one sample Kolmogorov-Smirnov test to assess the normality of the measured fungal colony radius. The Mann-Whitney test was used to compare the differences between the control and treated samples. A p value of < 0.05 was considered statistically significant. Statistical analysis was performed using a statistical software program (SPSS Version 12.0, Chicago, USA).

A.2.3.2 DISEASE INCIDENCE AND SEVERITY ASSESSMENT

Disease incidence and severity were analysed based on the Kolomogorov-Smirnov tests, followed by a Mann-Whitney test, in order to determine normality and to compare the differences between the control and treated samples. A p value of < 0.05 was considered statistically significant. Statistical analysis was performed using a statistical software program (SPSS Version 12.0, Chicago, USA).

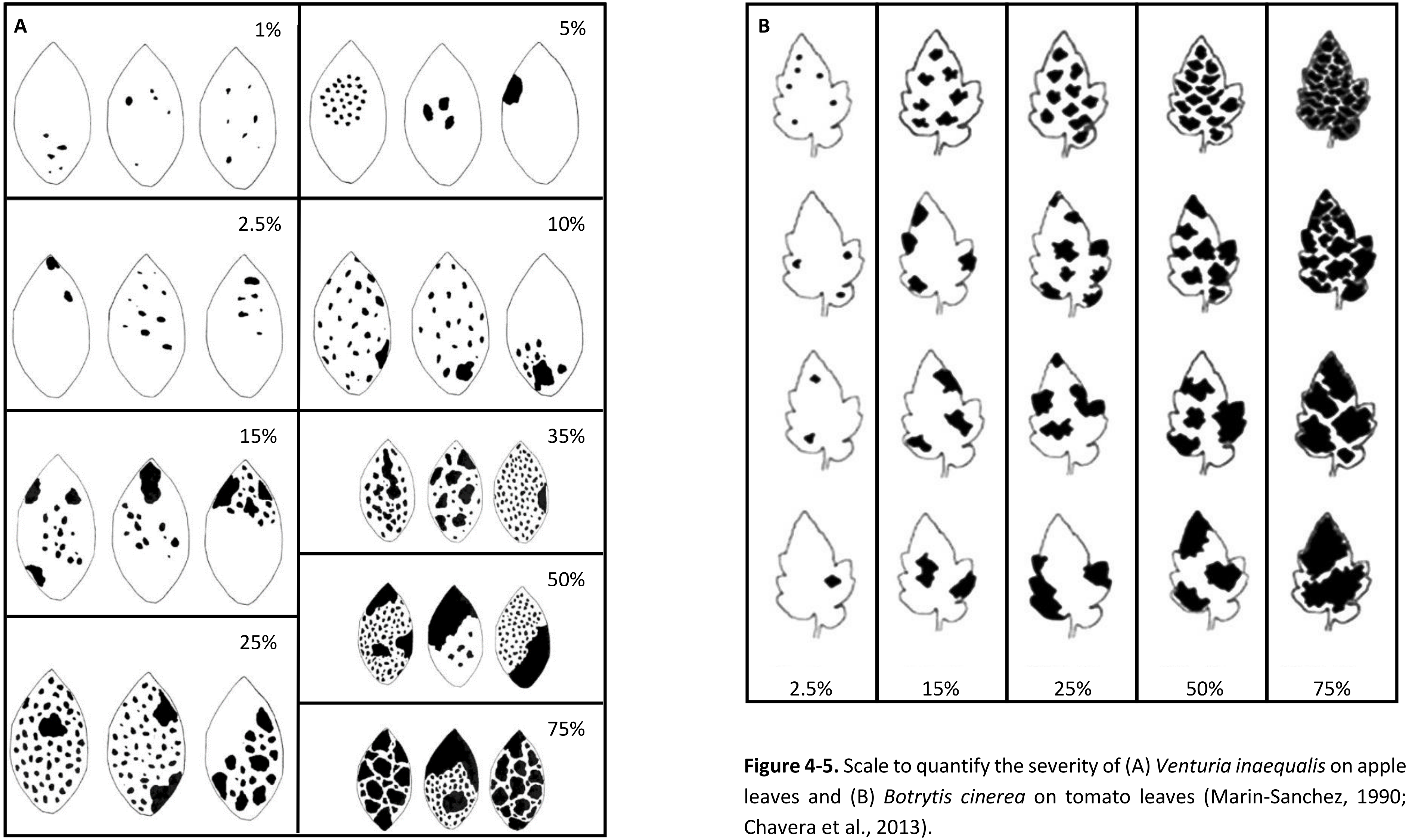


Figure 4-5. Scale to quantify the severity of (A) *Venturia inaequalis* on apple leaves and (B) *Botrytis cinerea* on tomato leaves (Marin-Sanchez, 1990; Chavera et al., 2013).

A.3 RESULTS

A.3.1 ANTIFUNGAL ACTIVITY IN VITRO BIOASSAY

The inhibition effects of 9 zeolites and 1 chemical product (**Table 4-3**) were evaluated against two fungi, *V. inaequalis* and *B. cinerea*. The diameter of the growth inhibition zone was measured after the exposure of these fungi to different concentrations of the toxicants (**Figure 4-6**). The obtained results are listed in **Table 4-4.** Significant differences were observed for almost all zeolites and used concentrations compared to the control. More information about the data analysis can be found in **Appendix E** (**Tables E-1** and **E-2**).

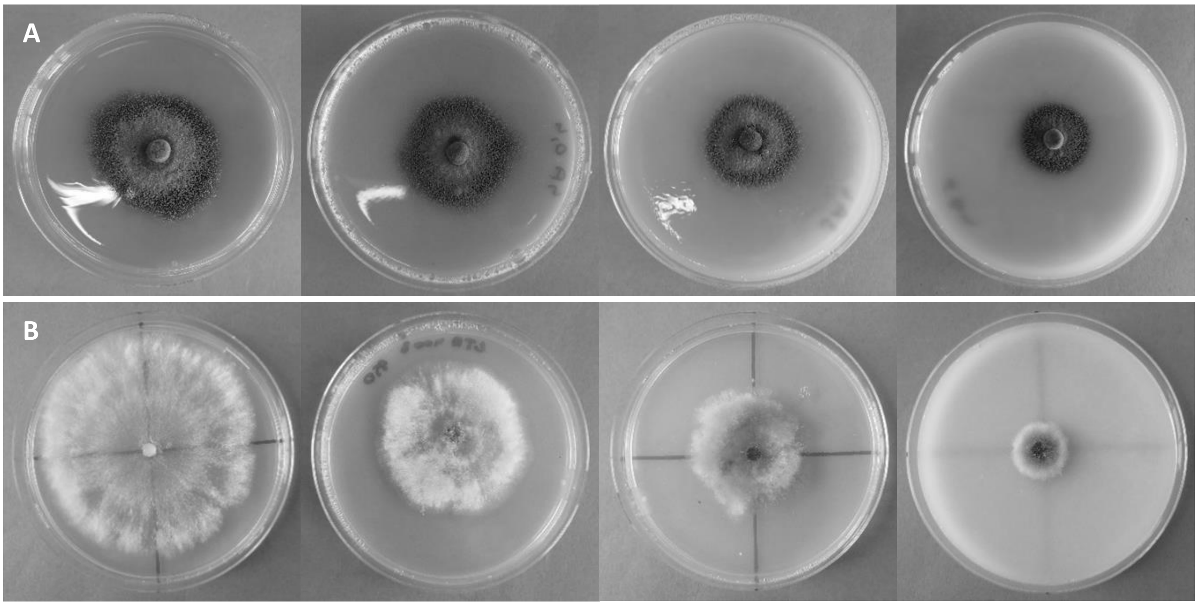


Figure 4-6. Antifungal activity of BEA 950 WP on the hyphal growth of (A) *Venturia inaequalis* and (B) *Botrytis cinerea*. The used concentrations were from left to right 0, 400, 4000 and 20000 mg.l^{-1}.

Control treatments in all experiments revealed that all the studied fungi grew extensively. Inhibition of all fungi was observed with increasing zeolite concentrations, as shown in **Figure 4-6**. Data in **Table 4-4** showed that the mycelium growth of both fungi was most affected by zeolite LTA and its formulations. These products reached a growth inhibition by about 100% at the highest concentrations of 4000 and 20000 mg.l^{-1}.

Table 4-4. Growth inhibition (± SE) of the fungicide materials (**Table 4-3)** against *Venturia inaequalis* and *Botrytis cinerea* compared to the control (n=8).

	Inhibition (%)			
Concentration (mg.l^{-1})	**0**	**400**	**4000**	**20000**
Venturia inaequalis				
BEA	0.00 ± 1.08	2.51 ± 0.97	6.92 ± 1.23	7.80 ± 1.08
BEA 850 WP	0.00 ± 1.08	3.79 ± 0.99	7.61 ± 1.55	28.19 ± 0.71
BEA 950 WP	0.00 ± 1.08	8.00 ± 1.99	18.39 ± 0.64	36.31 ± 0.99
FAU	0.00 ± 1.08	4.02 ± 0.88	20.74 ± 1.73	27.78 ± 1.00
FAU 850 WP	0.00 ± 1.08	13.68 ± 1.57	28.33 ± 0.68	39.30 ± 1.06
FAU 920 WP	0.00 ± 1.08	14.04 ± 1.36	28.35 ± 1.81	49.44 ± 0.93
LTA	0.00 ± 1.08	30.12 ± 0.58	100.00 ± 0.00	100.00 ± 0.00
LTA 800 SC	0.00 ± 1.08	42.96 ± 0.60	100.00 ± 0.00	100.00 ± 0.00
LTA 850 WP	0.00 ± 1.08	45.77 ± 0.60	100.00 ± 0.00	100.00 ± 0.00
Hermosan 80	0.00 ± 1.08	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
Botrytis cinerea				
BEA	0.00 ± 1.45	4.68 ± 2.25	8.83 ± 3.80	39.63 ± 2.60
BEA 850 WP	0.00 ± 1.45	5.44 ± 4.32	70.68 ± 1.49	72.67 ± 2.78
BEA 950 WP	0.00 ± 1.45	34.46 ± 0.71	54.24 ± 1.39	73.78 ± 1.00
FAU	0.00 ± 1.45	4.96 ± 2.30	5.30 ± 2.98	21.78 ± 2.22
FAU 850 WP	0.00 ± 1.45	16.56 ± 4.06	36.04 ± 0.72	61.05 ± 1.79
FAU 920 WP	0.00 ± 1.45	21.97 ± 1.70	31.18 ± 1.55	74.45 ± 1.31
LTA	0.00 ± 1.45	3.14 ± 4.15	91.12 ± 0.49	100.00 ± 0.00
LTA 800 SC	0.00 ± 1.45	29.63 ± 3.58	89.09 ± 1.99	100.00 ± 0.00
LTA 850 WP	0.00 ± 1.45	31.29 ± 1.84	88.06 ± 0.67	100.00 ± 0.00
Daconil 50	0.00 ± 1.45	90.53 ± 1.03	93.77 ± 1.04	94.59 ± 0.35

Zeolites BEA and FAU also resulted in inhibition of the fungi, especially for *B. cinerea*. However, compared to the inhibition percentages obtained by zeolite LTA and its formulations, zeolites BEA and FAU showed less effect on fungal growth. Inhibition percentages of *V. inaequalis* after exposure to 20000 mg.l^{-1} of zeolite BEA and its formulations were 7.80%, 28.19% and 36.31% respectively. The exposure to 20000 mg.l^{-1} of zeolite FAU and its formulations resulted in inhibition percentages of *V. inaequalis* of 27.78%, 39.30% and 49.44%, respectively. It can be noticed that similar growth inhibition rates were observed after the fungicidal treatment with zeolite LTA at a concentration of 400 mg.l^{-1}.

On the other hand, higher inhibition values were observed for *B. cinerea* after exposure to the same products. Zeolite BEA and its formulations reached inhibition percentages of 39.63%, 72.67% and 73.87%, respectively, at a concentration of 20000 mg.l^{-1}. Finally, zeolites FAU and its formulations reached inhibition percentages of 21.78%, 61.05% and 74.45%, respectively, at a concentration of 20000 mg.l^{-1}. Again, zeolite LTA obtained better results at lower concentrations, i.e. between 400-4000 mg.l^{-1}.

In general, all of the tested compounds showed antifungal activity. However, it can be concluded that the formulations resulted in higher growth inhibition values compared to the industrial product. For example, the inhibition percentage of *B. cinerea* resulted in 3.14% after exposure to 400 mg.l^{-1} of zeolite LTA. At the same concentration, the formulations of this pure technical zeolite, i.e. zeolite LTA 800 SC and LTA 850 WP, were about 10 times more effective at inhibiting the growth of *B. cinerea*. This was a general observation for most zeolite types and concentrations for both fungi.

Despite the observed inhibition of the fungi after exposure to the zeolites and especially after exposure to zeolite LTA and its formulations, the chemical products still revealed the highest inhibition effect (ca. 100%) at all concentrations against the tested fungi.

A.3.2 DISEASE INCIDENCE AND SEVERITY ASSESSMENT

Apple trees and tomato plants showed after the inoculation with *V. inaequalis* and *B. cinerea* incidence disease symptoms, such as chlorosis, wilting, root and foot rot, stem rot and necrosis. As shown in **Figure 4-7**, incidence of all the above mentioned symptoms ranged from 11.46-56.75% for apple trees and from 8.00-92.00% for tomato plants, respectively. Data analysis showed that there were significant differences in the incidence of the inoculated plants treated with the zeolite (**Table 4-3**) compared to the positive untreated inoculated control. This was especially the case for the apple trees. The lowest incidence for the apple trees was obtained after using the LTA zeolites and the chemical product. Besides the chemical product, the industrial zeolites BEA and FAU indicated less incidence for the tomato plants. However, this reduction was not significantly and no clear dose-response relationship was seen for all treatments.

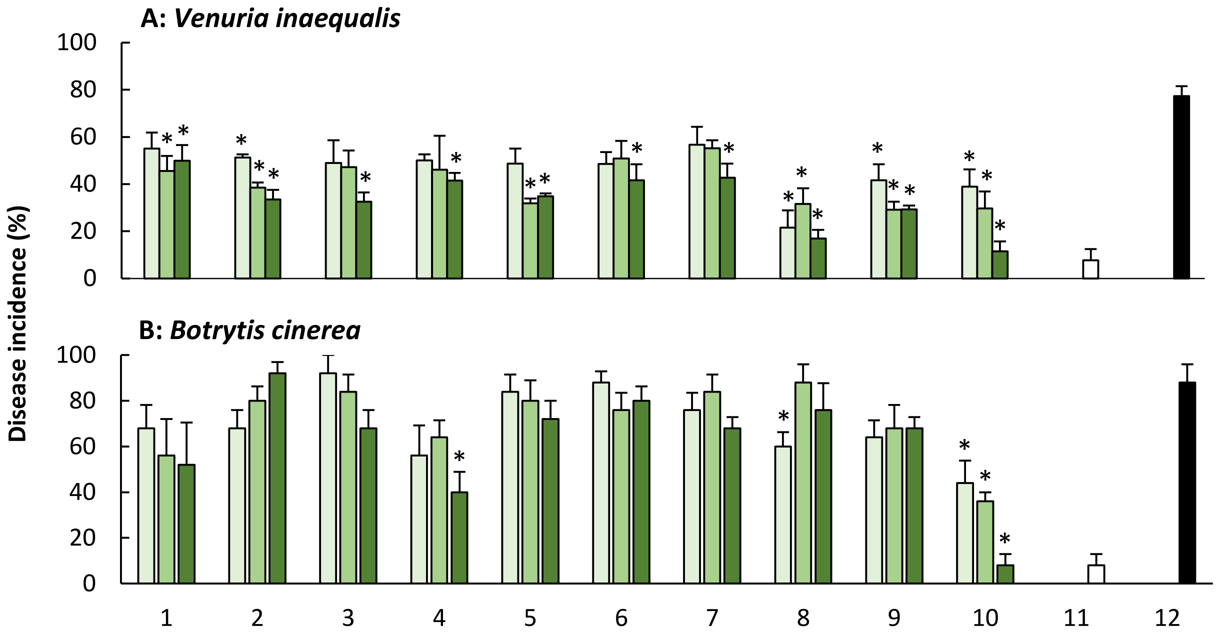


Figure 4-7. Effect of fungicide spray treatments (**Table 4-3**) on *Venturia inaequalis* (A) and *Botrytis cinerea* (B) incidence on leaves (0-4 scale). The used concentrations were 400 (□), 4000 (▨) and 20000 (▩) mg.l^{-1}. The white bar (□) represents the disease incidence of control plants that were not inoculated (negative control), while the black bar (■) represents the disease incidence of control plants that were inoculated (positive control). Asterisks indicate significant differences in disease incidence between sample and positive control treatments (n=5).

Apple scab and grey mould can cause varying degrees of damage to the apple trees and tomato plants. That is why the symptoms on the leaves were scored using a scale, ranging from 0 (no disease symptoms) to 4 (lesions covered more than 75% of leaf surface or dead leaf). The observed disease severity and the disease class distribution are shown in **Figures 4-8 and 4-9**, respectively.

As demonstrated in **Figure 4-8**, the proportion of the affected plant area was lower for the apple trees than for the tomato plants. Data analysis illustrated that the disease severity showed significant differences between the inoculated plants treated with the zeolite (**Table 4-3**) compared to the positive control. This was especially the case for the apple trees, for which all the treatments showed significant differences. The disease severity of the tomato plants treated with the chemical product and the industrial zeolites BEA and FAU was significant lower compared to the positive control.

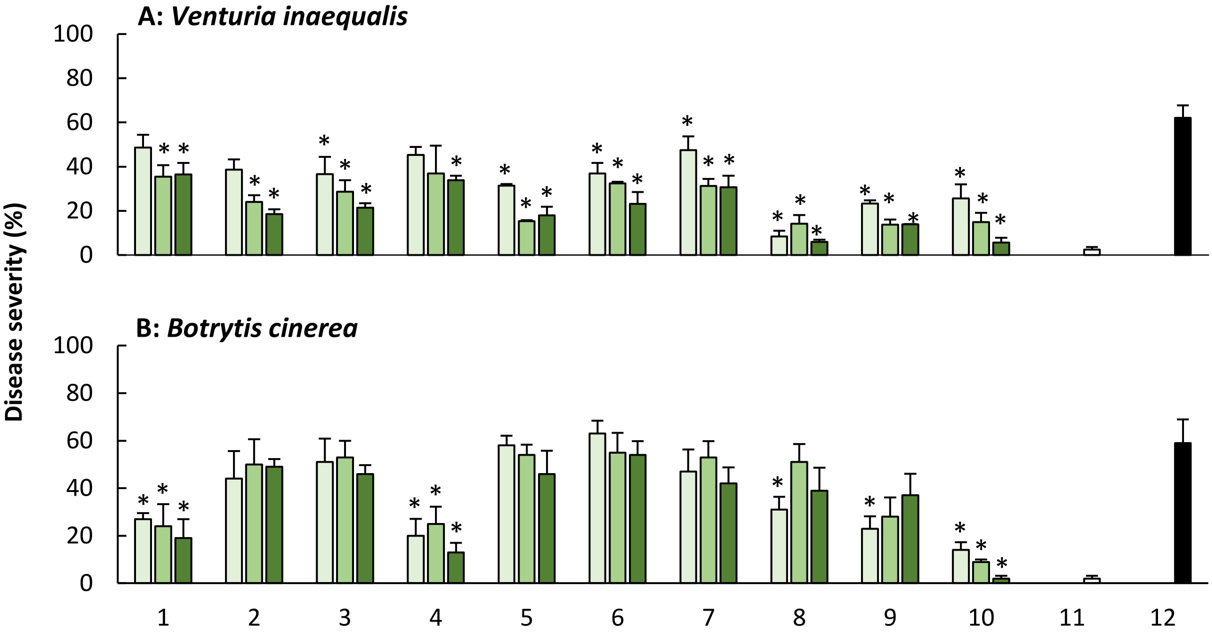


Figure 4-8. Effects of fungicide spray treatments (**Table 4-3**) on *Venturia inaequalis* (A) and *Botrytis cinerea* (B) severity on leaves (0-4 scale). The used concentrations were 400 (□), 4000 (🟩) and 20000 (🟩) mg.l^{-1}. The white bar (□) represents the disease severity of control plants that were not inoculated (negative control), while the black bar (■) represents the disease severity of control plants that were inoculated (positive control). Asterisks indicate significant differences in disease severity between sample and positive control treatments (n=5).

More clarification for the obtained results is illustrated in **Figure 4-9**. The apple trees had more leaves without symptoms, which is also visible in **Figure 4-7**, and showed little signs of class 1. When symptoms of *V. inaequalis* were present, it was visible on more than 25% of the leaf surface area. Looking at the tomato plants, only the chemical product showed most leaves without symptoms. A lot of *B. cinerea* was noticed, but most symptoms were present on less than 75% of the leaf surface area.

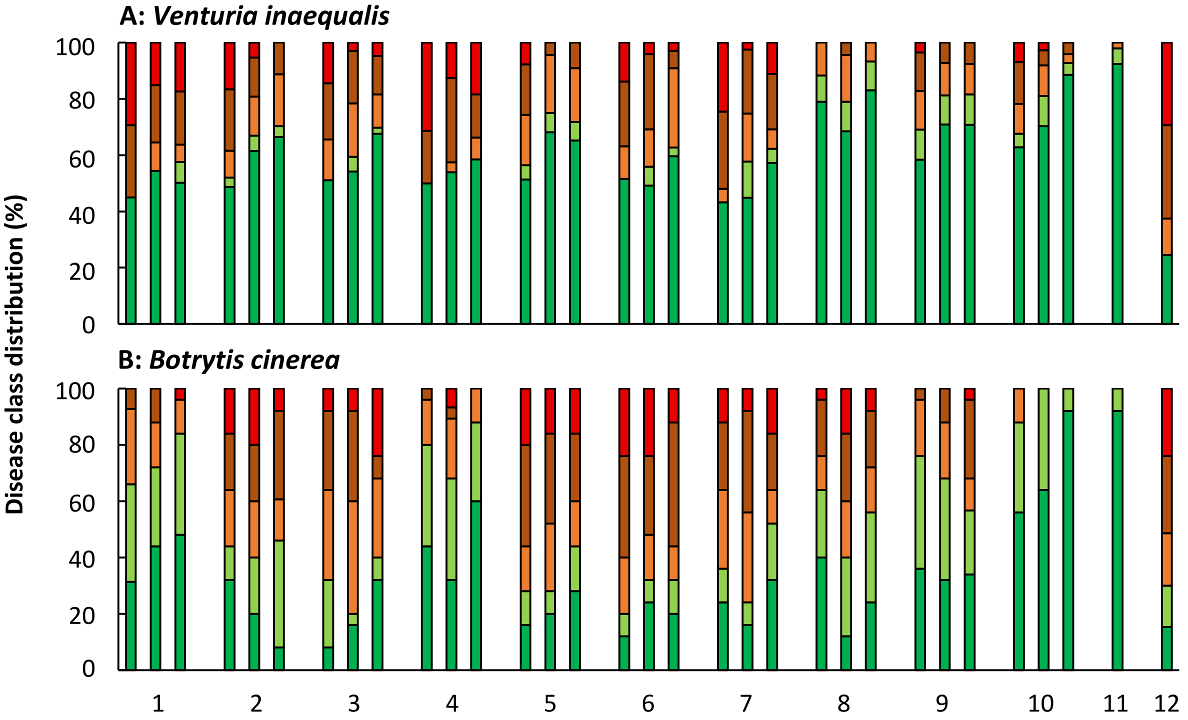


Figure 4-9. Bar stacks represent the disease severity of the fungicide materials (**Table 4-3**) as the relative frequency of occurrence of disease classes (0-4) for *Venturia inaequalis* (A) and *Botrytis cinerea* (B). For rapid visual evaluation of the data, a coloring scale with Class 0: DI = 0 (🟩), Class 1: 1 < DI ≤ 25 (🟩), Class 2: 26 < DI ≤ 50 (🟧), Class 3: 51 < DI ≤ 75 (🟫) and Class 4: DI > 75 (🟥) was used (n=5).

A very close relationship between incidence and severity is depicted in **Figure 4-10**. The best linear relationship between pairs of transformed incidence and severity was obtained by logarithmic (ln) transformation, which provided the highest coefficient of determination. The regression equation obtained from ln-transformed data and untransformed data showed a very high correlation between incidence and severity for both fungi. The variance of disease severity increased, as general means of disease incidence also increased. The general regression equations obtained from the analysis of pooled data for *V. inaequalis* and *B. cinerea* respectively, were ln (DS) = 0.6492.ln (DI) + 1.5905 and ln (DS) = 0.6206.ln (DI) + 1.4572.

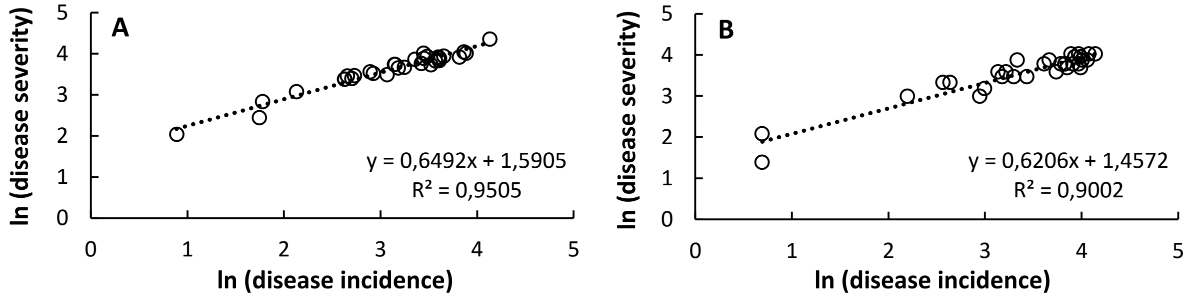


Figure 4-10. Relationship between incidence and severity of *Venturia inaequalis* (A) and *Botrytis cinerea* (B) with logaritmic (ln) transformation.

A.4 DISCUSSION

In the present work, antifungal activity bioassays were performed using only non-systemic products, i.e. both zeolites and conventional products were based on inhibition by contact. The results showed that zeolite LTA and its formulations obtained the highest effect in terms of growth inhibition. As described in Chapter 2, this zeolite is a hydrophilic zeolite, in contrast to zeolites BEA and FAU. Zeolites BEA and FAU obtained high growth inhibition values, but only when a concentration of 20000 mg.l^{-1} was used. However, in comparison to chemical products, the control of both diseases still needed very high concentrations of zeolite. Both chemical fungicides inhibit the fungi by multi-site contact activity (Yang et al., 2011).

Multi-site activity fungicides are widely used in agronomic activities due to the broad spectrum of disease control activity, but may have side effects on other microorganisms due to their multiple biochemical sites impacts (Yang et al., 2011). Chlorothalonil (tetrachloroisophthalonitrile), a widely used phthalonitrile fungicide, can reduce the activity of an antioxidant enzyme that plays an important function in the cellular respiration process. This can have a deleterious effect on the PPP degrading microbial community so that inhibition of degradation becomes apparent (Singh et al., 2002). Previous research found that chlorothalonil can influence bacterial growth in soil, which may have ecological consequences on N cycling (Chen et al., 2001). Other multi-site activity fungicides such as thiram (bis(dimethylthiocarbamoyl) disulfide) inhibited the growth of denitrifying bacteria, perhaps due to their nonspecific effects on biochemical compounds that contain thiol in target cells (Milenkovski et al., 2010). Increasing interest is directed towards biological control.

In many parts of the world, farmers and growers are accustomed to very high levels of disease control achieved by using fungicides. Similar levels of disease control are unlikely to be achieved with many novel disease control methods, like polymer or particle films, or adhesion inhibitors. However, the problems of fungicide resistance, breakdown in host resistance and increased public concern for the environment means that the development of new disease control methods cannot be ignored (Walters, 2009). The concept of hydrophobic particle film technology for the control of diseases was introduced by Glenn et al. in 1999. The authors suggested that disease control was achieved because plants were enveloped in a hydrophobic particle film barrier that prevented pathogen propagules or water from directly contacting the leaf surface. Puterka et al. (2000) found that hydrophilic particle films can also provide plant disease control. However, it is not well understood how particle films influence infection by disease organisms. Years of research have shown that hydrophobic kaolin particle films failed to control some diseases like Cladosporium carpophilum or Podosphaera leucotricha on peach, although it did control Monilinia fructicola (Lalancette et al., 2005). In contrast, hydrophilic kaolin particle films did not control any of the peach pathogens, leading the authors to suggest that hydrophobicity and deposit density may be important factors for effective disease management (Lalancette et al., 2005). Puterka et al. (2000) also observed that neither hydrophobic nor hydrophilic particle films controlled pear scab.

However, based on the results of the antifungal activity bioassays, it can be deduced that the multi-site contact activity mode of action of the conventional PPPs had more effect compared to the desiccating mode of action of the zeolites. This non-specific action of the conventional fungicides implies that numerous metabolic steps in fungal organisms are affected. They bind to or irreversibly modify reactive groups of numerous enzymes and other biologically important compounds. The chemically most reactive group in biological systems is the thiol residue of cysteine. Blockage of essential cysteine residues is the major cause for the fungitoxic action of non-specific fungicides (Köller, 1999).

It is known that both conventional PPPs, thiram and chlorothalonil, are rapidly absorbed by spores of susceptible fungi and bind to thiol groups after uptake into fungal cytoplasm. The attachment to thiol groups with subsequent disruption of enzyme activity appears to be the major mechanism of action (Carlile and Coules, 2012). This mode of action is probably faster and more efficient.

Subsequently, higher growth inhibitions were observed for the tested formulations. This may be caused due to the adjuvants present in the formulations. Looking at the composition of the different adjuvants, listed in **Table 4-5,** it is noteworthy that three of the four discussed adjuvants are composed of sodium alkylnaphthalene sulfonate. On the other hand, Rhodorsil EP is composed of polydimethylsiloxane.

Table 4-5. Composition of the adjuvants present in the zeolite formulations based on the MSDS files.

	Adjuvants	**Composition**
a	Agrilan 789 Dry	Acrylic copolymer
b	Morwet EFW Powder	Sodium alkylnaphthalene sulfonate
c	Rhodorsil Antim EP	Polydimethylsiloxane
d	PVP K-30	Polyvinylpyrrolidone
e	Ultrasil VN 3	Silicon dioxide
f	Morwet D425	Sodium sulfonated aromatic polymer
g	SAG 1572	Polysiloxane
h	Supragil WP	Sodium di-isopropylnaphthalene sulfonate
i	Reax 88B	Sulfomethylated sodium lignosulfonic acid
j	Rhodopol 23	Xanthan gum
k	Amebact C	2,2,2- (Hexahydro-1,3,5-triazine 1,3,5 tryl) triethanol
l	Soprophor FL	Ethoxylated polyarylphenol phosphate amine

Based on the information at the PPP database, sodium alkylnaphthalene sulfonates are used as insecticide, fungicide, microbiocide or adjuvant (PAN, 2014). The most common use is first listed. This means that this substance, present in de tested formulations, is able to kill insects, fungi (molds, mildews, others) and microbes (bacteria, viruses, fungi). Therefore, it can be used as an adjuvant to increase effectiveness of an active ingredient. Subsequently, polydimethylsiloxane is used as insecticide and adjuvant (PAN, 2014). These findings confirm the possible antifungal activity of the adjuvants, especially against *B. cinerea*.

Nevertheless, laboratory studies can obtain different results compared to studies carried out on a whole plant. Glenn et al. (1999) applied hydrophobic kaolin to plants in order to develop an artificially hydrophobic plant surface that would repel water. Water-repellent surfaces facilitate the removal of particulate depositions (spores, conidia, hyphae) through the deposition and subsequent runoff of rain, fog or dew. In single-leaf laboratory studies, fungal infection could be completely eliminated; however, on the whole plant and field plot scale, studies found complete coverage by the hydrophobic kaolin was impossible and so failed to control apple scab (Puterka et al., 2000). Therefore, it was important to examine the effect of zeolites against *V. inaequalis* and *B. cinerea* on plants. In this study, incidence and severity were used as measures to estimate the effect of zeolites. Since measures of incidence are more easily acquired and more reliable than measures of severity and since severity is more useful than incidence for certain objectives, a combination of incidence and severity facilitates the evaluation of disease intensity.

It was observed that none of the zeolite totally prevents apple scab or grey mould. However, the apple trees treated with zeolites showed significant differences compared to the positive control. The LTA formulations also showed the lowest disease incidence and severity for the apple trees. Looking at the tomato plants, the zeolites showed less effect. With the exception of the industrial zeolites BEA and FAU, no significant differences compared to the positive control were observed. Finally, the chemical product exhibits a good effect against both diseases. However, a clear linear relationship between the disease incidence and disease severity was noticed.

The observed fungicidal acitivty of the zeolites can be explained by the plant surfaces that provide chemical and physical cues, which are important factors in the development of infection structures of many plant pathogenic fungi. It is therefore no surprise that disrupting these processes, by coating the leaf with polymer films or applying agents that interfere with spore adhesion, can reduce infection and provide disease control (Walters, 2009).

Additionally, adhesion of fungal spores to the plant surface is thought to be the essential first step in the infection process. Once a spore has successfully adhered to the leaf surface, germ tube formation is rapid, whereas if conidia fail to adhere to the substrate, viability is rapidly lost. Once formed, germ tubes must also adhere to the plant surface, since this process is important in the perception of signals for further formation of the appressorium. The importance of spore adhesion to successful infection of plant surfaces suggests that disruption of this process could be useful in disease control (Walters, 2009). Zekaria-Oren and Eyal (1991) found that surfaces coated with film-forming polymers interfered with fungal penetration of the leaf and that the efficacy was related to thickness and uniformity of the coat on the leaf surface. They also observed that both the orientation of the germinating spores towards the stomata and the formation of appressoria were altered on coated surfaces, which may be the result of the disruption of mechanisms responsible for orientation of the germinating spores towards the stomata and appressorium formation.

Regardless the obtained fungicidal activity of the zeolites, it was striking that the disease severity on the tomato plants treated with the industrial zeolites BEA and FAU was lower compared to the formulated zeolites. Even significant differences were observed between these industrial zeolites and the positive control. This may be caused by the presence of the adjuvants in the formulations. Many studies have demonstrated a strong negative correlation between the amount of epicuticular wax and permeability of agricultural chemicals. The epicuticular waxes are a significant barrier to the penetration of a PPP spray into plant organs (Jenks and Ashworth, 1999). However, most agricultural sprays are formulated with adjuvants like wetting agents, spreaders, or stickers to facilitate doplet retention, distribution, and penetration into the plant surface.

Subsequently, as described in Chapter 3 (Section 4), both leaf surfaces of the test species, apple and tomato, are hairy. However, apple leaves have a more smooth surface area compared to the more hairy tomato leaves. Additionally, the epicuticular layer of the apple leaves contained a higher amount of wax compared to the tomato leaves. This epicuticular wax layer prevents the formation of stable, macroscopic water phases and, hence, the germination of the spores of many plant pathogens (Allen et al., 1991; Juniper, 1991; Liakopoulos et al., 2001).

Based on this information, it is most likely that the adjuvants present in the zeolite formulations penetrated more easily into the tomato leaves due to the lower amount of wax present in the epicuticular layer. This can damage the tomato leaves and make them more vulnerable for fungal diseases, especially for a fungus that establishes itself on injured tissues and can persist as a saprophyte for long periods. Compared to *V. inaequalis*, *B. cinerea* is a necrotrophic fungal pathogen and it is known that many necrotrophs sporulate abundantly on lesions as well as on dead plant material (Elad et al., 1994).

Subsequently, the interaction with germination takes places on the plant surface, whereas the interaction with sporulation is in necrotic lesions (Elad et al., 1994). This causes the interaction time between zeolites and germinating propagules to be shorter as a results of the already damaged tomato leaves due to the adjuvants.

Despite the better antifungal activity of the industrial zeolites, no further research was carried out on this matter. As described in Chapter 3 (Section 1.1), a PPP is rarely used or applied in its pure form. First of all, these zeolites are not soluble in water and therefore require adjuvants to form stable suspensions or emulsions when these zeolites are mixed with water in the spray tank. This is necessary in order to prevent sedimentation of the active ingredient. Additionally, adjuvants also improve the physicochemical and spreading properties, the biological activity and the safety issues of the active ingredient.

PART B: EFFECT OF BUILT-IN ADJUVANTS

Adjuvants in formulations are compounds that modify the effects of other compounds. They can be used in PPPs e.g. to increase the performance of the active ingredients or to make the formulation chemically more stable (Chapter 3). Also, an adjuvant may improve the efficacy of a PPP, which may lead to a reduced concentration or total amount of PPP required to achieve a given effect. However, it can be questioned whether these adjuvants also have a biological effect on their own. In order to clarify the results obtained in Part A, the following sections present the results of antifungal activity and bacterial gene profiling assays of the different adjuvants present in the tested zeolite formulations.

B.2 MATERIALS AND METHODS

B.2.1 TEST ITEMS

B.2.1.1 MYCELIUM PRODUCTION

Mycelium of *Botrytis cinerea* (CBS 120092, Utrecht, The Netherlands) and *Venturia inaequalis* (CBS 121310, Utrecht, The Netherlands) was derived from fungal cultures grown on Potato Dextrose Agar (PDA). The PDA was purchased from Becton Dickinson and Company, Le Pont de Clair, France. *Botrytis cinerea* plugs were transferred routinely on PDA at 22°C for 6 days in the dark. New subcultures of *B. cinerea* were made weekly. The mycelium growth of *V. inaequalis* is very slow and was grown for 4 weeks (2 cm colony diameter).

B.2.1.2 BUILD-IN ADJUVANTS

The adjuvants present in the formulations used in this thesis are summarized in **Table 4-6**, together with the different compositions of the tested formulations. However, for reason of confidentiality, the exact concentrations of applied adjuvants in the formulations are not known. Nevertheless, an assumption was made of the percentages used in the formulations. With the exception of amebact C, which was present at 0.025%, all other adjuvants were assumed to be present at 3% of the tested formulation.

B.2.2 MEASUREMENTS

B.2.2.1 ANTIFUNGAL ACTIVITY IN VITRO BIOASSAY

In vitro tests are usually performed as a first stage in the selection of active substances against the fungal pathogens. A mycelial plug from *Botrytis cinerea* or *Venturia inaequalis* was placed at the centre of a PDA plate amended with the adjuvants to be tested (**Table 4-6**). The plates were incubated at 22°C for 3 days (*B. cinerea*) and 8 weeks (*V. inaequalis*) and examined for an inhibition zone. Fungal colony diameters were determined as a measure of inhibition of

Table 4-6. Adjuvants present in the used formulations.

Treatment	Adjuvants	Use	Manufacturers	BEA 850 WP[a]	FAU 850 WP	BEA 950 WP	FAU 920 WP	LTA 800 SC	LTA 850 WP
	Control	/	/						
a	Agrilan 789 Dry	dispersing agent	AkzoNobel	x	x	x	x		
b	Morwet EFW Powder	Surfactant	AkzoNobel	x	x	x	x		
c	Rhodorsil Antim EP	anti-foam	Rhodia	x	x	x	x		
d	PVP K-30	structuring agent	ISP	x	x				x
e	Ultrasil VN 3	Carrier	Evonik	x	x				x
f	Morwet D425	dispersing agent	AkzoNobel					x	
g	SAG 1572	anti-foam	Momentive					x	
h	Supragil WP	wetting agent	Rhodia					x	
i	Reax 88B	dispersing agent	MeadWestvaco					x	
j	Rhodopol 23	thickening agent	Rhodia					x	
k	Amebact C	Biocide	Ametech					x	
l	Soprophor	dispersing agent	Rhodia						x

[a] Colours according to the composition of the formulations.

fungal growth and were compared to control plates without adjuvants. The experiment was performed twice with 4 plates per treatment. The zeolite formulations in this study contained 3% of each adjuvant, with the exception of Amebact, C which was present at a concentration of 0.025%. The adjuvant concentrations tested in this bioassay were calculated taken into account a zeolite concentration of 4000 mg.l^{-1}.

B.2.2.2 TOXIC MODE OF ACTION

The following paragraphs describe the protocol of the experiment developed by Antwerp University.

Bacterial strains

A gene expression assay can be used as a screening tool for the presence of toxicity in complex and poorly characterized matrices. Therefore, the toxic mode of action of the different selected compounds was evaluated using a gene expression assay. The performance of a battery of stress promoter::reporter gene fusions in genetically engineered *Escherichia coli* strains was investigated. All bacterial strains used, except *SfiA*, are based on an *E. coli* K-12 derivative SF1, which contains the lac4169 mutations, deleting the complete lac operon, and rpsL. All reporter constructs are promoter::*LacZ* fusions, present as single copies on the bacterial chromosomal. The *SfiA* strain is part of the SOS chromotest derived from *E. coli* GC4436 with a deletion in the lac operon carrying a *SfiA::LacZ* fusion in order to measure the responses to DNA damaging agents. **Table 4-7** illustrates the selected list of 14 transgenic *E. coli* strains, belonging to different toxicological endpoint classes, e.g. DNA damage, oxidative stress, protein denaturation, membrane damage, osmotic stress, general cellular stress and heavy metal presence. The integration of 14 endpoints in one test enables a more complete characterization of the mode of action of the toxicants or mixtures compared to one endpoint test.

Growth, dosing, and lysis

Cultures were inoculated from frozen aliquots 15 to 18 h before use, in 2 ml volumes of Luria Bertani broth base (LB) (Difco Laboratories, Beckton Dickinson) supplemented with NaCl to 5 g.l^{-1}. Precultures were grown overnight in 50 ml centrifuge tubes at 37°C and 250 rpm. The turbidity at 600 nm of the overnight cultures was checked to exceed 2.0 before proceeding with the assay.

Bacterial plates - At the end of the incubation, 50 µl of all cultures was aseptically added - one strain per row - to 250 µl of LB in column 12 of a 96-well sterile flat bottomed polystyrene microtiter plate (Sterilin, International Medical Products, Belgium) (**Figure 4-11**). Columns 2 through 11 were filled with 200 µl of LB and received 25 µl from the prediluted strains in column 12. Column 1 was used as a blank and received 225 µl of LB. Strains were grouped on

Table 4-7. Stress gene promoters fused to the *LacZ* gene and their functional grouping (modified from Dardenne et al., 2007 and Orser et al., 1995).

Type of stress response	Promoter	Gene product/function	Responsive to
Oxidative stress	KatG	Hydrogen peroxidase I	Oxidative stress
	Zwf	Glucose-6-phosphate dehydrogenase	Oxidative stress
	Soi28	Superoxide inducible gene	Superoxide radical generating agents
	Nfo	Endonuclease IV	Ss and dsDNA breaks, oxidative DNA damage
Membrane damage	MicF	Antisense RNA to 5' OmpF	Membrane integrity, osmotic stress
	OsmY	Periplasmic protein	Osmotic stress
General cell lesions	UspA	Universal stress protein	Growth arrest
	ClpB	Proteolytic activation of ClpP	Protein perturbation
Heavy metal stress	MerR[a]	Regulation of the mercury resistance operon (mer)	Heavy metals
DNA damage	Nfo	Endonuclease IV	Ss and dsDNA breaks, oxidative DNA damage
	RecA	General recombination and DNA repair	SOS response
	UmuDC	DNA repair	Radiation and/or chemically induced DNA damage
	Ada	Adaptive response to alkylation	DNA damage, mainly methyl adducts
	SfiA	Inhibitor of cell division	SOS response
	DinD	Unknown function within the DNA damage inducible response	DNA damage

[a] The promoter *MerR* was not considered for further analysis since it strongly and specifically reacts to specific heavy metal ions, i.e. mercury and cadmium, and no such inductions were observed in the dataset.

two plates in accordance to their background and maximum expression level, i.e. one plate receiving the *KatG, MicF, OsmY, UspA, RecA* and *Zwf* fusions, whilst the second plate received the *ClpB, UmuDC, MerR, Ada, DinD, Soi28, Nfo*, and *sfiA* fusions. The assay was performed in triplicate in 96 well plates.

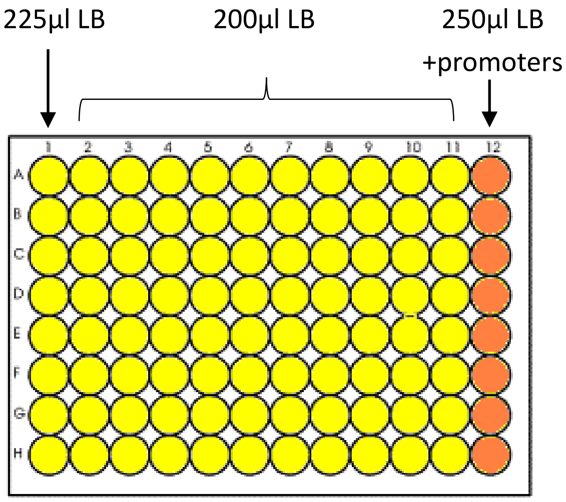


Figure 4-11. A 96-well sterile microtiter plate used for growth, dosing and lysis.

Chemical plates - New plates were prepared containing the compounds to be tested, i.e. 200 µl of all compounds (at a concentration 10 times higher than the highest test concentration) was added - one product per row - in column 11. Columns 2 to 10 received 100 µl of the used solvent (water). Columns 5 to 11 received an increasing concentration of the compound in a 1/2 serial dilution (**Figure 4-12**). Columns 2 to 4 served as negative controls for determining the basal β-galactosidase expression level for every promoter and were therefore only dosed with solvent.

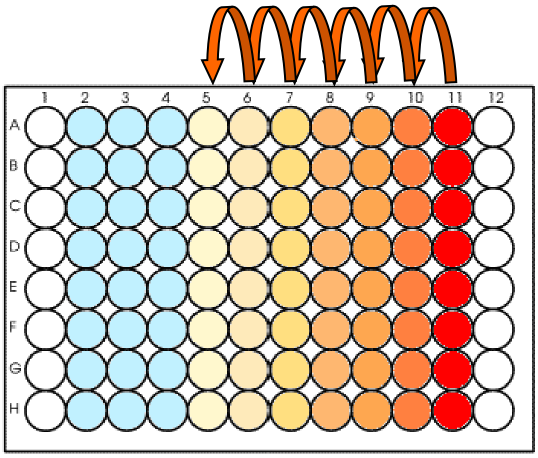


Figure 4-12. A 96-well sterile microtiter plate used for the 1/2 serial dilution.

After 90 minutes of resuscitation (37°C and 200 rpm) the optical density of the bacterial plates containing the *E. coli* strains was measured at 600 nm to check uniformity. Subsequently, these bacterial plates received 25 µl of the solutions present in the chemical plates. After 90 minutes of exposure (37°C and 200 rpm), optical density (600 nm) was measured again and the cells were lysed for β-galactosidase measurement, i.e. 30 µl of a 4% Triton X100, 0.5 mg.ml^{-1} Polymixine B solution was added as a lysing agent. The plates were then returned to the incubator for 15 min (37°C and 100 rpm) to allow lysis. The maximum test concentration used for each compound (**Table 4-6)** was 100 mg.l^{-1}.

B-galactosidase assay

Immediately after the 15 min lysis period, 60 µl of lysate was transferred to assay plates containing 200 µl of reaction buffer [10.70 g.l^{-1} $Na_2HPO_4{\cdot}2H_2O$; 5.50 g.l^{-1} $NaH_2PO_4{\cdot}H_2O$, 0.75 g.l^{-1} KCl; 0.25 g.l^{-1} $MgSO_4{\cdot}7H_2O$ adjusted to pH 7.0 and supplemented with 0.8 mg.ml^{-1} O-nitrophenyl-β-D-galactopyranoside (ONPG)]. All salts in the reaction buffer were purchased from UCB, Belgium. The assay plates were incubated at room temperature for 10 or 30 min for the first and second set of plates, respectively. The hydrolysis of ONPG (colorless) to ONP (O-nitrophenol) (yellow) by β-galactosidase was measured spectrophotometrically at 420 nm and was used as a measure for activity of the promoters. All measurements were performed on Biotek ELx808 spectrophotometers operated through the KC4 software distributed by the same supplier.

Calculations

Activity of the promoter was calculated taking into account the growth inhibition of the used strain. The results are presented as fold inductions at a given dose i, relative to the control values and were calculated through a set of formulas as follows (Nobels et al., 2011):

$$\text{Fold induction}_i = \frac{\text{Activity}_i}{\text{Average Activity}_{\text{negative controls}}}$$

$$\text{Activity}_i = 0.19\left[\frac{OD^{PE}_{420\text{ nm}} - OD^{SE}_{420\text{ nm}}}{\left(OD^{PD}_{600\text{ nm}} \text{ x } 90\text{ min}\right)\left(\left(OD^{PE}_{600\text{ nm}} - OD^{SE}_{600\text{ nm}}\right)\right)\text{x}\,\frac{90\text{ min}}{2}}\right]$$

with i the activity at a given dose, OD the optical density, PE the post exposure, SE the start exposure (= post dose) and PD the pre-dose.

B.2.3 DATA ANALYSIS

B.2.3.1 ANTIFUNGAL ACTIVITY IN VITRO BIOASSAY

A one sample Kolmogorov-Smirnov test was used to assess the normality of the measured fungal colony radius. The Mann-Whitney test was used to compare the differences between the control and treated samples. A p value of < 0.05 was considered statistically significant. Statistical analysis was performed using a statistical software program (SPSS Version 12.0, Chicago, USA).

B.2.3.2 TOXIC MODE OF ACTION

The presented fold inductions are the mean of three independent replicates. Fold inductions were considered significant when the following criteria were met: (a) presence of a concentration response relationship ($R^2 > 0.5$, significant at $p < 0.05$ for six degrees of freedom) and a positive slope different from 0 ($p < 0.05$) in a linear model, and (b) statistically significantly higher signal than the blank (Dunnett's test $p < 0.05$) (Nobels et al., 2010). The different stress responses were grouped into five major classes as defined in **Table 4-7**. The results after exposure to a certain stressor were evaluated starting from the bacterial gene expression profiles that met the statistical criteria as mentioned above. To take into account the dose response curves, the number of significantly induced responses per concentration range was used for each gene (maximum of seven). For each group, the results were represented as percentages of the number of significantly induced responses per concentration range for the relevant genes. A score of 100% stands for an induction at all the tested concentrations for all the appropriate genes.

B.3 RESULTS

B.3.1 ANTIFUNGAL ACTIVITY IN VITRO BIOASSAY

Based on the results obtained by the antifungal activity bioassays for zeolites in Part A of this chapter (Section A.3.1), it was concluded that the formulations might have an influence on the biological performance of the zeolites against the fungi. This was especially the case for the fungus *B. cinerea*. The formulations of zeolites BEA (🟨 🟩) and FAU (🟨 🟩) showed an increased inhibition percentage compared to the non-formulated zeolites BEA and FAU for all tested concentrations. The used adjuvants in these formulations, listed in **Table 4-8**, also show a clear effect on the inhibiton of both *V. inaequalis* as *B. cinerea*. The biggest effect on inhibition was found by Morwet EFW Powder and Rhodorsil, both present in the formulations of zeolites BEA and FAU.

At the concentration of 400 mg.l^{-1} the formulations (🟧 🟦) of zeolite LTA also showed an increased inhibition percentage compared to the non-formulated zeolite LTA. The influence of the used adjuvants became clear for formulation LTA 800 SC (🟧), of which the adjuvants Morwet D425 and Supragil WP obtained a higher inhibition percentage compared to the other adjuvants. However, for formulation LTA 850 WP (🟦), the influence of the used adjuvants was not clearly noticeable. More information about the data analysis is found in **Appendix E** (**Tables E-3** and **E-4**).

Table 4-8. Growth inhibition (± SE) of *Venturia inaequalis* and *Botrytis cinerea* by the adjuvants (**Table 4-7)** compared to the control (n=8).

				Inhibition (%)	
				Venturia inaequalis	***Botrytis cinerea***
			Control	0.00 ± 0.37	0.00 ± 0.18
a	🟨	🟩	Agrilan 789 Dry	22.38 ± 0.20	2.01 ± 0.23
b	🟨	🟩	Morwet EFW Powder	75.68 ± 0.32	73.15 ± 0.23
c	🟨	🟩	Rhodorsil Antim EP	43.49 ± 0.17	30.59 ± 0.69
d	🟨	🟦	PVP K-30	16.09 ± 0.27	8.34 ± 0.93
e	🟨	🟦	Ultrasil VN 3	15.74 ± 0.28	9.27 ± 0.69
f	🟧		Morwet D425	55.08 ± 0.78	80.33 ± 0.20
g	🟧		SAG 1572	12.61 ± 0.32	25.47 ± 1.37
h	🟧		Supragil WP	33.84 ± 0.27	51.90 ± 0.54
i	🟧		Reax 88B	8.03 ± 1.29	12.38 ± 1.01
j	🟧		Rhodopol 23	21.69 ± 0.14	0.75 ± 1.84
k	🟧		Amebact C	2.84 ± 0.18	2.72 ± 0.93
l	🟦		Soprophor	15.21 ± 0.41	26.47 ± 1.41

□ : Classification according to the composition of the formulations (see **Table 4-6**).

B.3.2 TOXIC MODE OF ACTION

Next to the fungal growth inhibition more, information regarding the toxicity of the selected adjuvants was obtained through a bacterial gene profile assay (BGPA) with 14 different toxicologically relevant stress genes. The dose-response profiles (**Figure 4-13**) illustrate inductions comparable to the results obtained with the antifungal activity in vitro bioassays for zeolites (Part A) and the used adjuvants (Part B). Subsequently, the gene expression profiles showed clear dose-dependent responses in a majority of stress genes for all the adjuvants.

Looking at the different zeolite formulations, it can be concluded that the formulations of zeolites BEA and FAU, 850/920/950 WP (🟨 🟩; see **Table 4-6**), were composed of two adjuvants that clearly exhibited toxicity. The dose-response profiles of Morwet EFW Powder and

Rhodorsil Antim EP show visible peaks of some stress promoters. The fold inductions of these adjuvants also showed significant inductions at the highest concentration of 100 mg.l^{-1} (**Figure 4-14**). For Morwet EFW Powder, all stress genes except for MerR and DinD showed significant induction. Rhodorsil EP observed significant inductions of six stress genes. The markers belong to different toxic modes of action, namely oxidative damage (*KatG, Zwf, Nfo*), membrane damage (*MicF, OsmY*) and little DNA damage (*Nfo, RecA*). The other adjuvants present in the formulations of zeolites BEA and FAU had no or little toxic effects. Based on the results, the markers show little or no inductions after exposure to Agrilan 789 DRY (UmuDC, SfiA) and PVP K-30 (-). Gene expression profiles of Ultrasil VN 3 presented six significant induced genes at the highest concentration, namely *KatG, Zwf, OsmY, RecA, UmuDC* and *SfiA*.

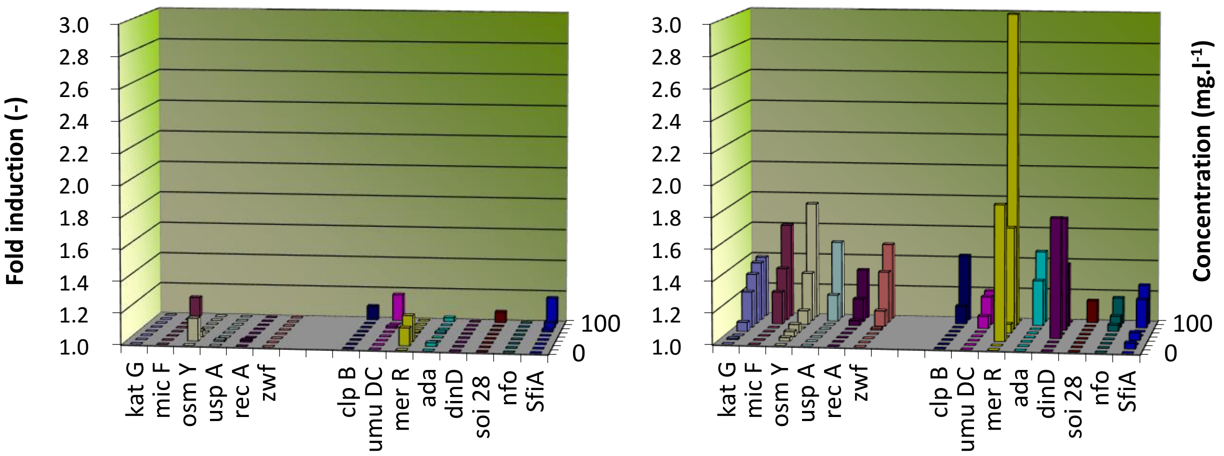
Considering all the above results and the induced stress promoter *uspA*, which especially reacts on cell growth, it can be concluded that the above discussed formulations contain adjuvants that have the potential to influence the antifungal activity bioassays.

The next paragraph discusses the results of the LTA formulations, LTA 800/850 SC/WP (🟧 🟦; see **Table 4-6**). Zeolite formulation LTA 800 SC (🟧) contained the adjuvants Morwet D425, SAG 1572, Supragil WP, Reax 88B, Rhodopol 23 and Amebact C. Only Supragil WP showed toxic effects. Its gene expression profile displayed inductions for most promoters. Looking at the fold inductions in **Figure 4-14**, significant induction was observed for all stress genes, except for *Soi28*, *merR, Ada* and *DinD*. Zeolite formulation LTA 850 WP (🟦), consisting of the adjuvants PVP K-30, Ultrasil VN 3 and Soprphor, exhibited the least toxic effect of all formulations. Nevertheless, Ultrasil VN 3 and Soprophor showed little toxic effects. After exposure to Soprophor FL, significant inductions at 100 mg.l^{-1} were found for genes responding to oxidative stress (Soi28, Nfo), membrane damage (OsmY) and DNA damage (Nfo, Ada, SfiA).

B.4 DISCUSSION

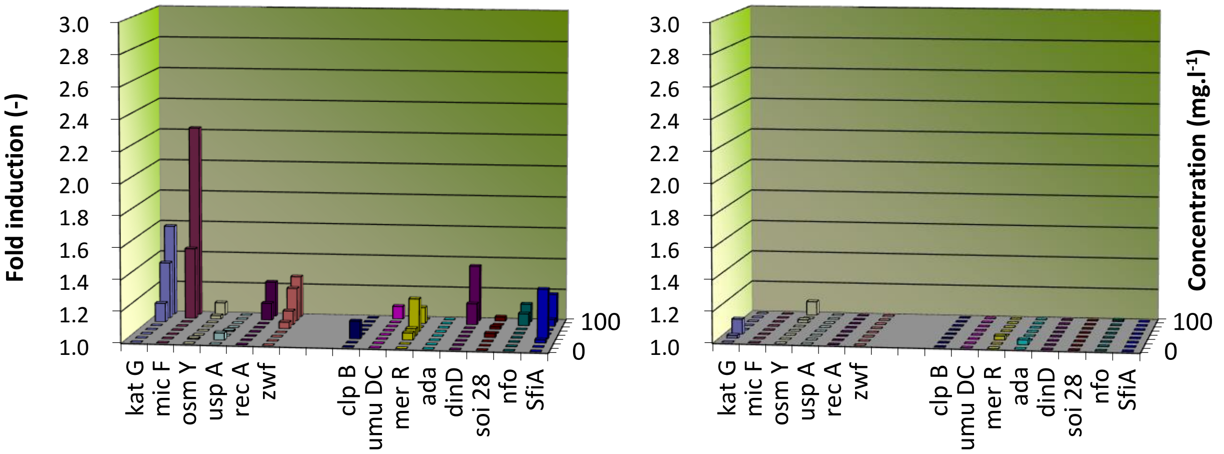
Adjuvants are referred as 'inert ingredients', meaning that they are harmless (US-EPA, 1997). However, this is definitely not the case, i.e. they can be toxic to humans and may have biological activity of its own (Cox and Surgan, 2006; Surgan et al., 2010). This activity of adjuvants is also proven by the current study and in a previous study by Nobels et al. (2011), which also illustrated the importance of reporting toxicity in different ways to characterize the toxicity of a compound.

Based on the results of the antifungal activity bioassays using zeolites as fungicide material, it can be concluded that the used adjuvants in the formulations also have an effect on the fungal growth of *Venturia inaequalis* and *Botrytis cinerea*. In order to examine this possible effect, a similar antifungal activity bioassay was carried out using the adjuvants as fungicide materials.



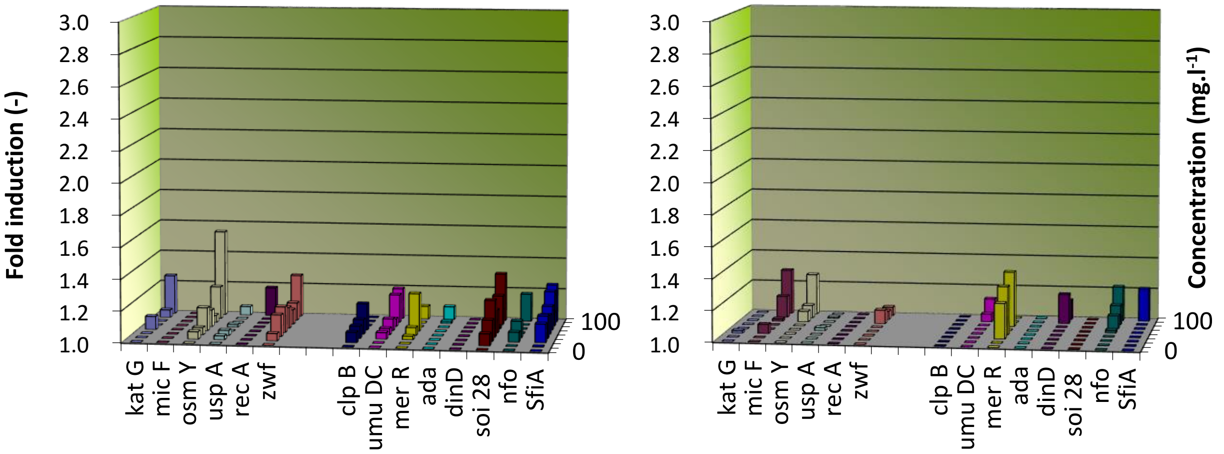
a: Agrilan 789 🟨 🟩

b: Morwet EFW Powder 🟨 🟩



c: Rhodorsil EP 🟨 🟩

d: PVP K-30 🟨 🟦

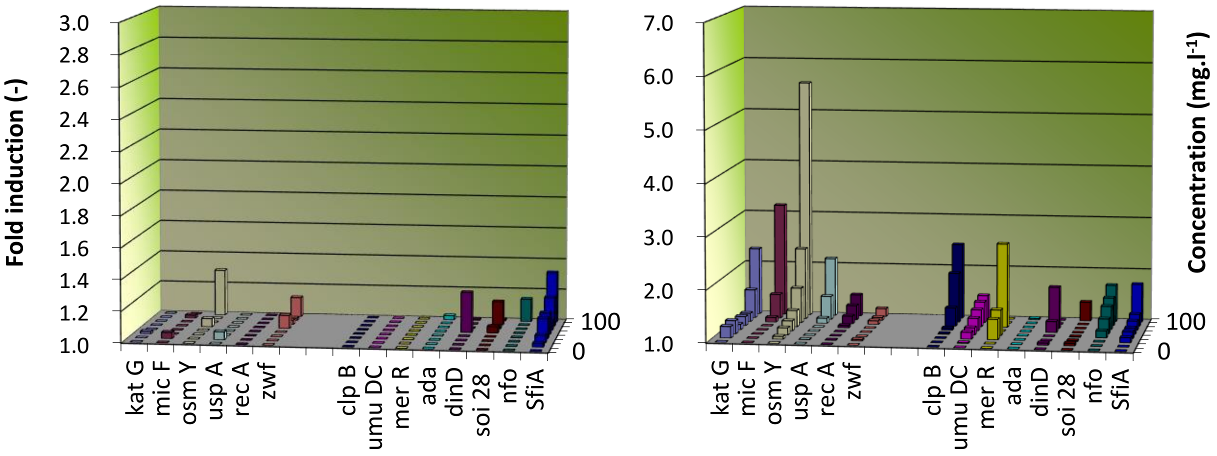


e: Ultrasil VN 3 🟨 🟦

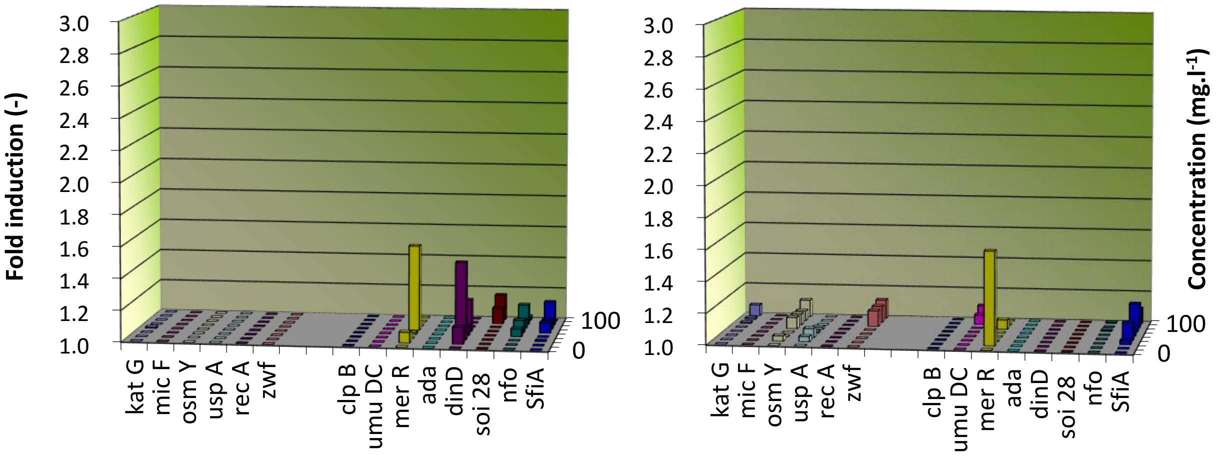
f: Morwet D425 🟥

Figure 4-13. BGPA after exposure to the different adjuvants, using a concentration range of 1.563-100 mg.l^{-1}. ☐ : Classification according to the composition of the formulations (see **Table 4-6**).

g: SAG 1572 🟧 **h: Supragil WP** 🟧



i: Reax 88B 🟧 **j: Rhodopol 23** 🟧



k: Amebact C 🟧 **l: Soprophor** 🟦

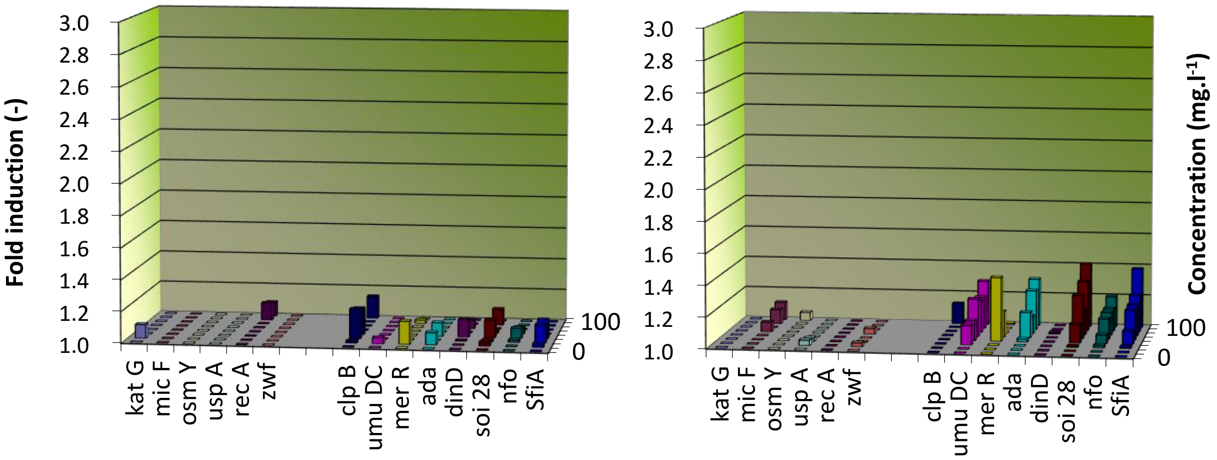


Figure 4-13. BGPA after exposure to the different adjuvants, using a concentration range of 1.563-100 mg.l^{-1}. □ : Classification according to the composition of the formulations (see **Table 4-6**) (continued).

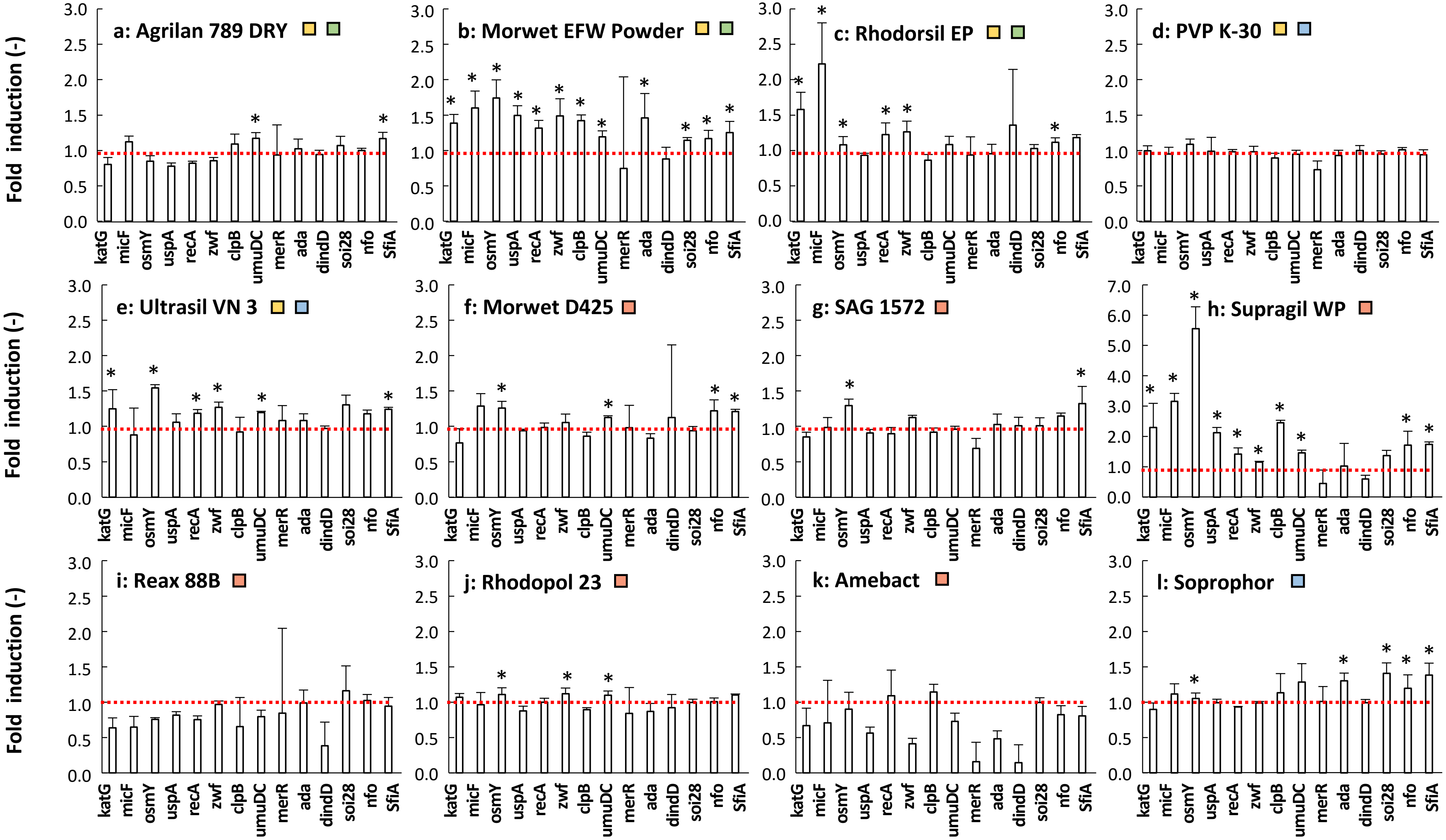


Figure 4-14. Fold inductions (± SD)observed at the highest concentration, i.e. 100 mg.l^{-1}, tested for each compound listed in **Table 4-6**. : Classification according to the composition of the formulations. Red dotted lines represent the fold induction of the control treatment and asterisks indicate significant differences in fold induction between sample and control treatments ($P < 0.05$, $R^2 > 0.5$ and slope > 0).

These results showed a clear dose-response effect of the adjuvants on fungal growth. The adjuvants Morwet EFW and Rhodorsil had an inhibition effect in the BEA and FAU formulations, while Morwet D425 and Supragil WP had an effect in the LTA 800 formulation. In this inhibition bioassay, the influence of the used adjuvants was not clear for formulation LTA 850 WP.

Subsequently, a bacterial multiple endpoint reporter assay was used to evaluate the different adjuvants at the toxic mode of action level. The results of the bacterial gene expression assays were categorised into five major modes of action mechanisms, oxidative stress, membrane damage, general cell lesions, specific stress caused by heavy metals and DNA damage. The results from this study demonstrated clearly at the gene expression level that inductions of stress responsive genes occur for the different adjuvants. Noticeably, membrane damage (*MicF, OsmY*) and DNA damage (*Nfo, RecA, UmuDC, Ada, SfiA, DinD*) were the main toxic modes of action since these genes were significantly induced. It is known that oxidative stress can cause severe cellular damage at different target levels ranging from protein perturbation to DNA damage. Therefore, oxidative stress is usually linked to DNA damage and is of major importance due to its long-term effects (Robbens et al., 2010).

Quantitative gene induction assays offer a bundle of information including not only the nature or identity of the genes induced, but when a dose-response approach is used, also the level of the induction in comparison to the reference system. Since each adjuvant can react differently to the different bacterial strains, no direct comparison was done at equal exposure concentrations between the gene induction profiles. It is also not possible to compare the fold induction values within a single gene expression profile, since the worst biological effect on the *E. coli* cell cannot be determined obviously. Nevertheless, the test clearly demonstrates that the most inductions of stress genes occurred for Morwet EFW Powder and Supragil WP.

Morwet EFW Powder and Supragil WP were the only adjuvants that induced *ClpB*, indicating protein degradation/perturbation. In *E. coli*, the heat shock protein ClpB performs its role by disaggregating and reactivating of insoluble aggregates and thermally aggregated proteins (Zolkiewski, 1999). This activity of *ClpB* is crucial for the ability to survive extreme heat stress in *E. coli* (Sanchez and Lindquist, 1990; Weibezahn et al., 2004). Because *ClpB* is the only known bacterial Hsp100 capable of suppressing and reversing protein aggregation, it is the ideal biomarker for investigating effects on protein integrity (Zolkiewski, 1999).

RecA regulates the SOS response usually as a result of DNA damage in prokaryotes (Smith and Walker, 1998; Diez et al., 2000). It has been proposed that upregulation of *RecA* results in the induction of the growth arrest gene, *UspA* (Diez et al., 2000). These compounds exhibited a tendency to induce DNA damage. The four adjuvants that induced *RecA* were Morwet EFW, Rhodorsil EP, Ultrasil VN 3 and Supragil WP. Only Morwet EFW and Supragil WP showed induction of *UspA* and resulted in DNA damage. It is most likely the result of inducing oxidative damage (Krivoshiev et al., 2015).

It is also interesting to note that *RecA* induction was accompanied by significant inductions in the stress genes *KatG* and *Zwf*. *KatG* encodes for hydrogen peroxidase 1, while *Zwf* encodes for glucose-6-phosphate dehydrogenase (Wolf et al., 1979; Tartaglia et al., 1989). Both are considered markers of oxidative damage through distinct pathways. This Oxidative damage was observed for all four adjuvants that induced the *RecA* stress gene. The results suggest that the compounds *RecA, KatG* and *Zwf* are able to induce DNA damage, most likely as a result of inducing oxidative damage.

The *MicF* gene encodes for an antisense RNA that post-transcriptionally regulates the outer porin protein F (OmpF) in response to stress stimuli. This downregulation of OmpF expression plays a central role in the cell's response to toxic and other environmental factors, since the ability to quickly control the permeability of molecules through the outer membrane is important to cell survival (Delihas and Forst, 2001). Only Morwet EFW, Rhodorsil EP and Supragil WP were able to induce this *MicF* gene. Noticeably, all significant inductions of *MicF* were accompanied by significant induction of *KatG, Zwf*, or both, indicating a general contribution of membrane damage by oxidative damaging-mechanisms. These results are in line with previous studies (Krivoshiev et al., 2015).

Despite the fact that the promoter *MerR* was not considered for further analysis, high fold inductions were noticed, which leads to a distortion. There high values are due to the very high sensitivity of this marker and are negligible in this experiment. It strongly and specifically reacts to specific heavy metal ions, i.e. mercury and cadmium, and no such inductions were observed in the dataset (Nobels et al., 2011).

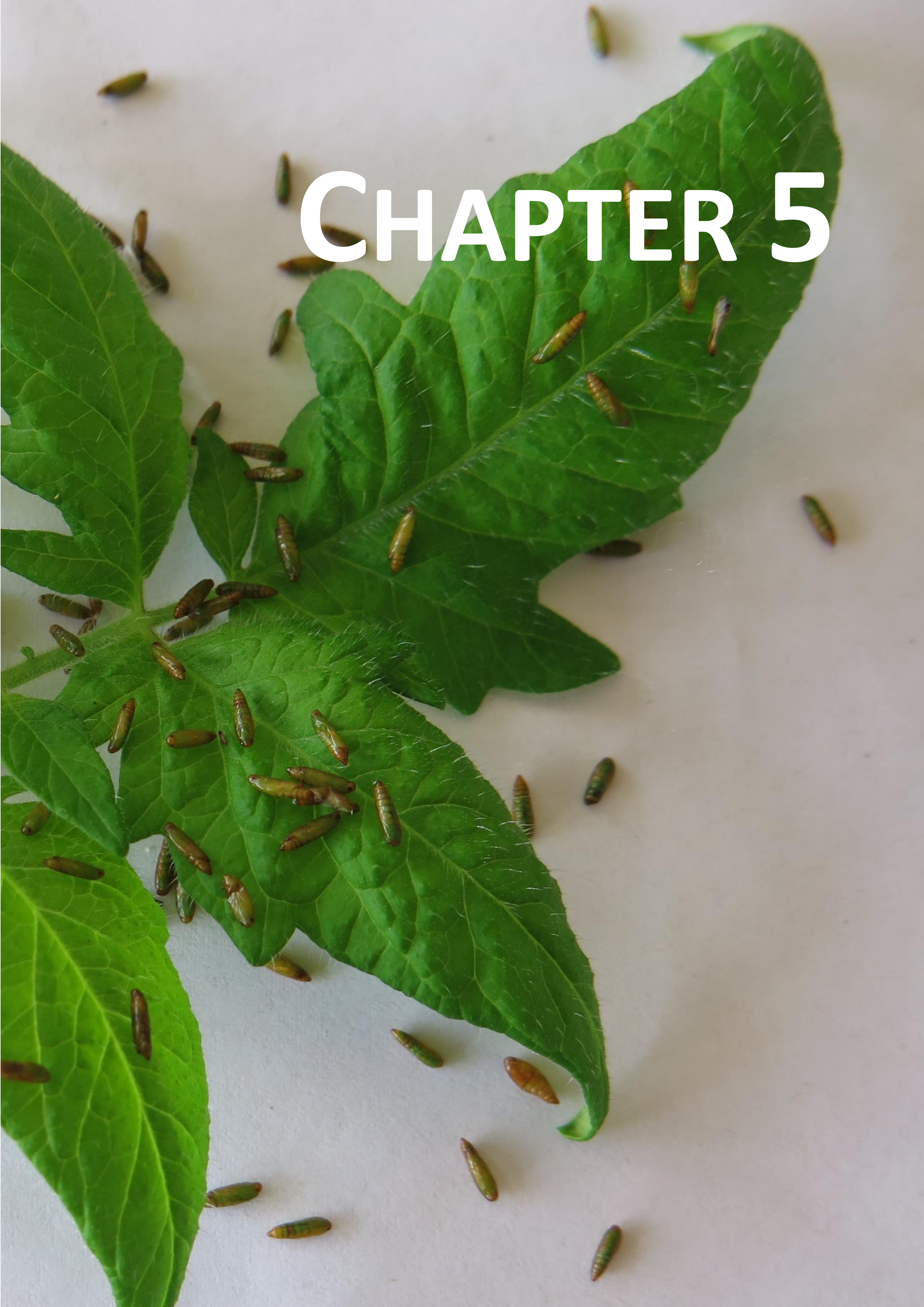
5 GENERAL CONCLUSION

Based on the results in Part A of this chapter, it can be concluded that the tested zeolite products had an antifungal activity and influence the growth of *V. inaequalis* and *B. cinerea*. The tested formulations obtained better results compared to the non-formulated zeolites, which raised the question whether the observed effect was due to the zeolite itself or the adjuvants added to the formulation. In order to answer this question properly, in Part B of this chapter, antifungal activity bioassays were provided for the adjuvants. Additionally, a bacterial multiple endpoint reporter assay with universally stress related endpoints was used to obtain more information about the toxicity and toxic mode of action of the used adjuvants in the zeolite formulations. From the twelve adjuvants used in the formulations, the assay correctly reflected the mode of action in an antifungal way. The results indicated that the tested substances showed toxic effects, suggesting that these adjuvants were responsible for the antifungal activity.

"Look deep into nature, and then you will understand everything better."

-Albert Einstein-

CHAPTER 5



Chapter 5

INSECTICIDAL ACTIVITY OF ZEOLITES

Since zeolites may have the following effects, i.e. dessicate the insect's body, induce lower hatching and oviposition rates, reduce the attractiveness of visual cues, etc., they can be used as a potential insecticide. This chapter focuses on the insecticidal activity of the zeolites on two chewing insects, i.e. *T. absoluta* and *L. decemlineata.*

1 INTRODUCTION

Insects are the most diversified group of Eukaryotes and are highly variable in many traits (Grimaldi and Engel, 2005). They are estimated to consume approximately 10% of all plant biomass produced annually. The exact amount of consumed plant biomass depend on a number of factors, such as the type of vegetation and the geographical location. A further consideration is the mode of feeding of the insect (Walters, 2015; Yoshizawa and Lienhard, 2016).

The types of mouthparts of an insect determine its feeding habits. Two groups can be distinguished, i.e. chewing insects and sucking insects (Sheaffer and moncada, 2009). Chewing insects have mandibles for chewing plant tissues whilst feeding. The damage from chewing insects can be readily seen as root damage, leaf mining, defoliation and boring of tissues. Sucking insects have a piercing mouthpart called a labrum that is used to puncture plant tissue and suck the fluid out of the plant. During feeding, these insects may cause additional damage because they can inject toxins or transmit plant pathogens, such as viruses or bacteria, into the plant (Sheaffer and moncada, 2009).

Insecticides are used to control insect pests. The more immediate pest control remedies that have been used over the years to reduce pests include cultural, chemical, physical, mechanical and biological control and the use of biopesticides (Fiola and Gill, 2011). Conventional insecticides are among the most popular chemical control agents because they are readily available, rapid acting, and highly reliable. A single application may control several different pest species and usually forms a persistent residue that continues to kill insects for hours or even days after application. Because of their convenience and effectiveness, insecticides quickly became standard practice for pest control during the 1960's and 1970's. Overuse, misuse, and abuse of these chemicals have led to widespread criticism of chemical control and, in a few cases, resulted in long-term environmental consequences (Philips, 2010). Alternative methods are needed in order to control the insect pests.

One of the alternatives is the use of zeolites as an insecticide. The use of zeolites as a biofilm matrix for controlling arthropod pests has not been sufficiently addressed in the literature. However, as mentioned in Chapter 1, similar physical control methods based on the use of kaolin have already been described. Physical controls are methods that physically keep insect pests from reaching their hosts. Kaolin acts as a physical barrier preventing insects from reaching the plant. It also acts as a repellent by creating an unsuitable surface for feeding or egg-laying. The dried clay particles can act as an irritant to the insect and can trigger an excessive grooming response. While there are other physical methods to remove pests, such as vacuuming, squashing or trapping them, none of these are very effective and are usually too time consuming or expensive on a large scale to be recommended (Brust, 2016).

Subsequently, zeolites will be used as contact insecticides on plants. Based on the fact that these zeolites also can have an abrasive effect, it was assumed that they could damage the mouthparts (mandibles) of chewing insects. This assumption lead to the assessment of the insecticidal effect of zeolites on two chewing insects, i.e. *Tuta absoluta* and *Leptinotarsa decemlineata*, in the current thesis.

Nowadays, biological control methods based on the predators *Nesidiocoris tenuis* (Hemiptera: Miridae) and *Macrolophus pygmaeus* (Hemiptera: Miridae) can be used to regulate the *T. absoluta* population (Urbaneja et al., 2009). Alternative control methods based on the use of insect's sex pheromones have also been developed for this purpose (Cocco et al., 2012; Guedes and Picanço, 2012; Megido et al., 2013). Nevertheless, in many countries (South America, Italy, Spain, etc.) chemical control methods are still considered as the main method to control *T. absoluta*. In order to decrease the damage caused by *T. absoluta*, horticultural growers applied insecticides (Moreno et al., 2012; Urbaneja et al., 2012). These chemical products were applied more than twice a week during a single cultivation period, which resulted not only in food and environmental contamination, but also in higher production costs and reduction of natural enemies of the pest (Filho et al., 2000). Furthermore, extensive use of insecticides led to the development of resistance in insect populations. An increase of developed resistance in *T. absoluta* populations was also reported in South America (Siqueira et al., 2000, 2001; Lietti et al., 2005; Urbaneja et al., 2012). These studies have illustrated that *T. absoluta* developed resistance to many classes of insecticides when resistance management strategies were not properly established. Accordingly, high risks were involved in the use of insecticides based on spinosyns, one of the few classes of insecticides still effective against *T. absoluta* in South America (Silva et al., 2011; Gontijo et al., 2013). Campos et al. (2015) detected already low levels of resistance of *T. absoluta* to spinosad.

Currently, diamides are also used against lepidopteran pests. Diamides are a new group of insecticides that have been classified as ryanodine receptor modulators (IRAC, 2014). However, after several years of field applications, cases of resistance development to diamides have been reported for some lepidopteran species. Especially in Italy, very high resistance levels to diamides were clearly detected (Roditakis et al., 2015). Subsequently, the introduction of the European Directive on the sustainable use of PPPs (2009/128/EC) requires that all professional users of PPPs follow the general principles of integrated pest management (IPM). Thus, the implementation of environmentally safe alternatives, reducing the use of chemicals, should contribute to the sustainability of tomato production.

The Colorado potato beetle (CPB) is the principal insect pest of potatoes in North America and Europe, and also well known for the high level of resistance to many insecticide classes (Casagrande, 1987; Weber and Ferro, 1994; Weber, 2003; Alyokhin, 2009). Many methods have been used to control CPB, including hand-picking, bird predation, introduction of natural enemies, trapping, border sprays, trench traps, propane flamers, crop rotation and resistant varieties containing glycoalkaloids and toxin genes from *Bacillus thuringiensis* (Mota-Sanchez et al., 2006). However, these methods have not been widely effective and growers still depend primarily on the use of chemical insecticides for CPB management (Casagrande, 1987). Due to this intensive use and the adaptability of CPB, whole classes of insecticides have failed because of the development of resistance (Bishop and Grafius, 1996). Since the introduction of dichlorodiphenyltrichloroethane (DDT), CPB resistance has followed a familiar pattern: new chemistries provided good to excellent initial control, but the CPB developed resistance within 1-3 years and newer compounds had to be introduced soon thereafter. The beetle has developed resistance to virtually every insecticide used for its control (Forgas, 1985). Currently, there are 42 active ingredients across several classes, including organophosphates, carbamates and pyrethroids, with reported resistance in CPB, which makes the CPB to be ranked among the top ten most resistant species to insecticides in the world (Mota-Sanchez et al., 2002; Whalon et al., 2005).

In addition to the applied product, insecticide susceptibility also varies during the life stage of an insect (Tomé et al., 2012). Although the egg stage is often perceived as the most vulnerable stage, it is a difficult target for insecticide application because of the sessile condition of the eggs at concealed sites (Beament, 1952; Smith and Salkeld, 1966; Martin et al., 2010; Koppel et al., 2011). In addition, the egg structure and physiology protect the developing embryos and minimises insecticide penetration (Beament, 1952; Zschintzsch et al., 1965; Koppel et al., 2011). The insect eggshell is a compound set of envelopes, remarkably effective in providing the oocyte protection against possible disadvantageous environmental influences like desiccation, water loss, bacterial infection and physical destruction. On the other hand, the eggshell enables gas exchange and maintenance of the water balance (Furneaux and Mackay, 1976; Al-Dosary et al., 2010).

In the current thesis, the insecticidal effect of zeolites on *T. absoluta* and *L. decemlineata* was assessed. The treatments on *T. absoluta* were mainly targeted against eggs and larvae. Ovicidal and larvicidal properties were studied by spraying the eggs directly (topically) or by treating leaves before oviposition (residually). An additional experiment was performed to assess whether the used products had repellent or attractant properties to adult females. This oviposition behaviour of females was determined by choice tests. Finally, a small bioassay was performed to determine the effect of the non-formulated zeolite LTA on the Colorado beetle *L. decemlineata*. Indicative toxicity and growth inhibition tests on larvae were carried out.

1.1 TUTA ABSOLUTA

Tuta absoluta (Lepidoptera: Gelechiidae), known as the South American tomato leafminer or pinworm, is one of the most devastating pests for tomato crops (Solanum lycopersicum L.), both in greenhouse and open field locations in different parts of the world (Moreno et al., 2012). It originated from South America and was first described in Peru in 1917 as *Phthorimaea absoluta* (Meyrick, 1917) (Desneux et al., 2010). Recently, *T. absoluta* has also become a serious threat to tomato production in the Mediterranean region (Seplyarsky et al., 2010). In Europe, this pest was first detected at the end of 2006 in the northern part of Castellón de la Plana in eastern Spain (Urbaneja et al., 2009). Since then, it has rapidly invaded other European countries and spread throughout the Mediterranean basin, including parts of North Africa and the Middle East where it immediately reached damaging levels on tomato production (Desneux et al., 2010, 2011; Kiliç, 2010; Tropea Garzia et al., 2012).

This pest attacks leaves, flowers, stems and especially fruits at any developmental stage, from seedlings to mature plants. In the absence of control strategies, fruit damage can reach 100% (Desneux et al., 2010). The damage is caused by the larvae mining the leaves and often also the tomato fruits. The larvae feed on the mesophyll, which affects the photosynthetic capacity of the crop, decreases the production and makes the tomatoes unsuitable for the market (Urbaneja et al., 2012; Van Damme et al., 2014).

1.1.1 TAXONOMY

The shortened taxonomic classification of the *Tuta absoluta* is presented in **Table 5-1**.

Table 5-1. Short taxonomy of *Tuta absoluta* (USDA, 2011).

Taxonomy of *Tuta absoluta*	
Kingdom	Animalia
Phylum	Arthropoda
Class	Insecta
Order	Lepidoptera
Family	Gelechiidae
Genus	Tuta
Species	*Tuta absoluta*
Preferred common name	tomato leafminer

1.1.2 LIFE CYCLE

During the life cycle, *T. absoluta* goes through four stages: egg, larva, pupa, and adult (**Figure 5-1**). The duration of these stages is directly related to the diet throughout development and environmental factors such as temperature.

Eggs: Eggs of the tomato leafminer are cylindrically shaped. They measure about 0.38 mm long by 0.21 mm wide. Newly laid eggs are creamy white and turn yellow to yellow-orange during development. As the embryo develops, eggs turn dark and the outline of the larval head capsule can be seen through the chorion; this is called the blackhead stage. Egg hatching takes place 4-6 days after egg lying (USDA, 2011).

Lavae: After hatching, young larvae penetrate leaves, aerial fruits (like tomato) or stems, on which they feed and develop. The tomato moth has four larval stages, well defined and different in size and colour (Desneux et al., 2010). The first two larval instar correspond to the critical phase of the species, which has a high mortality rate (79.8%), mainly due to predators and chemical control. The larval period is the most damaging period which is completed within 12-15 days.

The body of the larvae, which is cylindrical or slightly dorsoventrally flattened, and their head capsule diameter are the best characteristics to differentiate between larval instars. The classification of the different instar larvae, based on body length and head capsule diameter, is shown in **Table 5-2**. As mentioned above, their colour changes during development from creamy white to deep green. The last instar takes on a pinkish coloration.

Table 5-2. Larval measurements for *Tuta absoluta* (Vargas, 1970).

Instar	Body length (mm)		Head capsule diameter (mm)		Number of specimens
	Mean	Range	Mean	Range	
1	1.61	1.40-1.90	0.153	0.15-0.18	44
2	2.80	2.45-3.10	0.253	0.24-0.28	37
3	4.69	3.85-5.65	0.399	0.35-0.43	53
4	7.72	5.50-9.20	0.834	0.70-0.98	37

In tomato plants, young larvae can mine leaves, stems, shoots, flowers, and developing fruit; later instars can attack mature fruit (Vargas, 1970). As the larva develops and feeds, larval mines increase in length and width. In case of a severe attack, all leaf tissue is consumed leaving behind a skeletonized leaf and large amounts of frass (fecal pellets). These larvae also spin silk shelters in leaves or tie leaves together (Vargas, 1970).

When larvae are ready to moult they stop eating and purge their stomach contents, causing their coloration to return to creamy white. Fully-fed larvae usually drop to the ground on a silk thread and pupate in the soil, although pupation may also occur on leaves (Desneux et al., 2010).

Pupae: Pupation may take place in the soil, on the leaf surface or within mines, depending on environmental conditions. When *T. absoluta* does not pupate in the soil, a cocoon is usually built. Newly formed pupae are greenish and turn dark brown as they mature. Pupae have a cylindrical shape that is wider at the rear than at the front end. Its dimensions are about 5 to 6 mm long and 1 to 2 mm width. Male pupae are lighter (3.04 ± 0.49 mg) and smaller (length 4.27 ± 0.24 mm and width 1.23 ± 0.08 mm) than female pupae (4.67 ± 0.23 mg; 4.67 ± 0.23 mm and 1.37 ± 0.07 mm) (Fernandez and Montagne, 1990). After 8-15 days adults emerge fully formed.

Adults: Adults are micro Lepidopteran about 6-7 mm long. Their wings are narrow and long and they have long and filiform antennae. The moth has an indistinct wing pattern, being a variable mottled grey, brown and cream colour. There is no obvious sexual dimorphism, although males have a grey belly, while females have a creamy white belly. Females are also wider and more voluminous than the males. The wing span of females is 9.0 to 13.0 mm while that of males is 8.5 to 12.0 mm. The sex ratio of males and females is 1 to 3.

Tuta absoluta mainly tends to have nocturnal habits, and adults usually remain hidden during the day. This pest has a high reproductive potential and there may be 10-12 generations per year. The first mating usually occurs the day after adults emerge. The oviposition period lasts an average of 4 days and the average number of eggs per female is 52. Females usually lay eggs on the underside of leaves or stems, and to a lesser extent on fruits. A single female can lay a total of about 260 eggs during her lifetime. Adult males live longer than females. In the laboratory, mated males lived 26.47 ± 7.89 days while virgin males lived 36.17 ± 6.55 days. Mated females lived 23.24 ± 5.89 days while virgin females lived 27.81 ± 10.78 days (Fernandez and Montagne, 1990). Both genders mate multiple times.

The total life cycle is completed within 29-38 days, depending on environmental conditions. Studies have shown that development takes 76.3 days at 14°C, 39.8 at 19.7°C and 23.8 at 27.1°C (Barrientos et al., 1998).

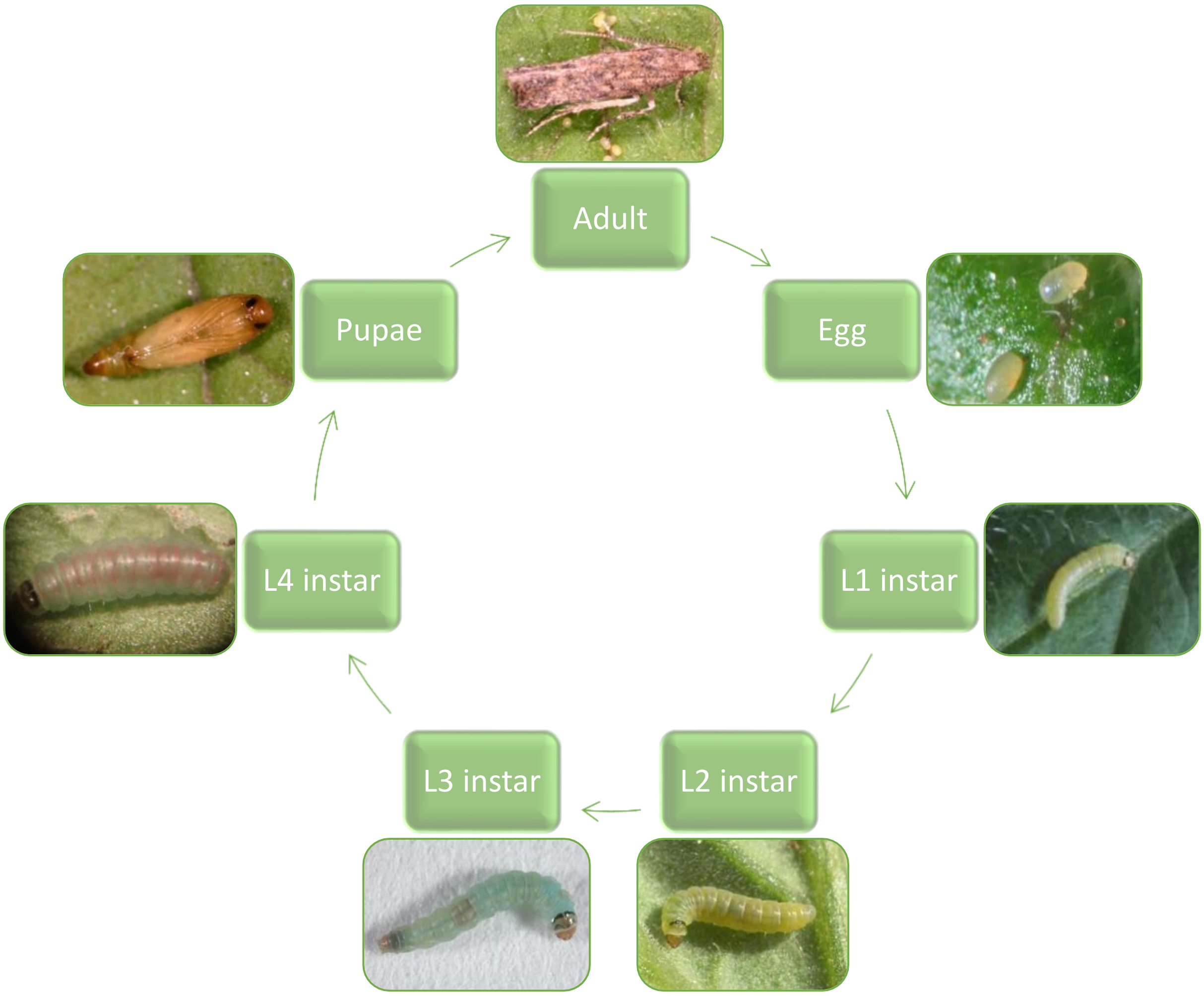


Figure 5-1. *Tuta absoluta* life cycle (Arnó and Gabarra, 2010).

1.2 LEPTINOTARSA DECEMLINEATA

Leptinotarsa decemlineata (Say) (Coleoptera: Chrysomelidae) is a phytophagous beetle that feeds predominantly on *Solanum tuberosum* (L.), better known as the potato plant. The yellow-orange beetle with ten black stripes on the elytron is generally present in all potato-growing areas (EPPO, 2004). The CPB was first discovered in the U.S. in 1811 by Thomas Nuttal and subsequently described in 1824 by Thomas Say. These wild specimens, collected in the Rocky Mountains, feed mostly on buffalo bur, *Solanum rostratum*. The insect's association with the potato plant, *Solanum tuberosum* (L.), was not known before 1859 when it began destroying potato crops about 100 miles west of Omaha, Nebraska. The insect began its rapid spread eastward, reaching the Atlantic Coast by 1874 (Jacques, 1985). In 1877 the first European population of CPBs was discovered in Germany. By the end of the 20th century, the pest had become a problem all over Europe, Asia and Iran (Jolivet, 1991; Weber, 2003).

The evolution of the name Colorado potato beetle is curious because the origin of the beetle is believed to be in central Mexico, not Colorado. However, Walsh (1865) observed a considerable beetle population feeding on *S. rostratum* in Colorado, which resulted into the generally accepted common name 'Colorado potato beetle'.

The CPB is considered to be the most important insect defoliator of potatoes. Both adults and larvae devour entire leaves without discriminating among leaf tissues. Approximately 40 cm^2 of potato leaves are consumed by a single beetle during the larval stage (Ferro et al., 1985; Logan et al., 1985), and close to 10 cm^2 of foliage per day are consumed during the adult stage (Ferro et al., 1985). Once the foliage is gone, beetles can feed on stems and tubers. Colorado potato beetle is very prolific, with one female laying 300-800 eggs. If left uncontrolled, the beetles can completely destroy potato crops (Alyokhin, 2009).

1.2.1 TAXONOMY

The shortened taxonomic classification of the *Leptinotarsa decemlineata* is presented in **Table 5-3**.

Table 5-3. Short taxonomy of *Leptinotarsa decemlineata* (EPPO, 2004).

Taxonomy of *Leptinotarsa decemlineata*	
Kingdom	Animalia
Phylum	Arthropoda
Class	Insecta
Order	Coleoptera
Family	Chrysomelidae
Genus	Leptinotarsa
Species	*Leptinotarsa decemlineata*
Preferred common name	Colorado beetle

1.2.2 LIFE CYCLE

The complete life cycle of *L. decemlineata* consists of four stages: egg stage, larva, pupa and adult (**Figure 5-2**).

Eggs: Following the copulation, the yellow-orange eggs are deposited on the underside of plant leaves in batches of 20-30. This is mainly on potato or other crop plants, nearby weeds or sometimes directly on the soil. The eggs have a typical elongate-oval shape with a length of 1.5 mm and a width of 0.7 mm.

Larvae: The larvae of *L. decemlineata* have a large red arched abdomen with black spots on both flanks. The abdomen consists of nine segments. The last segment (posterior) has a tubelike structure that has an adhesive function. The colour and size of the head and the pronotum, a sclerotized structure that is situated above the first pair of legs, are the main characteristics to distinguish between the different developmental stages. In **Table 5-4**, an identification key is given to recognize a specific larval stage.

Table 5-4. Larval measurements for *Leptinotarsa decemlineata* (Rombouts, 2011).

Character	(top view)	(top view)	(side view)
			
Larval instar	**Head width (mm)**	**Pronotum**	**Average body length (mm)**
1	0.6		1.5
2	1.0		3.0
3	1.6		5.0
4	2.4		8.0

When the larvae emerge from their eggs, they are called neonates. They are about 1.5 mm in length with a black head and pronotum. In this first larval stage, they stay mostly around the egg mass and cause little damage to the plant. The second-instar larvae are about 3 mm in length with a small head and black pronotum. They are dispersed on the plant, but still cause only small damage. In the third larval stage, the larvae are about 5 mm long. The head is clearly bigger and the pronotum is not full black. The larvae eat non-stop and cause severe damage. The fourth-instar larvae are about 8 mm in length. This stage is clearly recognizable, because the pronotum is more orange than black. These larvae, together with the third-instar larvae, do significant damage to the plant leaves.

The time it takes an organism to complete a larval stage is dependent on the temperature (optimal temperature: 30°C) (EPPO, 2004). It varies from 2.5-4 days for the first 3 stages and the last stage can take up to 9 days (Boiteau and Blanc, 1992; EPPO, 2004).

Pupae: At the end of the fourth-instar stage, the larvae burrow themselves into the soil. The pre-pupal stage can take as long as the fourth-instar stage. The larvae are called pupae as soon as a moult encapsulates them. This stage is called the metamorphosis and takes 5-7 days before the transformation into adults takes place. In this stage, the sex of the beetle can be determined (Boiteau and Blanc, 1992).

Adults: After the metamorphosis, adult beetles emerge from the soil. Their body is about 10 mm in length. The elytron shows a characteristic pattern with 10 black stripes on a yellow background. In the adult stage, the beetles cause severe damage to plants. Copulation and subsequent deposition of eggs is their most important occupation all year round, except for the winter. The adult beetles hibernate in the soil down to 10 cm under the surface. When temperature conditions get better (68 days a temperature above 10.5°C) they emerge from the soil, feed and deposit their eggs on the plants (EPPO, 2004).

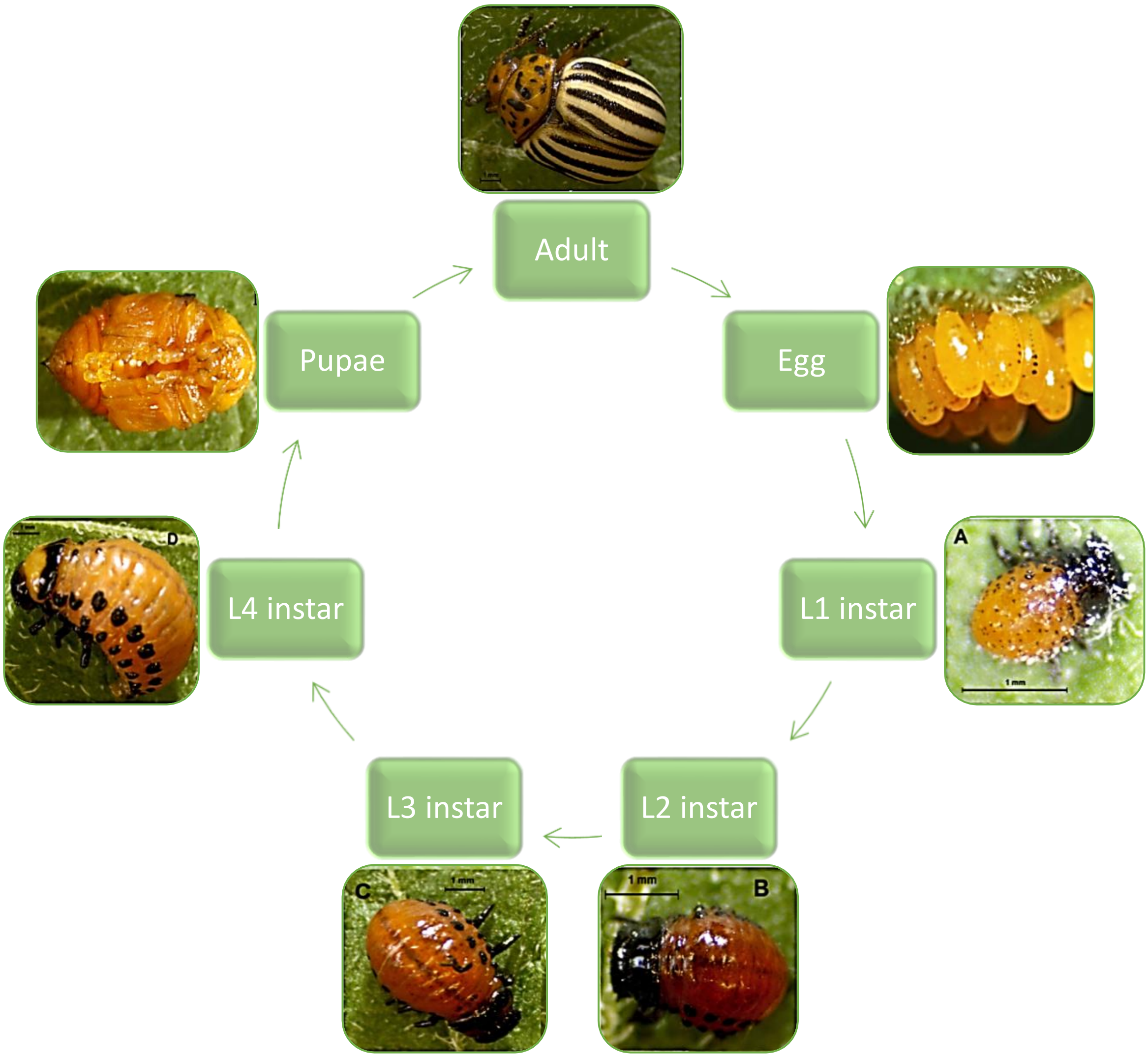


Figure 5-2. *Leptinotarsa decemlineata* life cycle (Rombouts, 2011).

2 MATERIALS AND METHODS

2.1 *TUTA ABSOLUTA*

2.1.1 PLANTS

Tomato seedlings (*Solanum lycopersicon* L. cv. Madison F1) were obtained from the company BPK Duffel N.V. (Duffel, Belgium) and placed in a greenhouse at 25 ± 1°C, 65 ± 15% RH and 16:8 (L:D)h.

2.1.2 INSECTS

Eggs of *T. absoluta* were obtained from a laboratory colony maintained on tomato plants in controlled conditions (25 ± 1°C, 65 ± 15% RH, 16:8 (L:D)h) at the Institute for Agricultural and Fisheries Research (ILVO) in Merelbeke, Belgium. This colony was started in 2011 with individuals collected in commercial fields of an organic tomato cultivation in Sint-Martens-Lennik, Belgium.

2.1.3 INSECTICIDE MATERIALS

The three zeolite types, BEA, FAU and LTA, together with two formulations of each type were compared with a commercial insecticide and a control treatment. Additionally, two biological equivalents of the zeolites, namely kaolin and its commercial formulation 'Surround' (Tessenderlo Kerley, US) were taken into account (**Table 5-5)**.

Table 5-5. Insecticide materials used in this study.

Treatment	Material name	Formulation type	Manufacturers
Z-1	BEA	Technical product	Clariant
Z-2	BEA 850[a]	WP	Fitofarmacia
Z-3	BEA 950[b]	WP	Fitofarmacia
Z-4	FAU	Technical product	Zeolyst
Z-5	FAU 850[a]	WP	Fitofarmacia
Z-6	FAU 920[b]	WP	Fitofarmacia
Z-7	LTA	Technical product	FMC
Z-8	LTA 800	SC	Fitofarmacia
Z-9	LTA 850	WP	Fitofarmacia
C-1	Spinosad (Conserve Pro)	SC	Dow Agrosciences B.V.
B-1	Kaolin	Technical product	Sigma Aldrich
B-2	Kaolin (Surround)	WP	Tessenderlo Group

[a, b] The formulations with a similar letter contain the same adjuvants.

2.1.4 TOPICAL AND RESIDUAL BIOASSAYS

To evaluate the toxicity of zeolites against *T. absoluta* eggs and larvae hatched from these eggs, a test was designed, based on topical and residual exposure bioassays. For the topical exposure bioassays, eggs were collected from tomato leaves in the laboratory colony after 24 hours of oviposition and gently transferred to tomato leaf discs. To keep the tomato leaf discs turgid for more than one week, they were deposited on top of an agar layer (1.5% concentration) in petri dishes of 35 mm diameter. Subsequently, the test products (**Table 5-5**) were sprayed on the leaf discs with eggs in the open dishes. A fine nozzle sprayer, connected to a 100 ml erlenmeyer, was used at a pressure of 1 bar. The spray was applied for 5s until the leaf discs became uniformly moist. The residual exposure bioassays followed a similar protocol, with the exception that the eggs were placed on the tomato leaf discs 24 hours after spraying. For both topical and residual bioassays, three concentrations were tested, i.e. all test products were dispersed in distilled water to obtain concentrations of 400, 4000 and 20000 mg.l^{-1}. Control treatments consisting of distilled water were also included. There were eight replicates per treatment and one replicate consisted of one leaf disc with 5 eggs. All treated petri dishes were kept in controlled conditions (25 ± 1°C, 65 ± 15% RH, 16:8 (L:D)h) until they were evaluated. Egg mortality was determined by scoring the number of non-hatched eggs 9 days after treatment and larval survival was determined by scoring the number of living larvae in the leaf discs 9 days after treatment.

2.1.5 CHOICE TESTS FOR OVIPOSITION BEHAVIOUR

Oviposition site preference of *T. absoluta* female adults was studied by using a simultaneous choice arrangement. For these tests, groups of 5 adult females and 5 adult males (distinguished using morphological characteristics of pupae under a stereomicroscope) were released within a cage (30 x 30 x 30 cm) and allowed to mate (van der Walt et al., 2008). After 24 hours, two plants were placed inside the cage, i.e. one that had been exposed to one of the test products (**Table 5-5**) and one untreated plant. Three concentrations were tested, i.e. all test products were dispersed in distilled water to obtain concentrations of 400, 4000 and 20000 mg.l^{-1}. Control treatments, i.e. a cage containing two untreated plants, were also included. Each product was tested in four separate cages.

The experiment was completely randomized. After 24 hours, the number of eggs deposited in each plant was counted to assess the oviposition preference of the *T. absoluta* females. This short time period was chosen to exclude the possibility that suitable oviposition sites would become saturated during the course of the experiment.

2.2 *LEPTINOTARSA DECEMLINEATA*

2.2.1 PLANTS

Potato plants (*Solanum tuberosum* L. cv. Bintje) were grown in a small greenhouse compartment (2 x 2.5 x 4m) at the faculty of Bioscience Engineering at Ghent University, Belgium.

2.2.2 INSECTS

Eggs of *L. decemlineata* were obtained from a laboratory colony maintained on potato plants in controlled conditions (25 ± 1°C, 65 ± 15% RH, 16:8 (L:D)h) at the laboratory of agrozoology at Ghent University in Ghent, Belgium. Every other day, potato leaves containing deposited eggs were placed in a petri dish to hatch after 4-9 days. These eggs were laid by the female beetles at the backside of the leaves in clusters of 30 eggs.

2.2.3 INSECTICIDE MATERIAL

The non-formulated zeolite 4A of type LTA (FMC, Spain) was selected for the insecticidal bioassays and growth-inhibitory assays against *L. decemlineata*.

2.2.4 INSECTICIDAL AND GROWTH-INHIBITORY BIOASSAYS

First-instar larvae of *L. decemlineata* emerging from the eggs (also called neonates) were put on potato leaves treated with zeolite LTA. Three concentrations were tested, i.e. zeolite LTA was dispersed in distilled water to obtain concentrations of 10, 1000 and 10000 mg.l^{-1}. Control treatments, consisting of distilled water, were also included. An amount of 0.1% of a spreader sticker (Tween 20) was added to all solutions.

The potato leaves were sprayed until run-off with a manual commercial sprayer. After drying, the leaves were placed in petri dishes, i.e. three replicates for each treatment and control. Each replicate contained ten larvae. The experiments were performed in a growth chamber at 25 ± 1°C, 65 ± 15% RH and 16:8 (L:D)h.

The larvae were weighted before and after the experiment. Larval growth inhibition and mortality were scored after 8 days of continuous feeding on treated leaves, assayed relative to the control.

2.3 DATA ANALYSIS

Normality of the data was first tested using the Kolmogorov-Smirnov test. When a Levene's test indicated homoscedasticity, the means were separated using Tukey's multiple comparison test to identify which treatments were significantly different. If the conditions for ANOVA were not satisfied, the non-parametric Mann-Whitney test was used, followed by multiple comparisons of mean ranks for all groups.

Mortality data obtained from concentration-response bioassays were corrected by the mortality observed in the control treatment (Abbott, 1925) using the formula:

$$\text{Corrected mortality (\%)} = \frac{P - P_0}{100 - P_0} \cdot 100$$

where P is the percent mortality of treated insects and P_0 is the percent mortality of insects in the control treatment. This adjusted value is permissible when the mortality in the control treatment does not exceed 20 percent or when mortality data are based on a sufficiently large number of replications.

The mortality values were analysed using a four-way analysis of variance (ANOVA) with the following factors: product, dose, life stage and application method. When a Levene's test indicated homoscedasticity, the means were separated using Tukey's multiple comparison test to identify which treatments were significantly different. If the conditions for ANOVA were not satisfied, the nonparametric Mann-Whitney test was used, followed by multiple comparisons of mean ranks for all groups.

The oviposition activity of adult females was expressed by an oviposition activity index (OAI) calculated using the formula:

$$\text{OAI} = \frac{N_t - N_c}{N_t + N_c}$$

where N_t is the number of eggs laid on plants exposed to the test solution and N_c is the number of eggs laid in control treatment (Kramer and Mulla, 1979). The OAI ranges from -1 to +1, meaning that 0 indicates a neutral response, a negative value indicates deterrence and a positive value indicates a stimulant effect. A Kolmogorov-Smirnov was used to indicate the normality. A Student's t-test ($P < 0.05$) was used to test for significant differences between the number of eggs laid on the treated and the control plants.

The individual larval weight was determined for each treatment and control, which made it possible to calculate the growth inhibition as follows:

$$\text{Growth inhibition (\%)} = \frac{C_L - T_L}{C_L} . 100$$

where T_L is the larval weight gain of the treated larvae and C_L is the larval weight gain of the larvae in the control treatment.

3 RESULTS

3.1 *TUTA ABSOLUTA*

3.1.1 TOPICAL AND RESIDUAL BIOASSAYS

Results from bioassays testing the effects of topical and residual exposure are shown in **Figure 5-3**. The mortality registered in the control treatments was lower than 10% in all assays (**Appendix F**, **Tables F-1** and **F-2**). Therefore, the corrected mortality was used to present the results.

In general, and particularly in the topical application method, a trend was visible with higher mortality values for the formulated zeolites as compared to the mortality values for the non-formulated zeolites. Comparing the different types of zeolites, zeolite FAU (Z-4, 5, 6) resulted in the highest egg mortality (ovicidal effect), followed by zeolite BEA (Z-1, 2, 3) and zeolite LTA (Z-7, 8, 9). Zeolite FAU (Z-4, 5, 6) showed high mortality rates of eggs + larvae. Despite the low egg mortality of the zeolite LTA formulations (Z-8, 9) in the topical application method, the egg + larval mortality of these zeolite formulations yielded better results compared to the formulated products of the other zeolites BEA and FAU. This indicates a more larvicidal effect of the LTA formulations. However, all these observations were not significantly different.

Despite no significant differences between the zeolite products, it can be deduced that the outcome of this bioassay was affected by the application method. To verify this statement, a four-way analysis of variance (ANOVA) was performed (**Table 5-6**).

No four- or three-factorial interactions were observed between the factors dose, application method, product and insect life stage for the mortality of eggs and eggs + larvae. However, significant interactions ($P < 0.05$) were obtained for application method x product and application method x life stage, which indicates that the product and the life stage influenced the effect of the application method on the mortality of *T. absoluta*. The main factors application method ($P < 0.01$) and life stage ($P < 0.001$) also had a significant impact on the mortality. Therefore, the data was split and analysed separately (**Table 5-7**). Nevertheless, the effect of application method and life stage on the mortality of *Tuta absoluta* showed no clear interactions between the different factors.

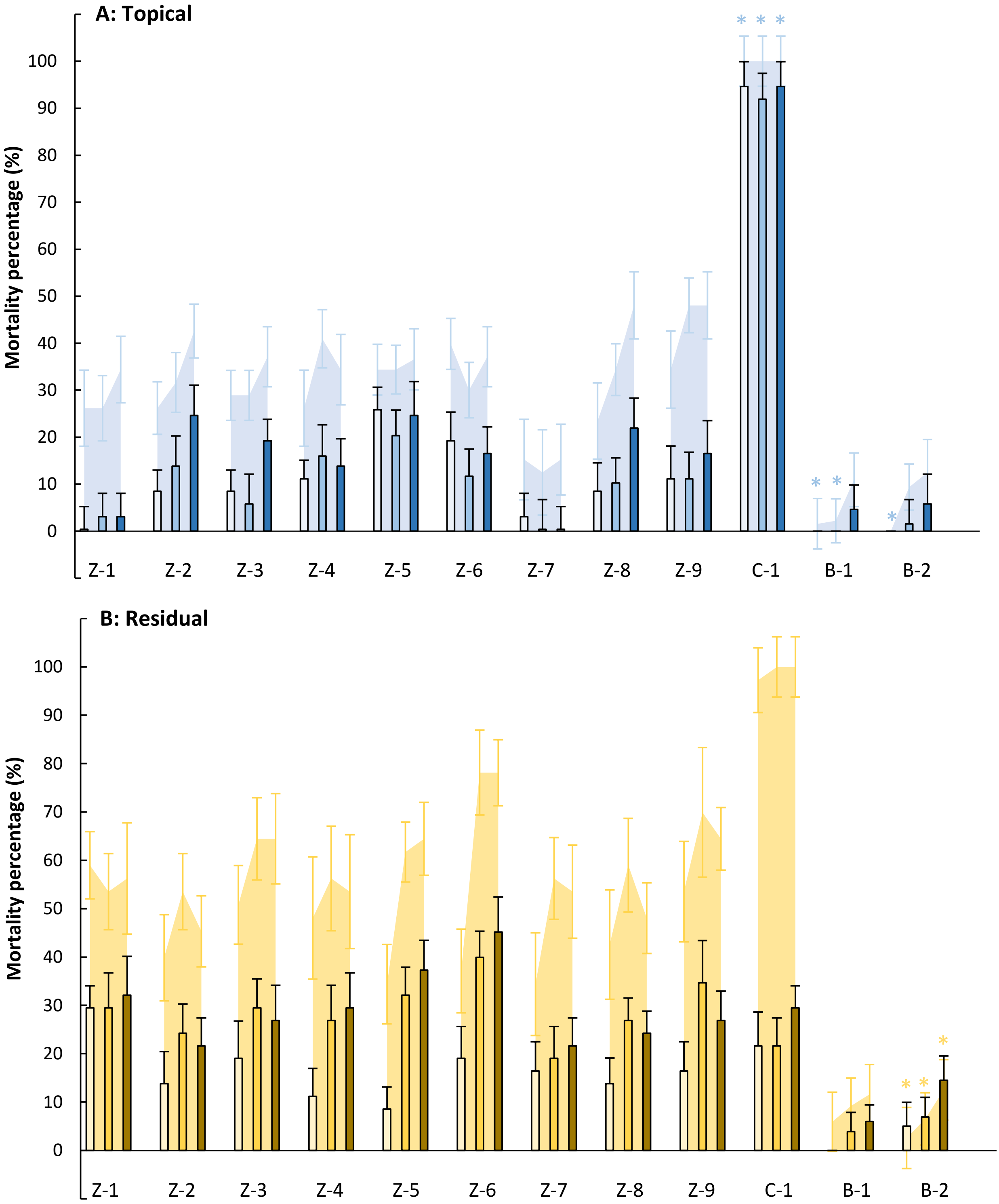


Figure 5-3. Percentage of corrected mortality (± SE) of *Tuta absoluta* eggs (bars) and eggs + larvae (area) in topical (A) and residual (B) exposure bioassays, using different concentrations of the insecticide materials (**Table 5-5**), i.e. 400 (□,□), 4000 (🟦,🟨) and 20000 (■,■) mg.l^{-1}. Asterisks indicate no significant differences ($P < 0.05$) between eggs and eggs + larvae mortality (n=8).

Table 5-6. Four-way ANOVA results indicating the effect of dose, application method, product and life stage on the mortality of *Tuta absoluta*.

Factor	**F**	**df**	**P**
Dose	3.234	1	0.074
Application method	6.925	1	0.010[b]
Product	1.057	1	0.306
Life stage	45.472	1	0.000[c]
Dose x application method	0.043	1	0.836
Dose x product	0.001	1	0.971
Dose x life stage	0.004	1	0.947
Application method x product	4.883	1	0.029[a]
Application method x life stage	3.938	1	0.049[a]
Product x life stage	0.772	1	0.381
Dose x application method x product	0.000	1	0.996
Dose x application method x life stage	0.043	1	0.836
Dose x product x life stage	0.003	1	0.958
Application method x product x life stage	0.144	1	0.705
Dose x application method x product x life stage	0.000	1	0.989
Error		128	

[a, b, c] Significant differences, with a: $P < 0.05$, b: $P < 0.01$ and c: $P < 0.001$.

Table 5-7. Three-way ANOVA results taking into account the effect of application method and life stage on the mortality of *Tuta absoluta*.

Factor	**F**	**df**	**P**	**F**	**df**	**P**
Application method		**Topical**			**Residual**	
Dose	0.977	1	0.327	2.854	1	0.096
Product	0.539	1	0.465	7.433	1	0.008[b]
Life stage	8.745	1	0.004[b]	54.011	1	0.000[c]
Dose x product	0.001	1	0.980	0.001	1	0.980
Dose x life stage	0.029	1	0.866	0.014	1	0.906
Product x life stage	0.611	1	0.437	0.177	1	0.675
Dose x product x life stage	0.002	1	0.967	0.001	1	0.974
Error		64			64	

Table 5-7. Three-way ANOVA results taking into account the effect of application method and life stage on the mortality of *Tuta absoluta* (continued).

Factor	**F**	**df**	**P**	**F**	**df**	**P**
Life stage	**Eggs**			**Eggs + larvae**		
Dose	1.990	1	0.163	1.395	1	0.242
Application method	0.278	1	0.600	8.548	1	0.005[b]
Product	0.015	1	0.904	1.458	1	0.232
Dose x application method	0.114	1	0.736	0.000	1	1.000
Dose x product	0.000	1	0.989	0.003	1	0.956
Application method x product	4.446	1	0.039[a]	1.345	1	0.251
Dose x application method x product	0.000	1	0.995	0.000	1	0.990
Error		64			64	

[a, b, c] Significant differences, with a: $P < 0.05$, b: $P < 0.01$ and c: $P < 0.001$.

Notwithstanding the difference between the application methods, little biological effect on eggs was observed when a zeolite particle film was sprayed topical or residual onto leaves. Although *T. absoluta* egg viability was little affected by the zeolites, the mortality of neonate larvae hatching on treated leaves was significantly higher for all zeolites. Larval mortality increased sharply to 60%, indicating that the zeolites have larvicidal properties. These larval survival results were also significantly different from the control.

Nevertheless, differences of the mortality rates were observed when using zeolites compared to the alternative products. First of all, the hatching percentage recorded for kaolin was slightly reduced compared to that of the control treatment for both application methods (7.14% and 4.29%). Moreover, kaolin had very little or no larvicidal effect compared to the zeolites. The opposite effect was observed when using the commercial formulation of spinosad. In both exposure treatments, it was observed that almost all of the larvae that hatched out of egg masses treated with spinosad died within 48 hours. Spinosad was the least active in its indirect ovicidal activity at all concentrations tested, yielding 21.64-29.48% mortality for concentrations ranging between 400-20000 mg.l^{-1}. **Figure 5-3** clearly points out the significant differences between the zeolites and their formulations regarding the chemical plant protection product.

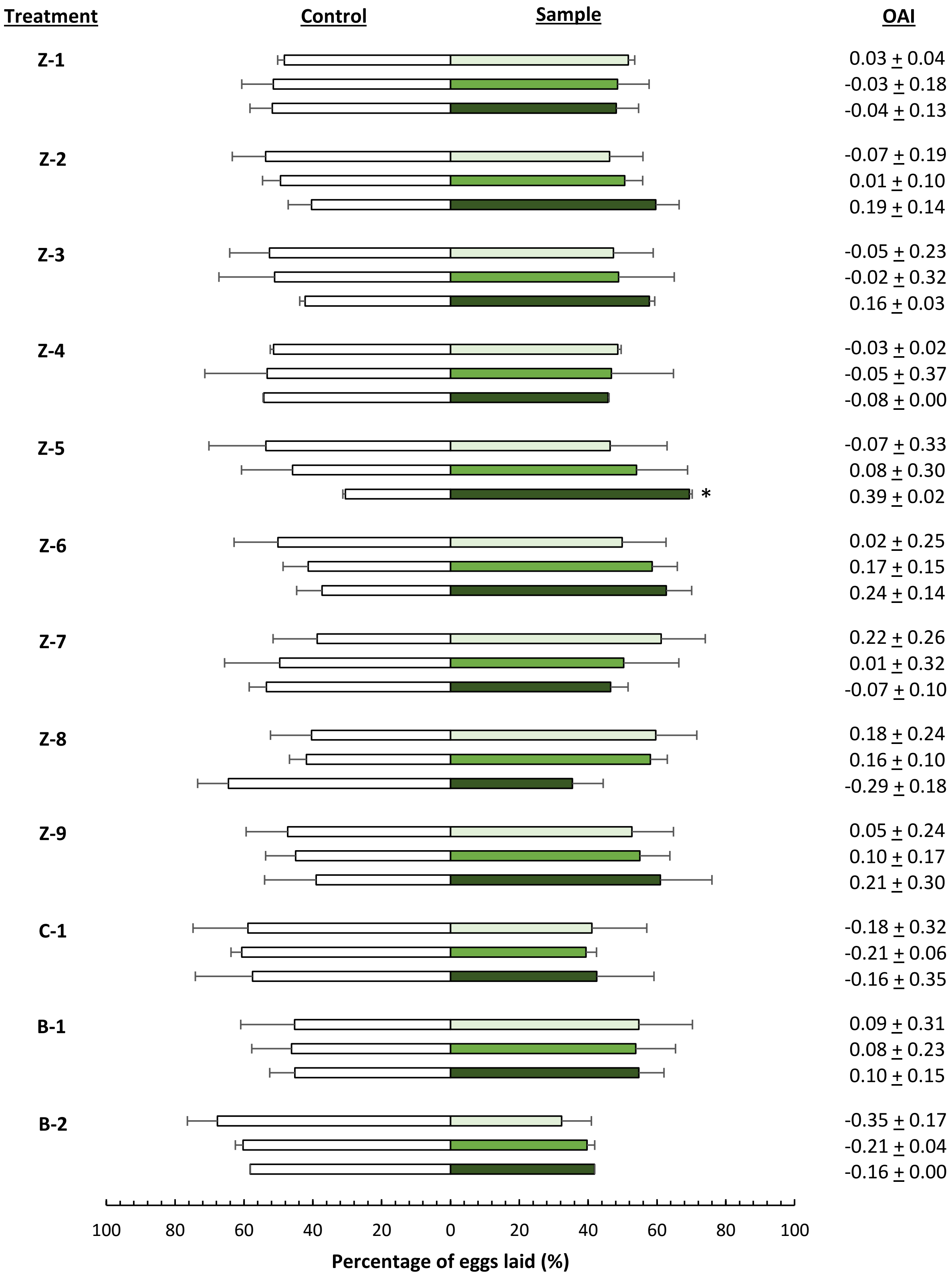


Figure 5-4. Oviposition response of *Tuta absoluta* to plants treated with different concentrations of the insecticide materials (**Table 5-3**). White bars indicate the mean percentage of eggs laid on control leaves, green bars indicate the mean percentage of eggs laid on leaves treated with a concentration of 400 (□), 4000 (🟩) and 20000 (■) mg.l^{-1}. Asterisks indicate significant differences ($P < 0.05$) in oviposition activity between treated and control leaves (n=4).

3.1.2 CHOICE TESTS FOR OVIPOSITION BEHAVIOUR

The results of the oviposition bioassays are illustrated in **Figure 5-4**. Although *T. absoluta* females laid significantly more eggs on the leaves treated with 20000 mg.l^{-1} of zeolite 5, the overall number of eggs did not differ significantly from the control. On the other hand, a slight trend was observed in the oviposition activity of the wettable powder (WP) formulations. The WP zeolites showed a positive OAI response at concentrations of 4000 and 20000 mg.l^{-1} compared to the industrial products. Spinosad and Surround elicited a negative OAI response at the three tested concentrations. Our findings also indicate that *T. absoluta* females manifested a little preference for ovipositing on kaolin surfaces.

3.2 LEPTINOTARSA DECEMLINEATA

Zeolite LTA was screened for insecticidal activity against first instar larvae of *L. decemlineata*. The obtained mortality and weight gain after 8 days of continuous feeding on leaves treated with different concentrations of zeolite LTA are presented in **Figure 5-5**. Based on these results it was concluded that zeolite LTA had a significant insecticidal activity at 10000 mg.l^{-1}. Larvae feeding on potato leaves treated with 10 and 1000 mg.l^{-1} of zeolite LTA showed no significant differences with the control.

A significant decrease in weight gain was also noticed for larvae feeding on leaves treated with 10000 mg.l^{-1} of zeolite LTA. Larvae feeding on potato leaves treated with 10 and 1000 mg.l^{-1} of zeolite LTA showed no significant differences with the control.

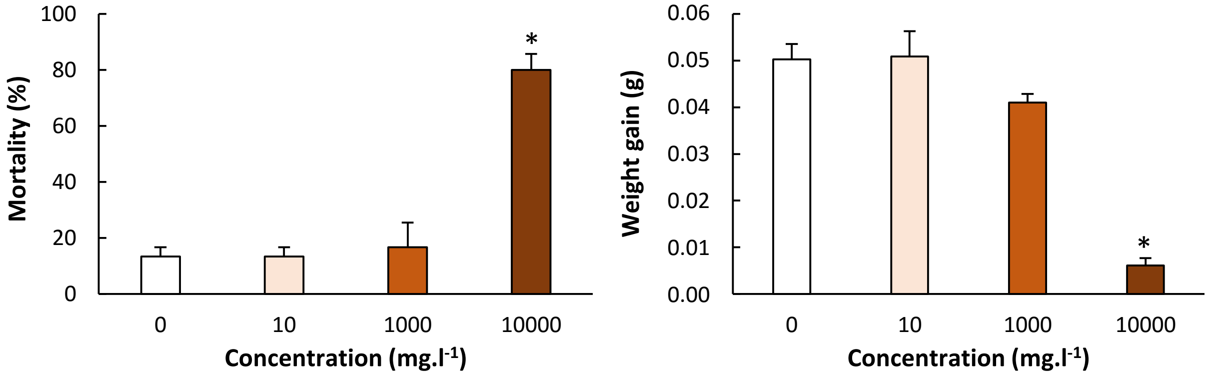


Figure 5-5. Mortality and weight gain of *Leptinotarsa decemlineata* larvae after 8 days of continuous feeding on leaves treated with different concentrations of zeolite LTA, i.e. 0 (☐), 10 (☐), 1000 (■) and 10000 (■) mg.l^{-1}. Asterisks indicate significant differences ($P < 0.05$) in mortality and weight gain between sample and control treatments (n=3).

4 DISCUSSION

Zeolits can have several modes of activity as insecticide. First, it can act as a physical barrier, preventing insects from reaching vulnerable plant tissue, and it can act as a repellent by creating an unsuitable surface for feeding or egg-laying. The uniform white film may also disrupt the insect's host-finding capability by masking the color of the plant tissue. Furthermore, particles of zeolites can act as an irritant to the insect. After landing on a treated surface, particles of zeolites break off and attach to the insect's body, triggering an excessive grooming response that distracts the pest. Finally, zeolites can absorb and adsorb epicuticular lipids, causing death by desiccation.

4.1 *TUTA ABSOLUTA*

4.1.1 TOPICAL AND RESIDUAL TOXICITY

Topical and residual toxicity tests were performed on the eggs of *Tuta absoluta* in order to examine whether zeolites could have a desiccating effect on eggs or not. Egg exposure to zeolites is of great importance against the tomato leafminer. Although the chorion surface layer has limited permeability to ovicidal and toxic substances, some chemicals can pass through it. These compounds can adversely affect embryonic development or causing death (Trisyono et al., 2000; Consoli et al., 2001; Galvan et al., 2005; Nation, 2008; Fogel et al., 2013). In particular, before egg hatching, egg exposure to zeolites seemed to significantly affect the development process by weakening the first instar larvae and increasing their mortality. Exposure of larvae to insecticidal compounds occurs through direct body contact as well as ingestion of residues on leaf surfaces (Dagli and Bahsi, 2009). There should be no difference between the numbers of surviving larvae for both application methods because the larvae were not treated after hatching.

Based on the Si/Al composition, described in Chapter 2, zeolite 1 (Si/Al: 11.84) and zeolite 4 (Si/Al: 15.40) can both be classified as hydrophobic zeolites, while zeolite 7 (Si/Al: 1.15) can be classified as hydrophilic. It was noticed that the ovicidal effect of the tested zeolites correlated well with their hydrophobic properties. This is in contrast with the phenomenon observed by Hoffmann et al. (2008). They noticed a correlation between the ovicidal activity of neonicotinoids against the plum curculio *Conotrachelus nenuphar* (Coleoptera: Curculionidae) and the octanol-water partitioning coefficient (log K_{ow}) of these compounds.

Hydrophilic compounds are unlikely to reach target sites within the embryo, since the lipid layers of the insect chorion provide a general barrier to hydrophilic materials (Hoffmann et al., 2008). However, the results of the adsorption experiment, described in Part B of Chapter 2, indicated that hydrophobic zeolites preferentially adsorb the intermediate and non-polar PPPs. Therefore, it can be presumed that the more hydrophobic zeolites are more attached to the hydrophobic egg surface and are more able to desiccate the eggs and the leaf surface. Low relative humidity can prevent embryo development and egg hatching (Guarneri et al., 2002). Norhisham et al. (2013) found that dehydration of the *Dinoderus minutus* Fabricius (Coleoptera: Bostrychidae) egg leads to contraction and shrinking of the chorion and the embryo. This effect on eggs caused by loss of water has also been reported by Woods and Singer (2001) on Lepidoptera. Eggs of Lepidoptera in general have no special morphology or physiology equipments for water uptake, although the eggshell of some species may be more porous over its ventral surfaces than on lateral or upper surfaces (Woods and Singer, 2001).

The lower hatching rates of the *T. absoluta* eggs for the residual application were rather unexpected. A decrease in adhesion of the insect eggs on the leaf surface can be an explanation for the higher mortality in the residual trials. Adhesive fluids have been repeatedly reported to surround insect eggs and glue them to substrates (Adiyodi and Adiyodi, 1976; Hilker et al., 2005). The eggs of many lepidopteran species are tightly glued onto the lower leaf surface (Hilker and Meiners, 2006).

Plants can also have an impact on the mortality, which explains the higher mortality for the residual bioassay. The eggs of herbivorous insects are usually closely associated with leaf surfaces. As a consequence of living in these microclimates modified by their host plants, these eggs may take advantage of the leaf physiology (Potter et al., 2009). However, eggs laid on a leaf are enclosed by the leaf's boundary layer, consisting of leaf volatiles and atmospheric gases, what may affect embryonic development (Woods, 2010). A plant may respond to insect eggs by the formation of necrotic tissue at the site of egg deposition, where humidity decreases and local temperature increases (Balbyshev and Lorenzen, 1997; Little et al., 2007; Shapiro and De Vay, 1987). As a result, the egg will probably desiccate and the stressed embryo will die (Woods, 2010).

Subsequently, it is possible that larvae hatch at the side of the leaf surface and directly penetrate into the leaf. The probability that the zeolite particles and newly emerged larvae come into contact with each other increases for the residual method.

In addition to the zeolites, other products were also tested, such as kaolin, Surround and spinosad. Kaolin and Surround are products that control insects by creating a particle barrier on plant surfaces that irritates and repels them, rather than actually killing these insects (Glenn and Puterka, 2005). Although the low effect on hatching rate, there appeared to be a slight tendency to reduced hatching when eggs are laid onto kaolin residues. Similar results were also observed in other studies (Unruh et al., 2000; Larentzaki et al., 2008; Bengochea et al., 2014).

As for spinosad, the obtained results were in line with those obtained by Temerak (2005) and Hanan and Samya (2014), who found that spinosad produced 100% mortality of the egg masses of *Spodoptera littoralis* (Lepidoptera: Noctuidae) after hatching. Spinosad was the least active in its indirect ovicidal activity at all concentrations tested by Hanan and Samya (2014), recording 0-45% inhibition for concentrations ranging between 0.1-100 mg.l^{-1}. However, the high toxicity of spinosad on lepidopterous eggs was also supported by Boiteau and Noronha (2007), who found that spinosad residues caused high immediate (24h after exposure) contact mortality in the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Crambidae). Dagli and Bahsi (2009) also noticed that topical exposure of *Orius majusculus* (Hemiptera: Anthocoridae) adults to spinosad resulted in greater mortality compared to the mortalities during residue tests. These findings can be explained by the fact that spinosad is effective on target insects through both ingestion and contact exposure. It is more a potent larvicide, which is in line with our findings of 100% larval mortality. Nevertheless, it can also have an ovicidal effect, mostly when it is mixed into organic solvents (Boiteau and Noronha, 2007). It is not highly systemic, but does possess some leaf-penetrating characteristics, which clarifies the high egg mortality after topical exposure (DOW, 2015). This indicates that spinosad can penetrate the egg, causing the exposed egg to stop further development.

4.1.2 CHOICE TESTS FOR OVIPOSITION BEHAVIOUR

Zeolites work by creating a barrier film by covering the leaves with a white powdery film, which adheres and irritates insects. Additionally, using zeolites on plants can help to repel many types of insects. Identification of suitable oviposition sites is a critical feature of insects' life history because it ultimately influences the survivorship of their progeny (Huang et al., 2005). Oviposition behaviour is influenced by visual, tactile and olfactory cues, with the first considered being of primary importance when zeolites are used (Guha et al., 2012). As described in Chapter 1, the layer of particle film covering the leaves reduces the attractiveness of visual cues and prevents insects from recognizing and finding plant parts on which they prefer to lay eggs. Subsequently, odour of plants, the plant surface and the plant's interior guide egg-laying herbivorous insect females to their host plants and influence the choice of oviposition sites (Städler, 2002). To be effective, complete coverage of the plant is necessary. Hence, high levels of zeolite coverage over the leaves are needed to achieve any sort of control (Reddy, 2012).

In this study, no significant difference was observed between the number of eggs laid on the treated leaves and the control leaves. Despite these results, a slight trend was noticed in the oviposition activity of *T. absoluta* females exposed to leaves treated with the wettable powder formulations. However, this effect can be the result of the additives used in the formulations. Subsequently, olfaction also might have played a role in the increased amount of eggs onto the treated leaves. The effect of additives is possible here as well, given that some additives are responsible for a mild attractiveness to female insects.

The negative oviposition response towards spinosad and Surround can be clarified by the statements given above. Conversely, a slightly positive preference of the *T. absoluta* females for leaves treated with kaolin was noticed, which is in line with the findings of Porcel et al. (2011).

Nevertheless, all these findings did not significantly differ from each other. Therefore, it can be concluded that these products had no effect on the oviposition behaviour of *T. absoluta*. Even kaolin and its formulated product Surround, which is already commercially used in agriculture and has insect repellent properties, did not show repellent effects on *T. absoluta*.

4.2 *LEPTINOTARSA DECEMLINEATA*

Insects pass through a number of development stages from egg to adult. As a general rule, the larval stages are targeted at rapid growth through feeding, whilst the adult stages are involved in reproduction. Therefore, the larval stages are usually the crop destructive steps of the insect's life cycle and consequently the most targeted by insecticides.

As observed in the screening test, zeolite LTA was able to reduce the larvae with 80% when using a concentration of 10000 mg.l^{-1}. A trend was noticed between this larval mortality and larval weight, which indicates the possibility that the obtained mortality was the result of malnutrition. This can be explained by the following two statements, previously discussed in Chapter 1. Firstly, zeolite particles could be attached to the body of *L. decemlineata* larvae, which disrupts the insect's behaviour to such a degree that it is unable to feed and eventually starves (Glenn et al., 2001). Secondly, the zeolite layer covering the leaves reduces the attractiveness of visual cues and, as such, prevents insects from recognising and finding the plants on which they lay eggs (Leskey et al., 2010). Although there is no guaranteed certainty of the mode of action, this reduced recognition of the leaves also might have an influence on the eating behaviour of the insects.

Additionally, Glenn et al. (1999) reported that abrasion and sorption of various inert mineral particles on insects are considered to be key elements for insect killing activity. This may also be the cause of the reduction in weight. Beetles are chewing insects, with typically noticeable mandibles that consume the plant (Korth et al., 2006; Whiting, 2014). It is possible that zeolites have abrasive effects on these insect mandibles. Minerals in plants have also been shown to act as physical abrasives to chewing insects (Korth et al., 2006). Djamin and Pathak (1967) observed that the mandibles of the Asiatic rice borer, *Chilo suppressalis* (Lepidoptera: Crambidae) suffer significantly when feeding on high-silica varieties.

The fact that insecticidal activity was noticed at a zeolite concentration of 10000 mg.l^{-1} was not surprising. As described in the previous section, high levels of zeolite coverage are needed to achieve any sort of control. This also applies to other natural products used as biopesticide.

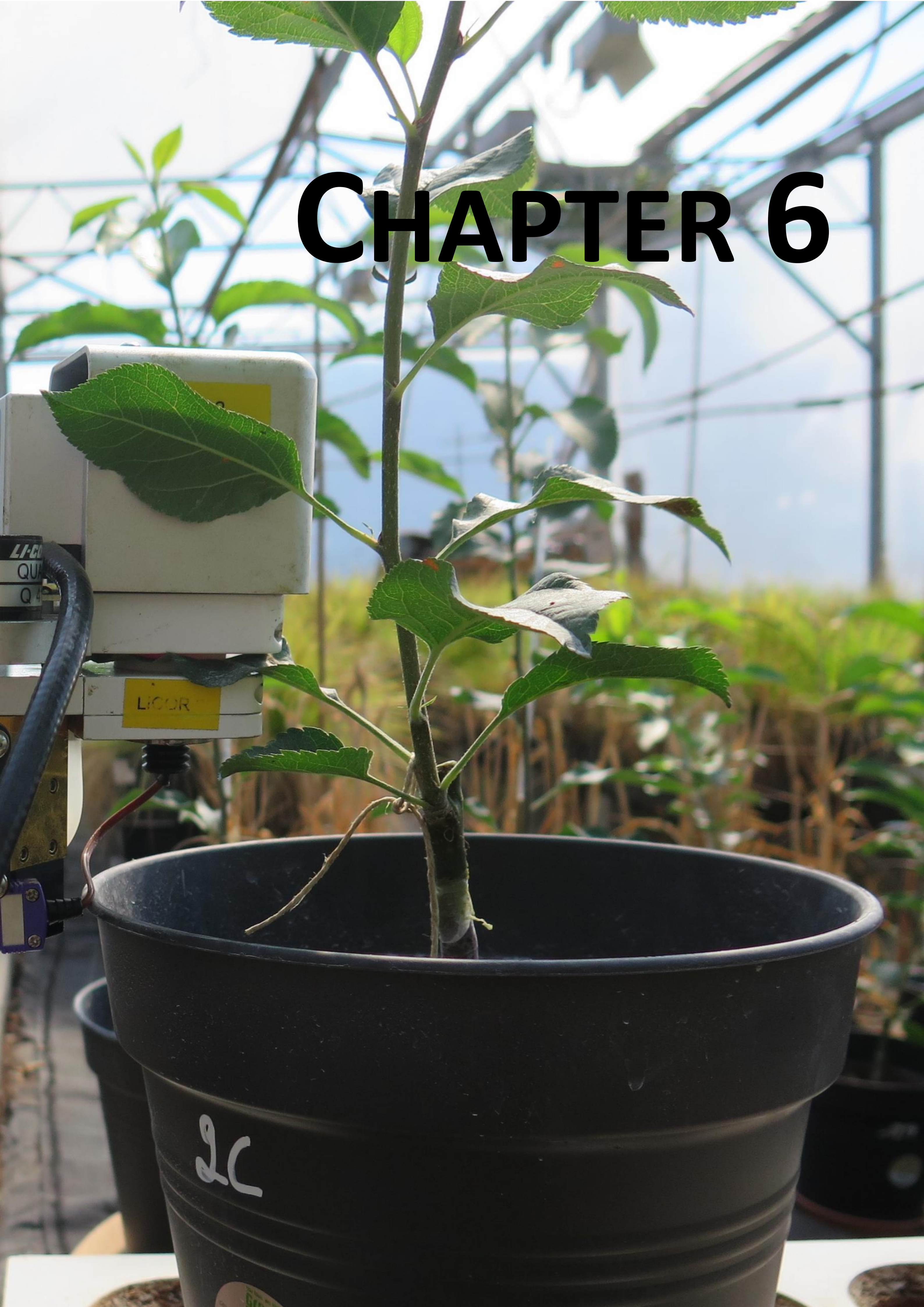
For example, Surround WP (Engelhart Corporation) contains 95% of the active ingredient kaolin. When Surround WP is applied to plants, concentrations between 25000-50000 mg.l^{-1} are used (Omri, 2004). Neem products, containing azadirachtin as active ingredient, have also been used at high concentrations as insecticides. For example, the ready to use product Ozoneem Trishul (Ozone Biotech) contains concentrations between 300-50000 mg.l^{-1} of the active ingredient azadirachtin (Ozone Biotech, 2012).

5 CONCLUSION

Based on the results of this chapter, it can be derived that the tested products, BEA, FAU, LTA and their formulations, had no real insecticidal activity against the eggs of *T. absoluta*. Nevertheless, egg exposure to zeolites seemed to affect the development process by weakening the first instar larvae and increasing their mortality. Subsequently, based on the choice test, no significant difference was observed between the number of eggs laid on the treated leaves and control leaves. The small bioassay, conducted in order to examine the effect of zeolite LTA against the CPB *L. decemlineata*, indicated mortality and growth inhibition at high concentrations of 10000 mg.l^{-1}.

> *"An experiment is a question which science poses to Nature, and a measurement is the recording of Nature's answer."*
>
> **-Max Planck-**

CHAPTER 6



Chapter 6

EFFECT OF ZEOLITES ON PLANTS

Besides the effect of zeolites against plant diseases and insect pests, zeolites may also have beneficial effects for the treated plant itself. Zeolites are able to adsorb CO_2, which may influence photosynthesis. Zeolites may also reduce leaf temperature by reflecting the infrared radiation. These properties lead to a reduction of transpiration rate, which may improve the water-use efficiency, the yield and the fruit quality. In the following chapter, the possible beneficial effects of zeolites on plant growth, dry weight, water uptake and photosynthesis are described.

1 INTRODUCTION

1.1 POSSIBLE EFFECTS OF ZEOLITES ON PLANTS

As described in the previous chapters, particle film technology can be used for controlling arthropod pests and diseases of agriculture crops. In addition, applying particle films can also have beneficial effects on the treated plants. Recent work carried out, using a kaolin particle film, suggests that it generally reduces heat stress, sunburn and drought stress (Jifon and Syvertsen, 2003; Glenn and Puterka, 2005; Rosati et al., 2007; Reddy, 2012). A higher photosynthesis rate can also lead to favorable effects on plant productivity, including an increase in yield and fruit size (Kaszab, 2008). Zeolites are able to adsorb carbon dioxide (CO_2) molecules, which results in an increased of the amount of CO_2 near the stomata (**Figure 6-1**). Because of these findings it is important to examine the influence of zeolites on photosynthesis and their possible mitigating effects on heat and drought stress.

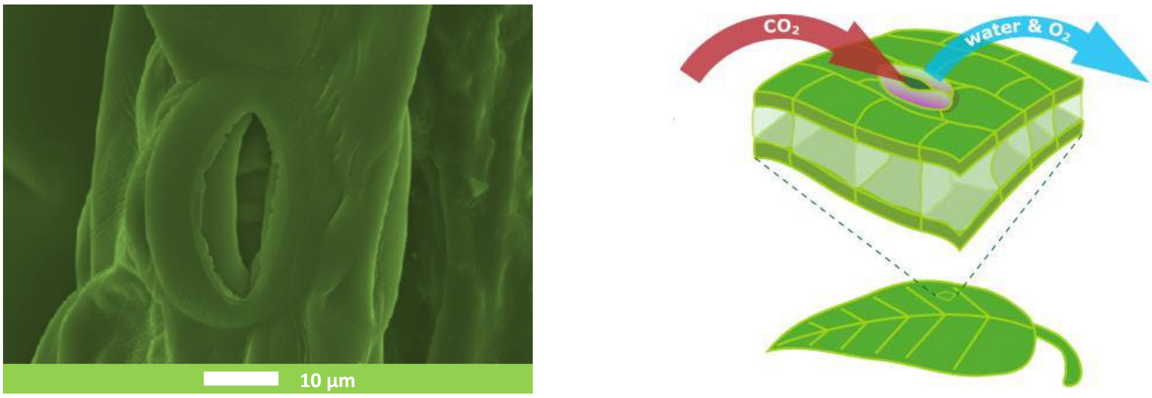


Figure 6-1. Cryo-SEM image of a stomata in the lower leaf epidermis of tomato (Garcia, 2014; Hanssens, 2014).

1.2 IMPORTANT FUNCTIONS OF FOLIAR PLANTS

Leaves perform important processes, such as photosynthesis, transpiration and chlorophyll a fluorescence. A transverse section of a typical leaf distinguishes three regions, being the epidermis, the mesophyll and the vascular bundles or veins. Cells of the mesophyll make up the bulk of the internal leaf tissue and contain large populations of chloroplasts (Pyke, 2007). Chloroplasts are the organelles where photosynthesis occurs and they provide all of the reduced carbon in higher plants, from photosynthesis during the day to starch degradation at night (Walker and Herold, 1977). **Figure 6-2** gives an overview of the plant and leaf anatomy.

1.2.1 PHOTOSYNTHESIS

Plants are able to fix atmospheric CO_2 through photosynthesis using light energy. This unique process provides the plant with organic compounds (primarily sugars) that are transported from the leaves (sources) to energy-demanding sinks (e.g. roots, fruits), where they can be used for storage, as building stones for synthesis of new components or as energy source.

1.2.2 TRANSPIRATION

Next to the important role of the stomata regarding photosynthesis, the stomata are also responsible for transpiration. Transpiration is the process by which sap flow, which typically is transported through the plant from roots to small pores on the lower side of the leaves, is changed into water vapour and is released into the atmosphere. It happens in a proportion of about 90% through the stomata and 10% through the cuticle (Duca, 2015). In other words, this process is an inevitable consequence of photosynthesis, because the entry of CO_2 molecules through open stomata also allows water vapour from the intercellular spaces and substomatal cavities to escape to the atmosphere by the same route (Steppe, 2004).

1.2.3 STOMATAL CONDUCTANCE

The stomata play an essential role in controlling both water losses by transpiration and CO_2 uptake for photosynthesis and plant growth (Damour et al., 2010). By closing the stomata plants retain water when sufficient CO_2 needs are met. The rate of passage of CO_2 entering the stomata is defined as the stomatal conductance. Stomatal conductance can also be defined as the rate of passage of water vapour exiting the stomata. The inverse of stomatal conductance is stomatal resistance which is directly related to the boundary layer resistance of the leaf and the absolute gradient of water vapour from the leaf to the atmosphere (Garcia, 2014).

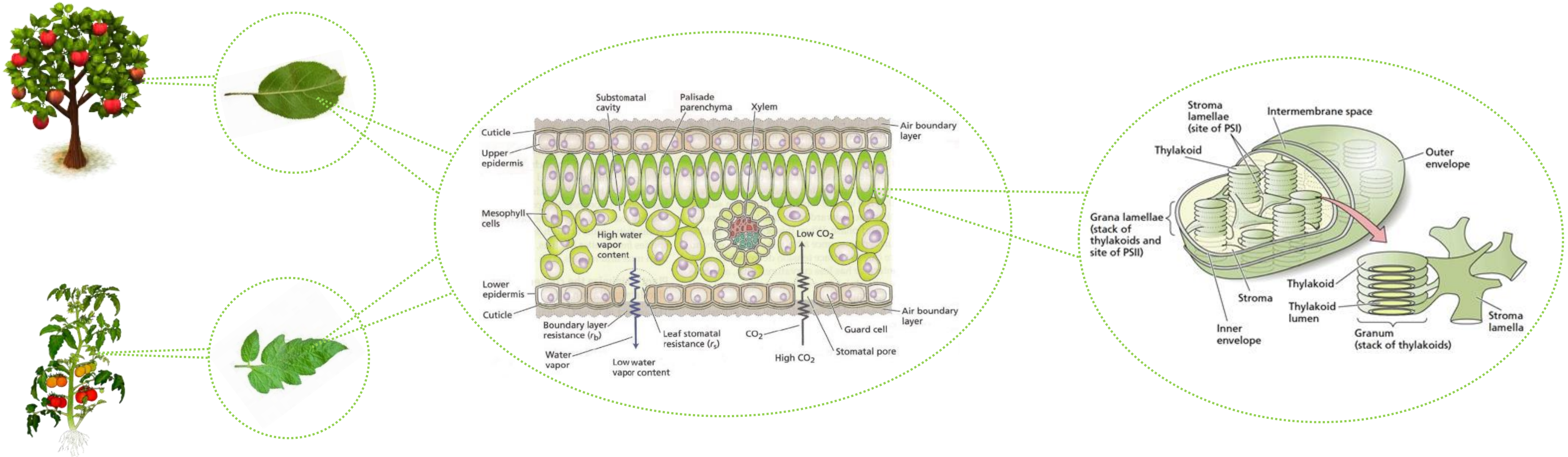


Figure 6-2. Apple tree and tomato plant anatomy (Leaf cross section from Taiz and Zeiger, 2006; Diagram of chloroplast from Taiz and Zeiger, 2010).

It is generally accepted that stomatal conductance decreases in response to rising CO_2 concentration. Stomatal response to CO_2 and the way this response affects photosynthesis and transpiration have effects on plant water regulation and growth, since CO_2 used by the plant mainly passes through stomata (Ainsworth and Rogers, 2007). Although the effect of CO_2 on photosynthesis is well characterized, the photosynthetic stimulation observed in CO_2 enrichment experiments does not always match theoretical expectations (Long et al., 2004; Nowak et al., 2004; Ainsworth and Long, 2005; Rogers et al., 2006). Similarly, while stomatal conductance at elevated CO_2 is typically reduced, the effect is variable and subject to environmental feedback (Ellsworth, 1999; Medlyn et al., 2001; Gunderson et al., 2002; Wullschleger et al., 2002; Naumburg et al., 2003; Leakey et al., 2006).

1.2.4 FLUORESCENCE

When a leaf is illuminated, a certain amount of light energy will be absorbed by the photosystems in the thylakoid membranes. Each photosystem contains a light harvesting complex (LHC) which consists of an assembly of pigment molecules, called antenna complex, and a reaction centre ‘chlorophyll a’-molecule. This green ‘chlorophyll a’-molecule, absorbing blue and red light, plays a central role in photosynthesis (Raven et al., 2005). Light energy, absorbed by the ‘chlorophyll a’-molecule, excite an electron of ‘chlorophyll a’ from the ground state (S_0) to a higher energy level (S_1, red light, 660 nm; S_2, blue light, 420 nm). Since the higher excited states are not stable, electrons will return to the ground state. The electrons first relax to the lowest vibrational energy level of the first electronic energy level, S_1. Subsequently, the light energy at this state can undergo three fates: it can be used to drive photosynthesis (photochemistry), it can be dissipated as heat or it can be re-emitted to the ground state as red fluorescence (Misra et al., 2012).

The three processes occur in competition. Since the sum of rate constants is constant, any increase in the efficiency of one process will result in a decrease of the other two. Therefore, determining the yield of chlorophyll fluorescence reveals important information such as the impact of environmental stresses on photosynthesis (Maxwell and Johnson, 2000).

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL

The experiment was carried out on two different plant species. Measurements were conducted on cuttings of apple trees (Malus *domestica* vc. Golden Delicious) obtained from Schrama Nurseries, Biddinghuizen, Netherlands. The trees were approximately 26 cm high and their stem diameter at soil surface was approximately 2.3 mm. Subsequently measurements were conducted on tomato plants (Solanum *lycopersicum* L. vc. Admiro) obtained from BPK, Duffel, Belgium. The plants were approximately 19 cm high and their stem diameter at soil surface was approximately 4.3 mm. All plant material was divided into twelve groups: one control and eleven treatment groups. Each group consisted of 5 plants.

2.2 PLANT COATING MATERIALS

The three zeolite types, BEA, FAU and LTA, together with two formulations of each type were compared with a control treatment. Additionally, two biological equivalents of the zeolites, namely kaolin and its commercial formulation 'Surround' (Tessenderlo Kerley, US) were taken into account (**Table 6-1)**.

Table 6-1. Plant coating materials used in this study.

Treatment	Material name	Formulation type	Manufacturers
1	BEA	Technical product	Clariant
2	BEA 850[a]	WP	Fitofarmacia
3	BEA 950[b]	WP	Fitofarmacia
4	FAU	Technical product	Zeolyst
5	FAU 850[a]	WP	Fitofarmacia
6	FAU 920[b]	WP	Fitofarmacia
7	LTA	Technical product	FMC
8	LTA 800	SC	Fitofarmacia
9	LTA 850	WP	Fitofarmacia
10	Kaolin	Technical product	Sigma Aldrich
11	Kaolin (Surround)	WP	Tessenderlo Group
12	Control	/	/

[a, b] The formulations with a similar letter contain the same adjuvants.

2.3 EXPERIMENTAL SET-UP

At the end of July, 60 apple trees and 60 tomato plants, respectively, were planted in 5l pots. The apple trees were placed in the open greenhouse facilities of the Laboratory of Plant Ecology, Faculty of Bioscience Engineering, Ghent University, Belgium. The tomato plants were placed in the growth chamber at the Laboratory of Phytopharmacy, Faculty of Bioscience Engineering, Ghent University, Belgium. Two days before starting the measurements, 5 plants per treatment were sprayed with 4000 mg.l^{-1} of the selected product until runoff (**Table 6-1**). The apple trees and tomato plants were respectively treated with the zeolites at the 3rd and 13th of August 2015.

2.4 MEASUREMENTS

2.4.1 GAS EXCHANGE AND CHLOROPHYLL A FLUORESCENCE MEASUREMENTS

Foliar gas exchange and chlorophyll a fluorescence parameters were measured using a portable photosynthesis system (model LI-6400; Li-Cor Biosciences, Lincoln, Nebraska, USA) equipped with a fluorescence head (6400-40 Leaf Chamber Fluorometer, Li-Cor Biosciences, Lincoln, Nebraska, USA). This device is an open differential infrared gas analyser (IRGA), shown in **Figure 6-3**.

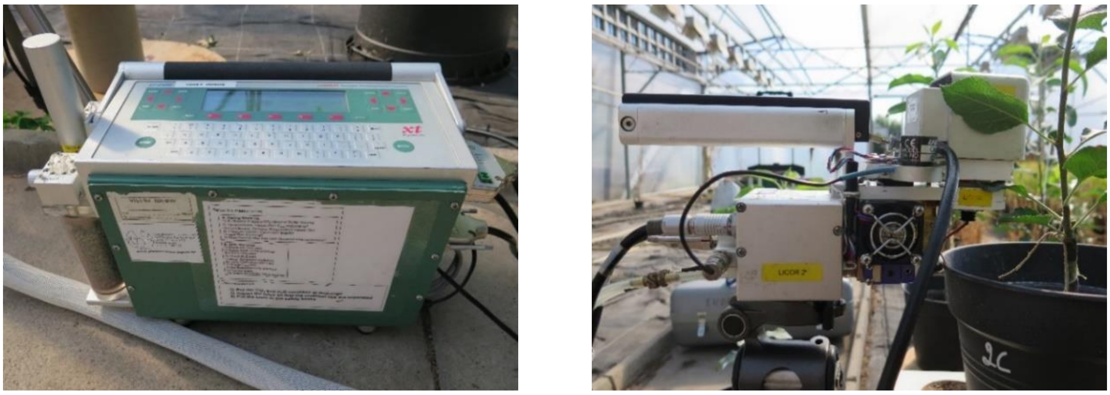


Figure 6-3. The Li-Cor LI-6400 Portable Photosynthesis System with Li-Cor 6400-40 Leaf Chamber Fluorometer as a sensor head.

First of all, light response curves were measured on the third fully developed leaf of three apple trees and tomato plants per treatment. This leaf was selected because a preliminary test showed that the obtained data remained stable. The light response curves were recorded using seven light intensities: 0, 50, 100, 250, 500, 1000 and 1500 µmol PAR.m^{-2}.s^{-1}. This was to detect the light intensity at which photosynthesis rates reached a saturation level. Further, a minimum and maximum waiting time of 5 respectively 7 minutes was applied before measurements on a leaf were recorded to allow stabilisation.

Subsequently, light saturated net photosynthesis (A_n, µmol CO_2.m^{-2}.s^{-1}), stomatal conductance (g_s, mol H_2O.m^{-2}.s^{-1}) and transpiration rate (E, mmol H_2O.m^{-2}.s^{-1}) were simultaneously measured on the third leaf of each apple tree and tomato plant. These measurements were performed at saturating red light (1500 µmol PAR.m^{-2}.s^{-1}) achieved with the red light emitting diode (LED) lamp of the fluorescence head, with an additional 10% of blue light to maximize stomatal opening, and 400 µmol CO_2.mol^{-1} in the cuvette. As temperature influences photosynthesis and transpiration rates, the chamber temperature of the fluorescence head was set to 25°C-26°C. Chlorophyll a fluorescence was quantified by measuring the light adapted photosystem II (PSII) efficiency (F_v'/F_m' = $1-F_0'/F_m'$), calculated from maximum fluorescence after a saturating light flash (F_m') and minimum fluorescence after a far-red pulse (F_0').

Simultaneous with the measurements of the light response curve, stomatal conductance, transpiration rates and chlorophyll a fluorescence parameters were measured. Ambient parameters, such as air temperature (T_{air}), leaf temperature (T_{leaf}), vapour pressure deficit (VPD), photosynthetic active radiation (PAR) and relative humidity (RH) were also recorded. Other variables were calculated, such as the intrinsic water use efficiency ($A_n.g_s^{-1}$) and the instantaneous water use efficiency ($A_n.E^{-1}$) (see Section 2.4.4).

These measurements were carried out twice a week during 4 weeks. The apple trees had eight examination points, while six examination points were sufficient for the tomato plants.

2.4.2 GROWTH MEASUREMENTS

Stem height and diameter at the base of each plant were measured at the beginning and at the end (after 4 weeks) of the experiment.

2.4.3 BIOMASS MEASUREMENTS

At the end of the experiment, all foliage and stem biomass were collected. Immediately after harvesting, the fresh weight (F_W, g) of this shoot material was determined. Subsequently, foliage and stem biomass were stored dried in an oven at 70°C for about 14 days and weighed. After dry weight (D_W, g) was obtained, the water content was calculated as the difference between F_W and D_W.

2.4.4 WATER USE EFFICIENCY

Water is the main abiotic factor limiting plant production. Water use efficiency (WUE) is a crucial parameter and can be measured at different scales, ranging from measurements on the leaf to more integrative ones at the plant and crop levels (**Figure 6-4**) (Medrano et al., 2015). WUE measurements on plant (integrated) and leaf (gas exchange) level were conducted in this research.

<u>Integrated water use efficiency</u> (= plant related characteristic)
The biomass WUE was determined as the total shoot biomass increase divided by the total water consumed by each plant. Just before the start of the experiment 5 plants were weighted and dried to estimate the initial dry weight. This process was also carried out at the end of the experiment (see Section 2.4.3). The total shoot biomass increase, during the experiment, was estimated as the difference between the dry weights at the beginning and at the end of the experiment. Plant water consumed over the four weeks was estimated from the sum of the daily water consumption. Plants were daily irrigated with a known amount of water avoiding water run-off. Containers were weighted two hours after irrigation and the day after just before irrigation. Water consumption was measured as a difference between both weights, which include water evaporation and transpiration.

Gas exchange water use efficiency (= photosynthetic related characteristic)
The intrinsic and instantaneous WUE were calculated based on the gas exchange measurements, as described in Section 2.4.1.

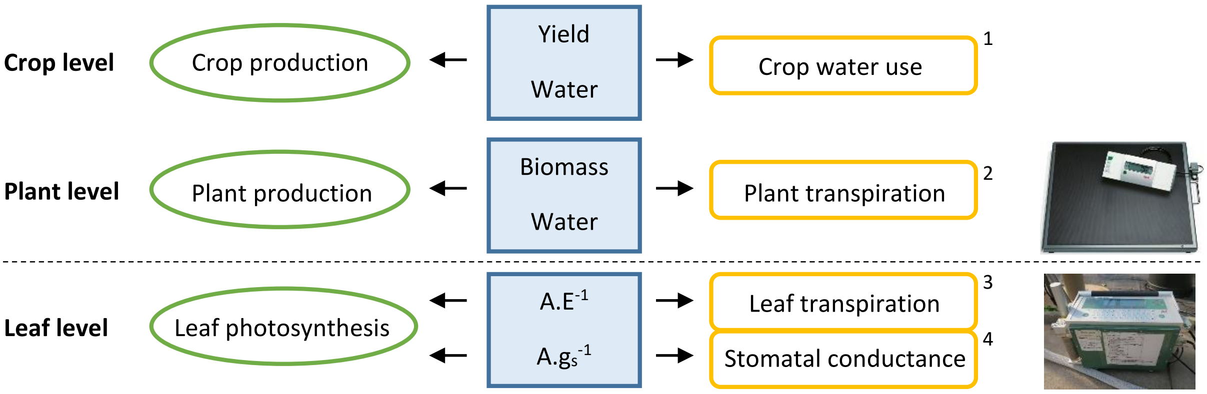


Figure 6-4. Different complexity levels for water use efficiency measurements, with 1: WUE_{yield}, 2: $WUE_{biomass}$, 3: $WUE_{instantaneous}$ and 4: $WUE_{intrinsic.}$ (adapted from Medrano et al., 2015). Above the line, the measurements were based on the whole plant, while below the line, the measurements were just based on some leaves of the plant. In this chapter the integrated WUE (2), using a balance, and the gas exchange WUE (3, 4) using the Li-Cor, were measured.

2.4.5 WATER UPTAKE LEAVES

In order to determine whether the leaves sprayed with the plant coating materials (**Table 6-1**) need more or less water, the four basic (non-formulated) treatments Z-1, Z-4, Z-7 and B-1 were tested. All products to be tested were dispersed in distilled water to obtain zeolite concentrations of 400, 4000 and 20000 mg.l^{-1}. Control treatments, consisting of distilled water, were also included. For each treatment and concentration, 4 apple and tomato leaves were sprayed until runoff. Subsequently, the leaves were placed in a known amount of water (**Figure 6-5**). Seven days later the residual amount of water was measured.

Figure 6-5. Set-up of the sprayed leaves in a known amount of water in order to calculate the water uptake of these leaves after seven days.

2.4.6 CARBON DIOXIDE ISOTHERM

Carbon dioxide isotherms were measured for the zeolites BEA, FAU, LTA and kaolin. Adsorption isotherms of pure CO_2 (purity of 99.99%) up to 1 bar were determined at 25°C, using a Belsorp Max instrument combined with BELCryo system.

2.5 DATA ANALYSIS

A one sample Kolmogorov-Smirnov test was used to assess the normality of the photosynthetic values. The Mann-Whitney test was used to compare the differences between the control and treated samples. Statistical analysis was performed with the Software Package for the Social Sciences (version 10.1 for Windows, SPSS Inc, Chicago, Ill, USA). A p value of < 0.05 was considered statistically significant.

3 RESULTS AND DISCUSSION

3.1 PLANT RELATED CHARACTERISTICS

3.1.1 GROWTH MEASUREMENTS

Plant height

The height of the apple trees and tomato plants was measured at the start and the end of this research (**Figure 6-6**). Visual observations prior to these measurements presumed little differences in height between test and control samples, which is also reflected in the results. The average heights of apple trees and tomato plants measured at the start and the end were respectively, 25.40 cm, 49.00 cm, 18.54 cm and 49.30 cm. For both apple trees and tomato plants no significant differences were observed. Dieleman et al. (2003) also noticed no effect of elevated CO_2 on plant height of pepper plants. These pepper plants were grown in greenhouses containing an increased CO_2 concentration. It was observed that the plants can adapt to being exposed for weeks to high CO_2 concentrations. This adaptation led to a less efficient use of the present CO_2. A possible method to avoid this adaptation was the use of pulsed CO_2.

Stem thickness

The stem thickness of the apple trees and tomato plants was also measured at the start and the end of this research (**Figure 6-6**). Visual observations prior to these measurements also suggested little difference in thickness between test and control samples, which is also reflected in the results. The average stem diameters of apple trees and tomato plants measured at the start and the end were respectively, 3.09 mm, 5.06 mm, 4.34 mm and 5.49 mm. For both apple trees and tomato plants no significant differences were observed. Dieleman et al. (2003) also noticed no effect of elevated CO_2 on stem thickness of pepper plants, which can be explained by CO_2 adaptation of the pepper plants.

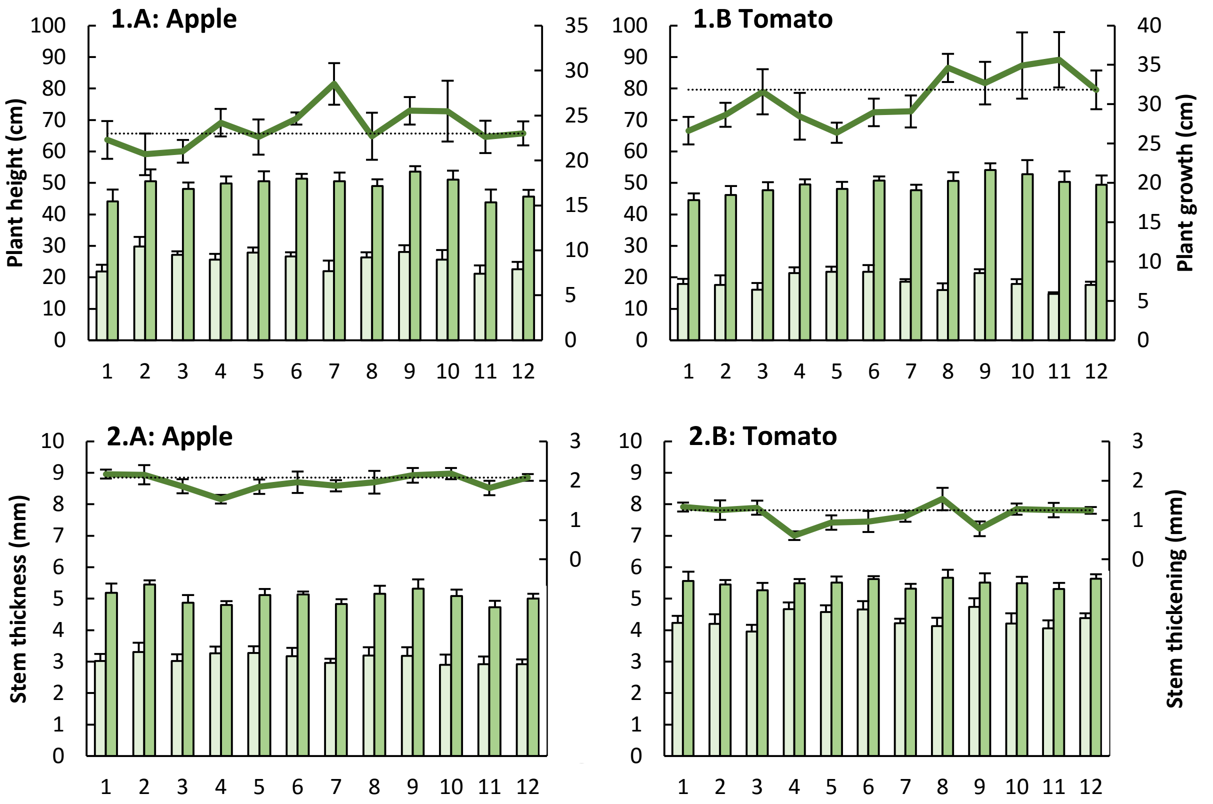


Figure 6-6. The primary y-axis shows both the initial (□) plant height (± SE; 1) and stem thickness (± SE; 2) as well as the plant height and stem thickness after four (tomato) or five (apple) weeks (🟩) of apple trees (A) and tomato plants (B) sprayed with the plant coating materials (**Table 6-1**). The differences between the initial and final thickness describe the plant growth (1) and stem thickening (2). The secondary y-axis presents the plant growth (± SE; 1) and stem thickening (± SE; 2) of the treated samples (▬) compared to the the control (···) (n=5).

3.1.2 BIOMASS MEASUREMENTS

The observed results in fresh weight, dry weight and moisture content of the different apple trees and tomato plants are presented in **Figure 6-7**. In general, an increased concentration of CO_2 provides a higher crop production (Kimball, 1986). The results of this research show that the plants treated with the products listed in **Table 6-1** produced more biomass compared to the control sample. However, no significant differences were noticed for the dry weight and moisture content of the apple trees and tomato plants. Nevertheless, Dieleman et al. (2003) did notice an effect of elevated CO_2 on the dry weight of leaves and stems of pepper plants and, in general, it is said that an elevated CO_2 concentration ensures a higher crop production (Kimball, 1986).

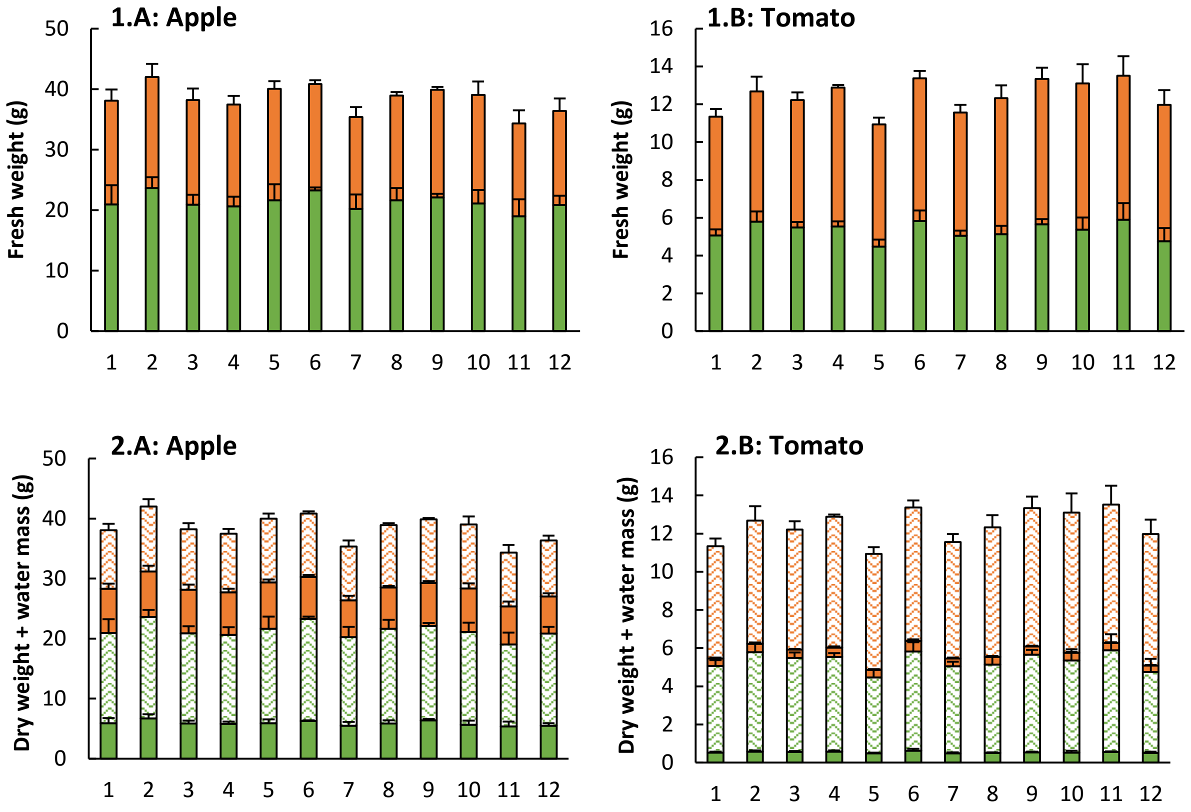


Figure 6-7. Fresh weight (± SE; 1) and composition of the fresh weight (± SE; 2) of apple trees (A) and tomato plants (B) sprayed with the plant coating materials (**Table 6-1**). The total shoot fresh weight consist of the fresh weights of leaves (1: 🟩) and stems (1: 🟧), and represents the dry weight (2: 🟩 and 🟧) and moisture content (2: ▨ and ▨) (n=5).

3.1.3 INTEGRATED WATER USE EFFICIENCY

The cumulative water consumption and the measured integrated water use efficiency of the samples are shown in **Table 6-2**, respectively. Agronomists and crop physiologists define WUE rather from an integrative approach, i.e. the accumulated dry matter divided by the water used by the crop in the same period (Abbate et al., 2004). Comparison between instantaneous/intrinsic (Section 3.2.5) and whole-plant values sometimes reveals a clear relationship, but often not (Flexas et al., 2010; Medrano et al., 2015).

Both, for the apple trees and tomato plants, significant differences were observed between the treated and control samples. This is mainly due to the higher dry weight values observed in the treated plants.

Table 6-2. Water use efficiency (WUE; ± SE) of the apple trees and tomato plants treated with the plant coating materials (**Table 6-1**) based on the produced biomass (increase of dry matter) per unit of water transpired (accumulated water consumption, kg) (n=5).

	**Initial dry weight shoot (g)**	**Final dry weight shoot (g)**	**Increase dry weight shoot (g)**	**Cumulated water consumption (kg)**	**$\mathrm{WUE}_{\mathrm{biomass}}$ ($\mathrm{g.kg^{-1}}$)**
			Apple		
1	4.11 ± 0.54	13.32 ± 1.65	9.21 ± 1.74	3.21 ± 0.02	**2.87 ± 0.54**
2	4.11 ± 0.54	14.32 ± 1.59	10.21 ± 1.68	2.79 ± 0.05	**3.66 ± 0.61**
3	4.11 ± 0.54	13.11 ± 1.25	9.00 ± 1.36	3.14 ± 0.11	**2.88 ± 0.44**
4	4.11 ± 0.54	12.86 ± 0.98	8.75 ± 1.12	2.76 ± 0.07	**3.18 ± 0.41**
5	4.11 ± 0.54	13.66 ± 0.98	9.55 ± 1.12	2.88 ± 0.08	**3.33 ± 0.40**
6	4.11 ± 0.54	13.27 ± 0.30	9.16 ± 0.62	2.88 ± 0.02	**3.18 ± 0.22**
7	4.11 ± 0.54	11.69 ± 1.30	7.58 ± 1.41	3.31 ± 0.12	**2.30 ± 0.43**[a]
8	4.11 ± 0.54	12.76 ± 0.68	8.65 ± 0.87	2.92 ± 0.15	**2.99 ± 0.33**
9	4.11 ± 0.54	13.58 ± 0.48	9.47 ± 0.72	3.51 ± 0.18	**2.72 ± 0.25**[a]
10	4.11 ± 0.54	12.89 ± 1.56	8.78 ± 1.65	3.28 ± 0.18	**2.71 ± 0.52**[a]
11	4.11 ± 0.54	11.76 ± 1.60	7.65 ± 1.69	2.72 ± 0.11	**2.83 ± 0.63**[a]
12	4.11 ± 0.54	11.66 ± 0.79	7.55 ± 0.95	3.06 ± 0.16	**2.49 ± 0.34**[a]
			Tomato		
1	0.23 ± 0.02	0.90 ± 0.11	0.67 ± 0.12	1.33 ± 0.01	**0.51 ± 0.09**
2	0.23 ± 0.02	1.02 ± 0.11	0.79 ± 0.11	1.35 ± 0.00	**0.58 ± 0.08**
3	0.23 ± 0.02	0.99 ± 0.09	0.76 ± 0.09	1.35 ± 0.01	**0.56 ± 0.07**
4	0.23 ± 0.02	1.05 ± 0.07	0.82 ± 0.07	1.21 ± 0.01	**0.68 ± 0.06**
5	0.23 ± 0.02	0.90 ± 0.06	0.67 ± 0.06	1.29 ± 0.02	**0.52 ± 0.05**
6	0.23 ± 0.02	1.18 ± 0.17	0.95 ± 0.17	1.32 ± 0.02	**0.73 ± 0.13**
7	0.23 ± 0.02	0.89 ± 0.10	0.66 ± 0.10	1.21 ± 0.01	**0.55 ± 0.08**
8	0.23 ± 0.02	0.92 ± 0.07	0.69 ± 0.07	1.34 v 0.03	**0.51 ± 0.05**
9	0.23 ± 0.02	0.97 ± 0.05	0.74 ± 0.05	1.35 ± 0.01	**0.55 ± 0.04**
10	0.23 ± 0.02	0.92 ± 0.14	0.69 ± 0.14	1.28 ± 0.02	**0.54 ± 0.11**
11	0.23 ± 0.02	0.94 ± 0.07	0.71 ± 0.07	1.30 ± 0.03	**0.55 ± 0.06**
12	0.23 ± 0.02	0.85 ± 0.09	0.62 ± 0.09	1.34 ± 0.01	**0.46 ± 0.07**[a]

[a] No significant differences between sample and positive control treatments (12).

3.1.4 WATER UPTAKE LEAVES

A small indicative tests was performed in order to examine the effect of zeolites and kaolin on plant leaves. Literature describes that zeolites and kaolin are able to reduce drought stress by reducing water vapour losses and absorbing condensed water (Lalancette et al., 2005; Reddy, 2012). **Figure 6-8** illustrates a clear effect of zeolite and kaolin use on water uptake. Leaves treated with higher concentrations of zeolite and kaolin needed more water during the test period. Both apple and tomato leaves showed the same trend.

First of all, the water holding capacity is one of the main and most important properties of zeolites (Tzia and Zorpas, 2012). The water holding capacities, obtained in Part B of Chapter 2 (Section B.3.2), illustrated that zeolites BEA (24%) and FAU (27.95%) were able to adsorb water, this in contrast to zeolite LTA (2.21%). A resemblance between those results and the results obtained in this chapter occurs. The leaves treated with zeolite FAU needed less water than the leaves treated with zeolites BEA, LTA and kaolin, which may be the results of two factors. First, zeolite FAU adsorbs more water, creating presence of water near the stomata which can reduce transpiration. Secondly, the coverage of the leaves with zeolites can have effects on photosynthesis what also can result in a reduced transpiration. However, all the values obtained at each concentration did not differ significantly from each other.

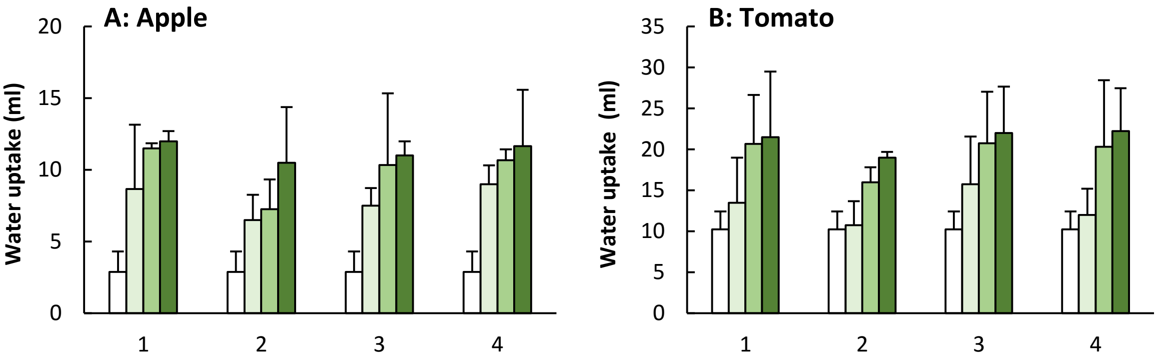


Figure 6-8. Water uptake (± SE) of apple (A) and tomato (B) leaves treated with the non-formulated plant coating materials, i.e. (1) BEA, (2) FAU, (3) LTA and (4) kaolin at a concentration of 0 (☐), 400 (🟩), 4000 (🟩) and 20000 (🟩) mg.l^{-1} (n=4).

Subsequently, the increased water uptake can possibly be explained as an effect of detached leaves. Injuries, as a result of detaching the leaves, induce a number of plant hormones, which may have an effect on the stomata.

Plant water loss is tightly balanced with water uptake to maintain beneficial water status. The most important control on water transport is the change of stomatal aperture (Kaiser and Legner, 2007). The pores of the stomata are flanked by specialized cells, known as guard cells. Within these guard cells, the plant hormone abscisic acid (ABA) is one of the most important chemical signals that triggers a signaling cascade leading to stomatal closure under abiotic conditions such as drought. Guard cell control of transpiration and water loss is strongly associated with drought tolerance (Hopper et al., 2014).

However, sometimes it is possible that the stomata fails to close as a result of their inability to synthesize ABA. Closure of stomata is not well correlated with the ABA content of leaves in all plants and stomata sometimes remain open in leaves with high ABA concentrations or stay closed or partly closed after the ABA concentration has decreased (Kramer and Boyer, 1995).

Trejo et al. (1993) reported that ABA is rapidly metabolized in mesophyll tissue, and this probably has contributed to the uncertainty concerning the role of ABA in stomatal closure.

However, detached leaves can also have an influence on these plant hormones and affect the transpiration rate. Halevy (1956) found that on mild autumn days the rates of transpiration of orange were similar for detached leaves and potted plants, but on hot, dry days rates of the latter were 25-50% lower than the rates of detached leaves.

In this study, all tested leaves were incubated at a constant temperature of 26°C. These conditions can be equated to hot days. Additionally, zeolites have a desiccating effect. They are able to absorb gaseous water from the air, creating a dry environment. These conditions can be equated to the conditions whereby Halevy (1956) observed a faster transpiration of detached leaves, which can explain the results in this tests. The zeolite concentration will also have an influence on the relative humidity. Therefore, an increase in zeolite concentration will result in a decrease in humidity, which ultimately will lead to an increase in transpiration rates.

3.2 PHOTOSYNTHETIC RELATED CHARACTERISTICS

3.2.1 CARBON DIOXIDE ADSORPTION ISOTHERM

As previously described in Chapter 1, zeolites are porous materials that are able to adsorb CO_2 molecules. The obtained CO_2 adsorption isotherms of the zeolites used in this research are displayed in **Figure 6-9**. As also observed by Honghong et al. (2012), it was found that the Langmuir equation (type I isotherm) was suitable for describing the adsorption of CO_2 on zeolites. Based on this equation it is possible to determine the maximum adsorption capacity (q_m) and energy (K_L). The obtained values of these constants q_m and K_L are presented in **Table 6-3**.

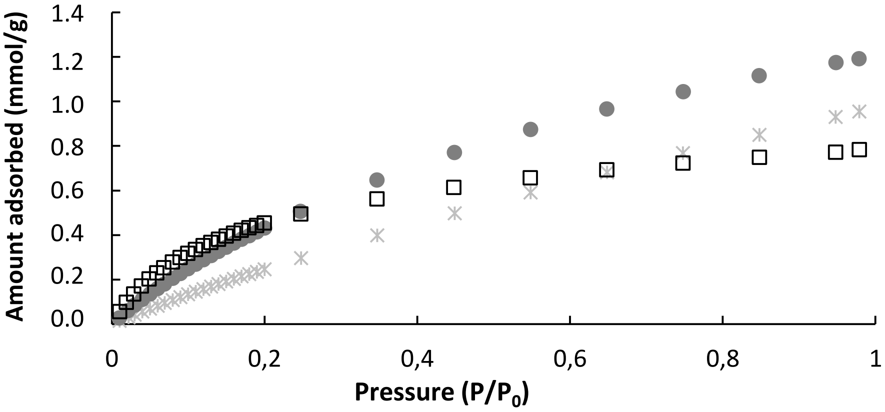


Figure 6-9. Carbon dioxide adsorption isotherms on the non-formulated zeolites BEA (●), FAU (✳) and LTA (☐) at 1 bar and 20°C.

The highest maximum adsorption capacity of CO_2 was obtained by zeolite FAU and decreased in the sequence FAU > BEA > LTA. This can be explained by the higher specific surface area (S_{Bet}) of zeolite FAU. Zeolite LTA showed a faster adsorption, what can be described by its lower Si/Al ratio. Zeolites with a low Si/Al ratio are more polar, what results in a faster interaction with CO_2 (Honghong et al., 2012).

Table 6-3. The obtained Langmuir isotherm parameters and zeolite properties of zeolites BEA, FAU and LTA.

	q_m (mmol.g^{-1})	K_L (kPa^{-1})	R^2 (-)	S_{Bet} (m^2.g^{-1})	Si/Al
BEA	1.4806	0.0200	0.9994	365	11.84
FAU	1.5306	0.0094	0.9979	661	15.40
LTA	0.7178	0.0800	0.9995	2	1.12

3.2.2 PHOTOSYNTHESIS LIGHT RESPONSE CURVE

Photosynthesis increases with higher CO_2 concentrations up to a saturation point, beyond which photosynthesis remains constant (Schulze et al., 2005). In order to determine this saturation point, a total of 36 light response curves were generated on apple trees and tomato plants for all the treatments listed in **Table 6-1**. A representative light response curve for both plants is shown in **Figure 6-10**. Apple and tomato leaf photosynthesis is of the C_3 type with a hyperbolic light response that typically saturates at 500-1000 µmol.m^{-2}.s^{-1}. The light compensation point, i.e. the light level for which the net CO_2 exchange is zero and respiration equals photosynthesis, is about 15 µmol.m^{-2}.s^{-1} for both plants. The maximum mean photosynthetic rates of apple trees and tomato plants were 8 and 6 µmol.m^{-2}.s^{-1}, respectively.

Based on these results it was concluded that the tested apple trees and tomato plants were in a healthy state. Good rates of photosynthesis per unit leaf area for healthy exposed apple leaves vary from 15 µmol.m^{-2}.s^{-1} in early summer to about 8 µmol.m^{-2}.s^{-1} at harvest time (Lakso, 1994; Wünsche et al., 1996; Greer, 2015).

Moreover, the maximum photosynthetic rate was also about 8 µmol.m^{-2}.s^{-1} in healthy tomato plants (Habermann et al., 2003). Similar results were found by Vu et al. (1986) and Machado et al. (1994).

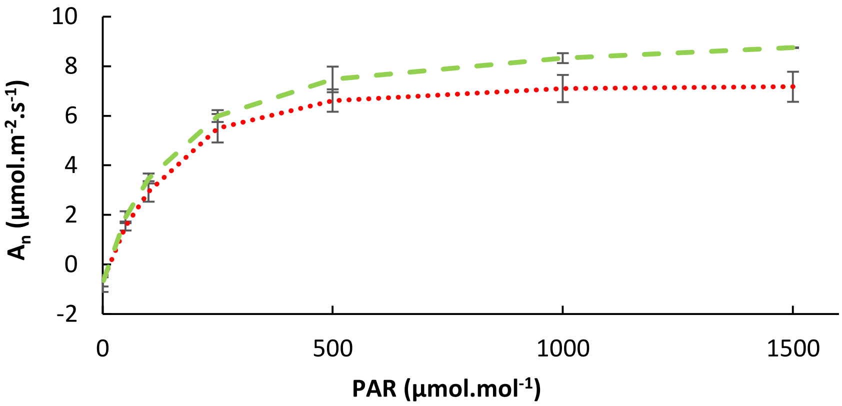


Figure 6-10. The average photosynthesis light response curve of apple trees (– –) and tomato plants (·····) (n=36).

3.2.3 LIGHT SATURATED NET PHOTOSYNTHESIS

The values of net photosynthesis (A_n) are summarized in **Figure 6-11**. A significant increase of net photosynthetic rate was observed for the treated apple trees compared to the control. This effect was noticed for the kaolin powder and most zeolites. However, there were no significant differences among the zeolite/kaolin treatments. A trend towards increased photosynthesis was noticed during the first two weeks after one treatment. Afterwards, photosynthesis started to decrease until it reached its initial value.

In contrast with these findings, no significant differences in net photosynthetic rate were noticed for the treated tomato plants compared to the control ones. However, a similar trend, as observed within the apple trees, was seen. Photosynthesis was increased during the first weeks for all sample treatments with the exception of the control, followed by a decrease until it reached its original rate.

In order to explain these findings, the various abiotic factors that influence the photosynthesis are taken into account. Photosynthesis responds to changes in many environmental parameters, such as light, temperature and CO_2 concentrations (Schroeder et al., 2001; Lombardozzi et al., 2012). These three main factors, also called the limiting factors, play a crucial role on leaf level controlling the stomatal aperture (Tuzet et al., 2003; Toole and Toole, 2004; Lombardozzi et al., 2012).

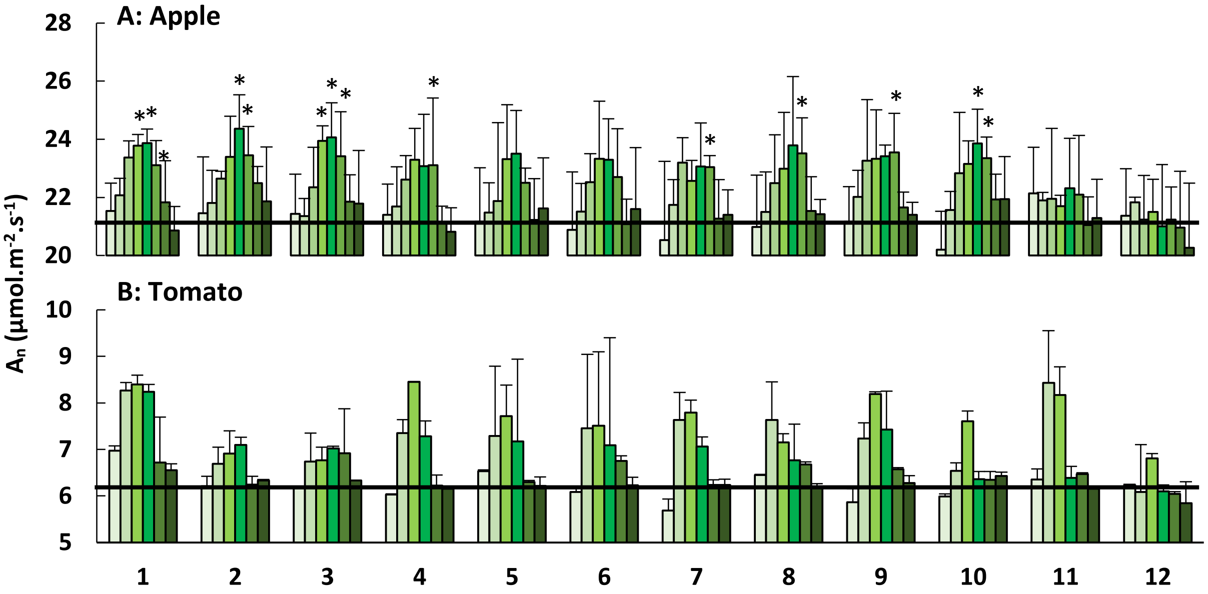


Figure 6-11. Net photosynthesis (A_n; ± SE) of apple trees (A) and tomato plants (B) treated with the plant coating materials (**Table 6-1**) as function of time. Asterisks indicate significant differences ($P < 0.05$) in A_n between sample and control treatments at a certain time examination point. The black line indicates the average A_n of the control treatments and the different colours indicate the different measurements during 4 weeks (n=5).

Light is directly involved in the photolysis of water and excitation of chlorophyll molecules. Indirectly, it controls stomatal movement, leading to diffusion of CO_2. As light intensity increases, photosynthesis increases up to a certain limit. At very high light intensity, photosynthesis is inhibited due to other factors. Light intensity requirement varies with the nature of the plant. However, photosynthesis can also increase in plants exposed to weak light for a longer period (Sinha, 2004). This depends on the weakness of the light and the exact exposure duration. In this research photosynthesis was measured at a constant light intensity of 1500 µmol.m^{-2}.s^{-1}. Therefore light intensity will not affect the observed results.

Additionally, temperature directly influences the rate of photosynthesis by stimulating the rates of activity of photosynthetic enzymes and the electron transport chain. At low temperature the rate of photosynthesis increases proportionally with the temperature until it reaches an optimum. Higher temperatures reduce photosynthesis (Toole and Toole, 2004). An increase in air temperature indirectly increases leaf temperature, which elevates vapour pressure deficit (VPD) and stimulates water loss by transpiration (Schulze et al., 2005). This can also be seen in the results displayed in **Figures 6-14** and **6-15**. Especially the apple trees showed a linear relation between the air temperature and the rate of photosynthesis. Additionally, these figures clearly point out that an increase in air temperature leads to an increase in leaf temperature. However, a higher leaf-to-air temperature was observed for the tomato plants.

As long as water is not the limiting factor, plants will diminish their leaf temperature by transpirational cooling (Salvucci and Crafts-Brandner, 2004; Damour et al., 2010). As evaporative demand increases, stomata tend to close to reduce the rate of water loss, resulting in reduced photosynthesis (Lloyd and Farquhar, 2008). Overall, the stomata are regulated to maximize carbon gain and minimize water loss (Bonan, 2002). **Figure 6-12** illustrates the measured transpiration rates for the apple trees and tomato plants, observed when measuring the net photosynthesis.

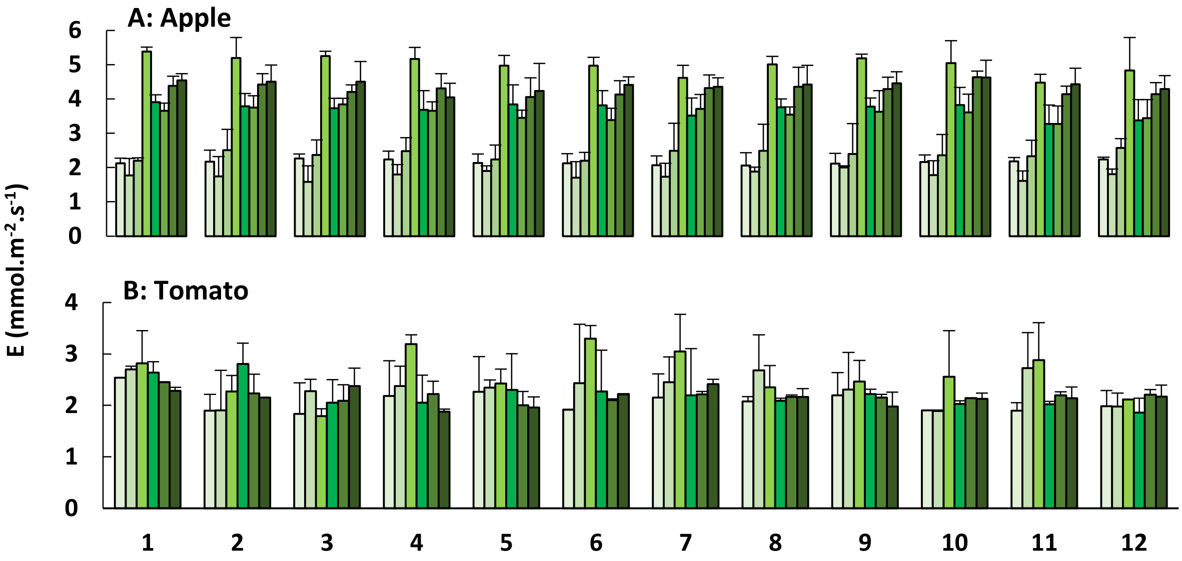


Figure 6-12. Transpiration (E; ± SE) of apple trees (A) and tomato plants (B) treated with the plant coating materials (**Table 6-1**) as function of time. The different colours indicate the different measurements during 4 weeks (n=5).

For both species, the observed transpiration rates were within the range reported by Saenger (2002). According to the latter, transpiration rates vary among species, but range from 0.5 to 6.69 mmol.m^{-2}.s^{-1}. The lower transpiration rates of the tomato plants can explain the higher leaf-to-air temperature that was observed in **Figure 6-15**. For the apple trees, it can be noticed that the transpiration rates increased when the air and leaf temperature increased. In contrast, the tomato plants were grown at a constant temperature of 26°C, what resulted in a constant transpiration of the leaves.

Furthermore, the transpiration rates of the apple trees showed very low values during the first two weeks compared to the subsequent measurements. Data on relative humidity, illustrated in **Figure 6-14**, confirmed a defect of the measuring device. The first three measurements of the relative humidity were too low, indicating that something was wrong.

Normally, higher temperatures reduce relative humidity, what leads to an increase in vapour pressure deficit (VPD). This is also illustrated in **Figures 6-14** and **6-15**. Consequentially, the stoma will tend to close what will reduce photosynthesis and transpiration. However, in some plants, stomata remain open at high temperature even when photosynthesis is significantly reduced and vapour pressure deficit is very high (Schulze et al., 1972; Lu et al., 2000). This response has been documented in only a few species and is not well understood, although it appears to be a strategy that uses transpirational cooling of the leaf to avoid or minimize heat stress.

The density, size and degree of opening of the stomata have an impact on the rate of photosynthesis and transpiration. This rate of passage is controlled by stomatal conductance. Under most environmental conditions, a close correlation between photosynthesis and stomatal conductance is expected. Stomata close to reduce water loss, resulting in reduced photosynthesis and transpiration. Alternatively, if biochemical limitations reduce photosynthesis, intercellular [CO_2] increases, resulting in partial stomatal closure. **Figure 6-13** represents the stomatal conductance for the apple trees and tomato plants, observed during the measurement of the net photosynthesis.

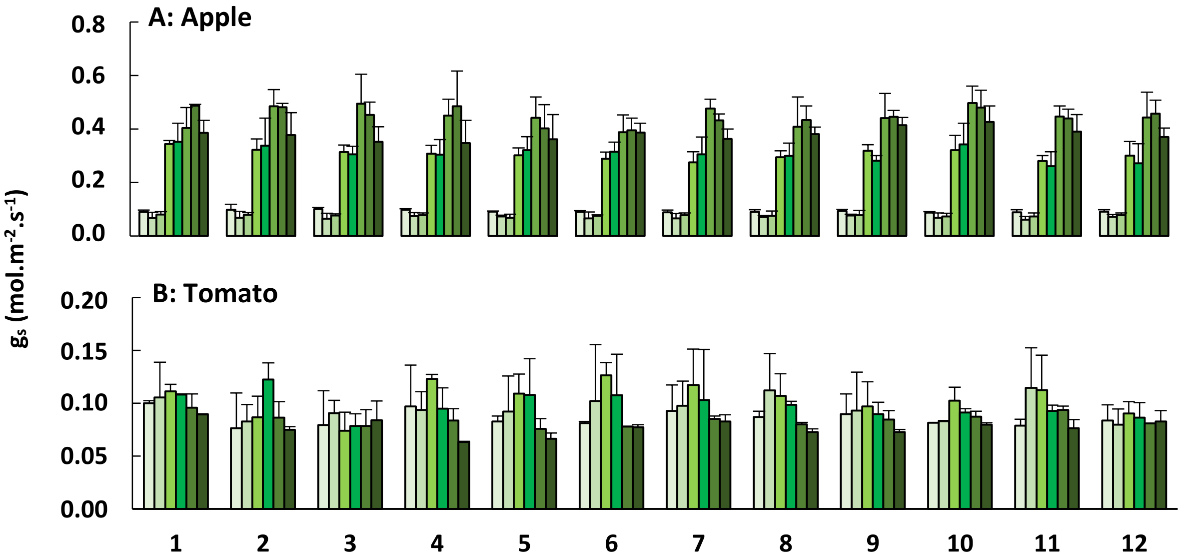


Figure 6-13. Stomatal conductance (g_s; ± SE) of apple trees (A) and tomato plants (B) treated with the plant coating materials (**Table 6-1**) as function of time. The different colours indicate the different measurements during 4 weeks (n=5).

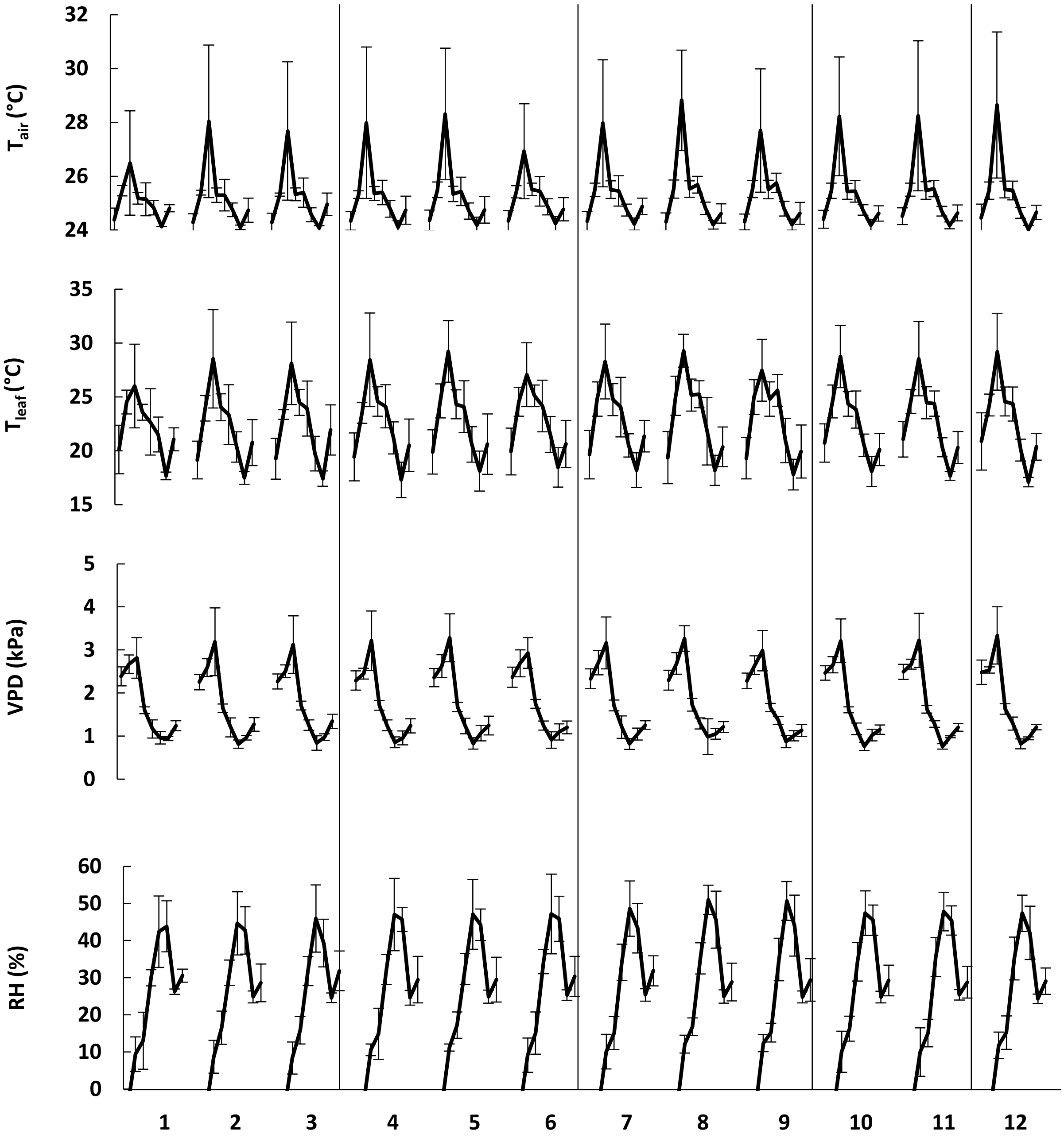


Figure 6-14. The ambient parameters, i.e. air temperature (T_{air}), leaf temperature (T_{leaf}), vapour pressure difference (VPD) and relative humidity (RH), involved during the measurements on treated apple trees (**Table 6-1**). The different data points per treatment indicate the mean (± SE) of different measurements during 4 weeks (n=5).

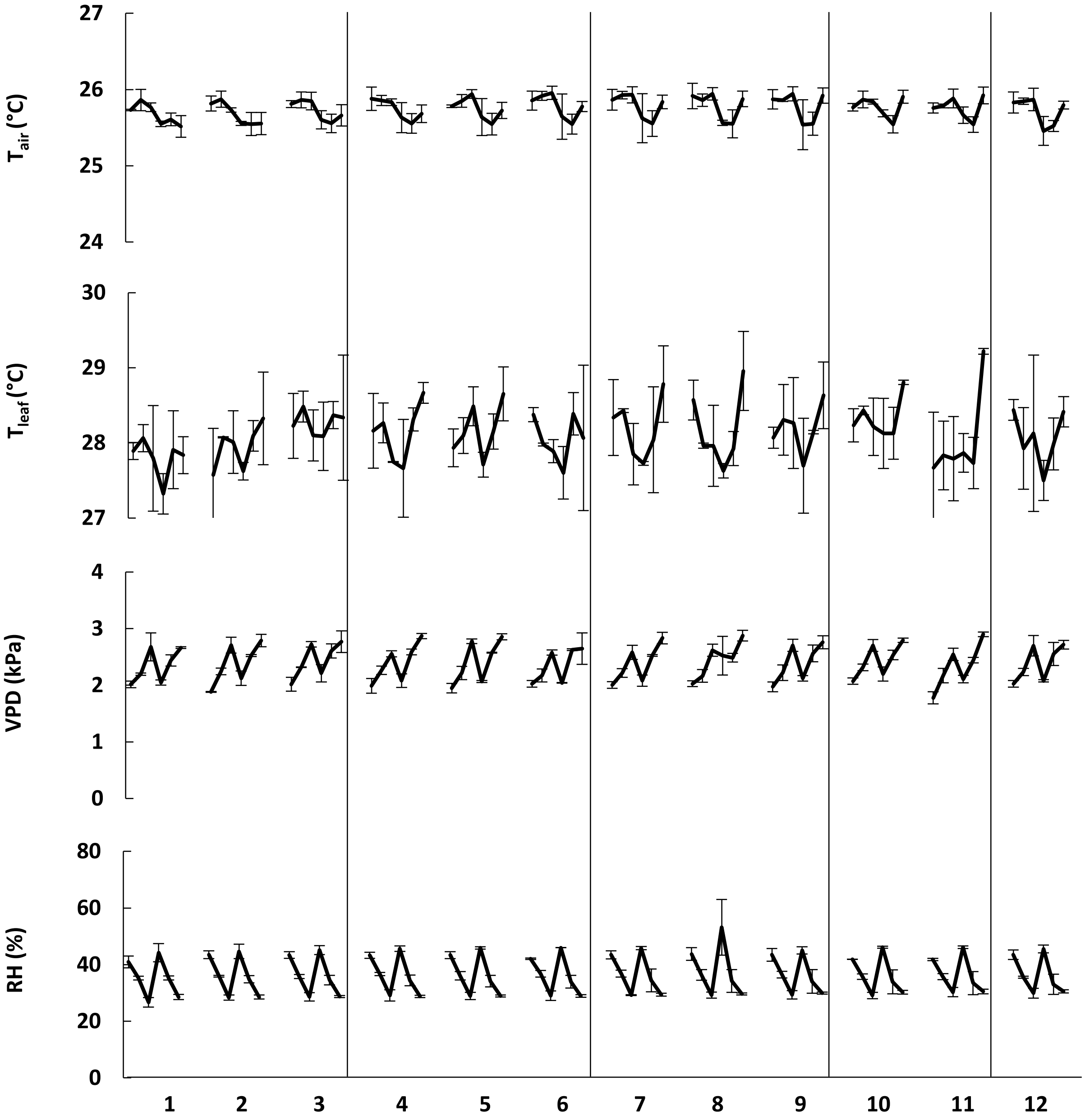


Figure 6-15. The ambient parameters, i.e. air temperature (T_{air}), leaf temperature (T_{leaf}), vapour pressure difference (VPD) and relative humidity (RH), involved during the measurements on treated tomato plants (**Table 6-1**). The different data points per treatment indicate the mean (± SE) of different measurements during 4 weeks (n=5).

Similar patterns in stomatal conductance and net photosynthesis were observed. This linear relation between g_s and A_n was also confirmed by Yu et al. (2004), who measured responses of photosynthesis and stomatal conductance of winter wheat to changes in light intensities. The stomatal conductance of the apple trees also showed very low values during the first two weeks compared to the subsequent measurements. Generally seen, the apple trees and tomato plants showed an increase in A_n and g_s during the first measurements. An increase of CO_2 concentration due to the adsorption of CO_2 molecules on the zeolites can be a possible explanation. After a while the elevated CO_2 concentration reduces g_s, which leads to a decrease in A_n. This latter has also been observed in previous research (Medlyn et al., 2001; Long et al., 2004; Ainsworth and long, 2005; Ainsworth and Rogers, 2007). However, the effect of elevated CO_2 concentration on A_n does not always match the theoretical expectations (Long et al., 2004; Nowak et al., 2004; Ainsworth and Long, 2005; Rogers et al., 2006). The effect is variable and subjected to environmental feedback (Ellsworth, 1999; Medlyn et al., 2001; Gunderson et al., 2002; Wullschleger et al., 2002; Naumburg et al., 2003; Bunce, 2004; Herrick et al., 2004; Long et al., 2004; Marchi et al., 2004; Morgan et al., 2004; Nowak et al., 2004; Leakey et al., 2006).

A relation between stomatal conductance and transpiration was also noticed. This can be linked to the interaction of climatic factors, i.e. temperature and relative humidity, as discussed before. An experiment conducted with kaolin instead of zeolites showed a similar increase in g_s, A_n and E of the treated leaves compared to control ones (Maletsika and Nanos, 2015).

3.2.4 CHLOROPHYLL A FLUORESCENCE

Finally, another potential limiting factor is chlorophyll content, which is qualitatively obtained as “leaf greenness”. Emission measurements of chlorophyll fluorescence has been used as a tool to understand the behaviour of plant growth. Water stress and cold stress in plants affect the normal operation of photosynthesis and these can also been detected by chlorophyll fluorescence measurements (Khanizadeh et al., 2000; Rennie et al., 2001).

The greater the value of F_v'/F_m', the greater the maximum efficiency of the PSII photochemistry (Olvera-Gonzalez et al., 2013). Values obtained for the apple trees and tomato plants varied between 0.6-0.7 and 0.4-0.5, respectively (**Figure 6-16**). These values indicate that the examined apple trees and tomato plants were healthy plants without stress. Mir et al. (1998) measured chlorophyll fluorescence from apple leaves with surface defects. For apple leaves with CO_2 injury, healthy regions had F_v'/F_m' of approximately 0.75 and decreased as low as 0.33 in the CO_2 damaged region. Loukehaich et al. (2011) found that healty regions of tomato leaves had F_v'/F_m' of approximately 0.4-0.6.

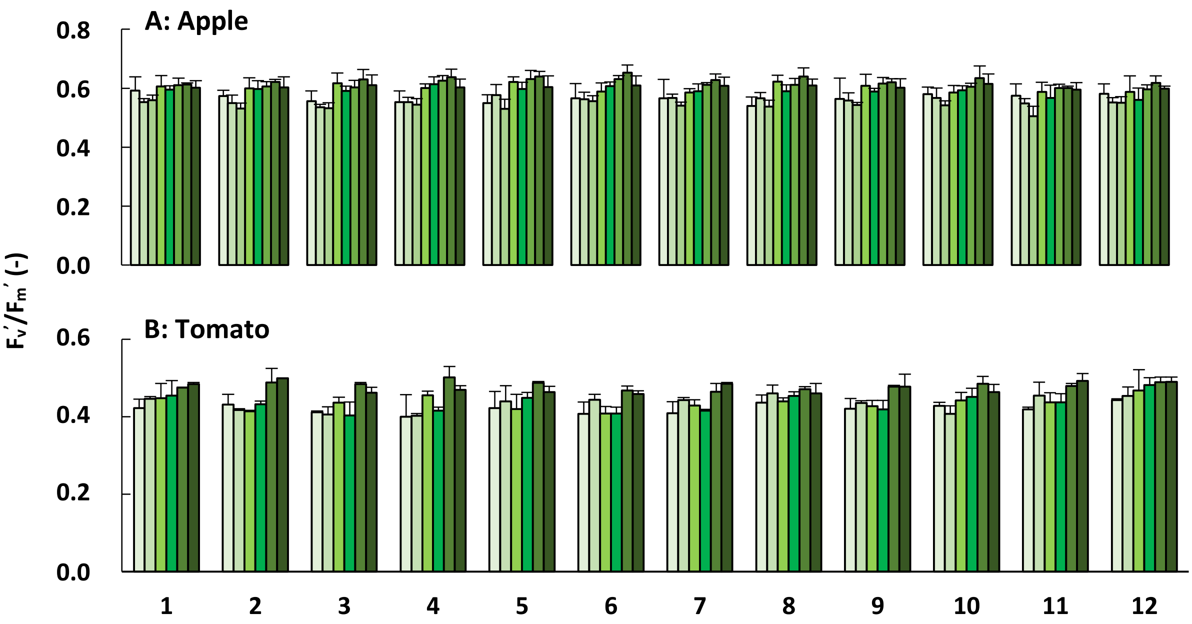


Figure 6-16. Chlorophyll a fluorescence (F_v'/F_m'; ± SE) of apple trees (A) and tomato plants (B) treated with the plant coating materials (**Table 6-1**) as function of time. The different colours indicate the different measurements during 4 weeks (n=5).

3.2.5 GAS EXCHANGE WATER USE EFFICIENCY

As mentioned in Section 2.4.4, gas exchange WUE includes $WUE_{instantaneous}$ and $WUE_{intrinsic}$. The results obtained for the apple trees and tomato plants are presented in **Figure 6-17**. From a methodological viewpoint, $WUE_{instantaneous}$ and $WUE_{intrinsic}$ are similar, but $WUE_{instantaneous}$ varies with atmospheric conditions (temperature and humidity) which affect vapour pressure deficit (VPD). Since this is not the case for $WUE_{intrinsic}$, this parameter gives a better approach of WUE (Medrano et al., 2015).

As a result of the constant difference in water vapour concentration between the leaf and the air, transpiration and stomatal conductance are linear related (Lambers et al., 2008). An improved and higher WUE can be achieved either through lower stomatal conductance and transpiration, caused by stomatal closure, or higher photosynthetic capacity or a combination of both (Condon et al., 2002; Ashraf and Bashir, 2003; Long et al. 2004). Therefore, the first three data points of the apple trees were not taken into account. In **Figure 6-17**, it can be seen that $WUE_{intrinsic}$ decreases when stomatal conductance and transpiration increases and vice versa. However, one can have a high stomatal conductance, but plant transpiration can still be low, because incoming solar radiation and water vapour deficit are for instance reduced.

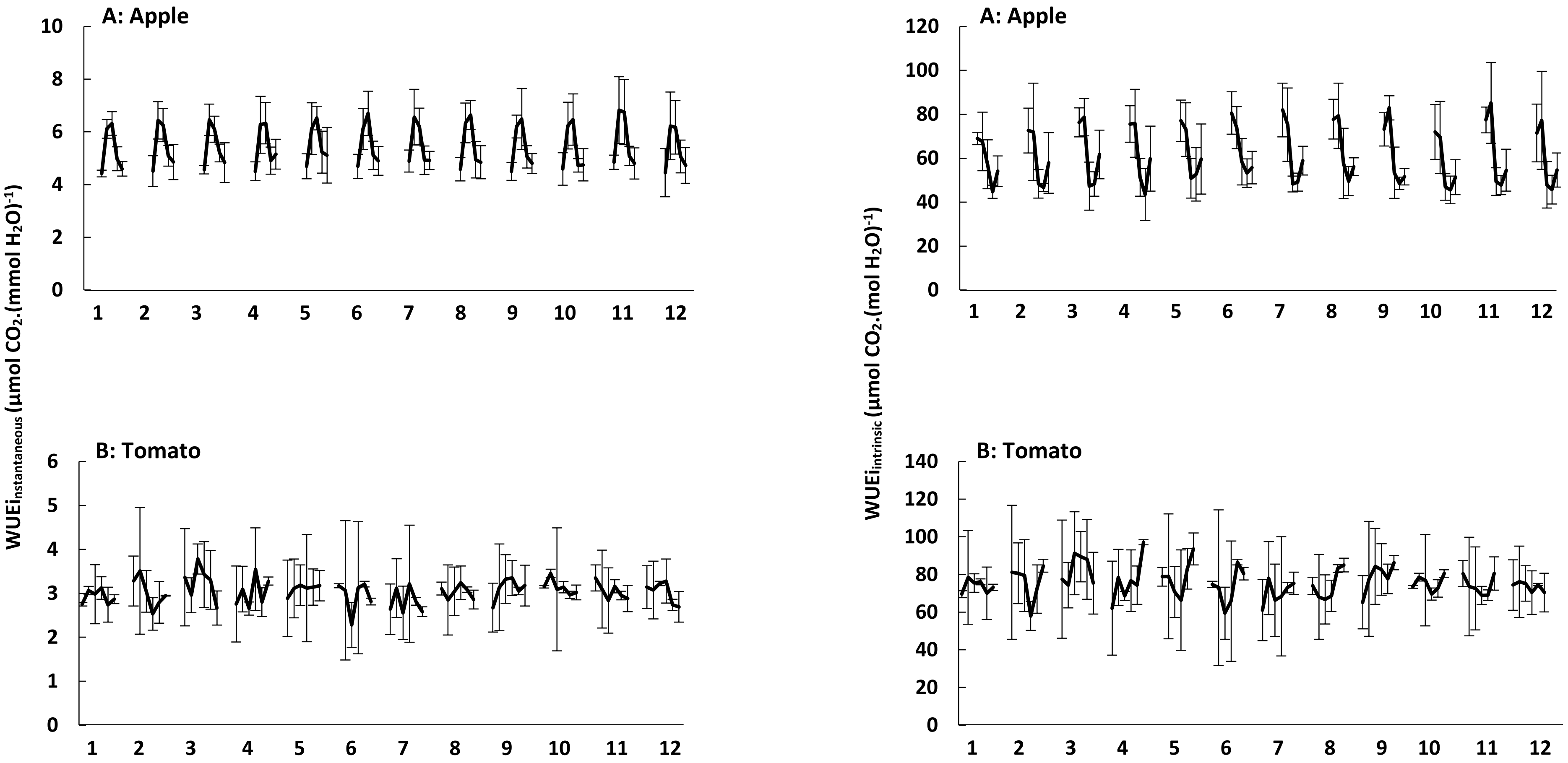


Figure 6-17. Instantaneous water use efficiency ($WUE_{instantaneous}$ or $A_n.E^{-1}$; ± SE) and intrinsic water use efficiency ($WUE_{intrinsic}$ or $A_n.g_s^{-1}$; ± SE) of apple trees (A) and tomato plants (B) treated with the plant coating materials (**Table 6-1**) as function of time. The different data points per treatment indicate the different measurements during 4 weeks (n=5).

Finally, WUE of treated leaves was usually similar to control leaves, suggesting that zeolites and kaolin did not increase water consumption without increasing CO_2 fixation (Chaves et al., 2004). This effect was also observed by Maletsika and Nanos (2015), when using kaolin on olive trees. Steiman and Bittenbender (2007) also observed that photosynthesis in kaolin leaves was significantly greater but WUE was not.

4 CONCLUSION

In general, zeolites have some effects on plant and photosynthetic related characteristics. The measured chlorophyll a fluorescence indicated that all tested plants were healthy and free from stress. Plant-related characteristics including stem height and thickness were not significantly influenced by the zeolite/kaolin treatments. Dry weight of the apple trees and tomato plants tended to increase, but this was not significantly different from the control. However, the increase had a positive influence on the measured integrated water use efficiency, which significantly differs from the control. Zeolites do not increase the water consumption of the whole plant, but increase the water uptake of individual leaves. Photosynthetic parameters also improved in the zeolite treatments. Photosynthesis of apple trees increased significantly, which was not the case for the tomato plants. The limiting and ambient factors showed no striking effects.

All these effects were observed during four weeks. It is noteworthy that the overall results increased during the first two weeks followed by a decrease during the last two weeks. This indicates that multiple treatments of the plants are required. This will be necessary for obtaining a better photosynthetic effect of the treated apple trees and tomato plants compared to the control.

"Science never solves a problem without creating ten more."

-George Bernard Shaw-

CHAPTER 7

Chapter 7

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

In this doctoral dissertation, a roadmap is provided towards the development of a new green PPP based on zeolites to be used in agriculture. In Chapter 1, the theoretical work is presented. The following five chapters present the experimental work, which investigated and connected different aspects related to the use of zeolites in agriculture. Subsequently, the current final chapter summarizes the main outcomes from this work and discusses the value of zeolites in commercial practice. Next to that, future perspectives and challenges are presented.

1 GENERAL DISCUSSION

1.1 GENERAL FINDINGS OF THIS STUDY

In the following paragraphs, the main outcomes from this doctoral thesis are summarized.

Chapter 1 summarizes the existing knowledge on zeolites. Since zeolites have important beneficial effectsonfungal diseases, insect pests and plant properties, they have the potential to become the future green alternatives that replace conventional PPPs. In **Chapter 2**, a theoretical deduction is presented describing the selection of zeolites appropriate for the use in agriculture. As conclusion, non-phytotoxic, water-holding zeolites with high adsorption capacities were selected. Seven different zeolite framework types were selected and further studied. Two zeolite framework types have met these requirements, i.e. zeolite type BEA and FAU. Additionally, a case-study was performed comparing the adsorption capacity of zeolites compared to other adsorbents, i.e. MOFs, activated carbon and resins. It could be concluded that activated carbon and carbonaceous resins exhibited higher adsorption capacities compared to zeolites and MOFs. However, the high affinity of activated carbon and carbonaceous resins for the PPPs makes regeneration of these materials difficult. Therefore, zeolites and MOFs are attractive materials when it comes to regeneration.

Chapter 3 describes the development of two different wettable powder formulations based on zeolite type BEA and FAU. The effect of these zeolite formulations, together with the ECO-ZEO formulations LTA 800 SC and 850 WP, on fungal diseases, insect pests and plants was studied in the next chapters (**Chapter 4, Chapter 5 and Chapter 6**).

From the results described in **Chapter 4**, it could be concluded that the test products had an antifungal activity. The inhibition test showed especially good results against *B. cinerea*. The disease incidence and severity assessments showed better results against *V. inaequalis*. Also, the results of the inhibition test made clear that the formulated zeolites obtained better results, from which it could be hypothesized that the antifungal activity was mainly caused due to adjuvants present in the formulation. This finding was confirmed by further research in this chapter.

In **Chapter 5,** it was demonstrated that the zeolites had no toxic effect on *T. absoluta* eggs and no repellent effect on *T. absoluta* females. In addition, no mortality of *L. decemlineata* eggs was observed. However, some growth inhibition occurred, suggesting that the feeding behaviour of *L. decemlineata* may have been affected.

Finally, in **Chapter 6,** the effect of zeolites on plant growth, water use efficiency and photosynthesis was evaluated. Only the plant photosynthesis was improved. The increase in photosynthesis was especially observed for the tested apple trees, but not for the tested tomato plants.

1.2 KEY FINDINGS OF THIS STUDY

Based on the general findings described in the previous Section 1.1, it can be concluded that there are three key findings in this doctoral thesis, i.e. (1) the adsorption capacity of the different PPPs by zeolites, (2) the antifungal activity of zeolites and the adjuvants present in formulations, and (3) the influence of zeolites on the increase of photosynthetic activity. Subsequently, these findings will be further discussed below.

1.2.1 ADSORPTION OF DIFFERENT CROP PROTECTION PRODUCTS BY ZEOLITES

Environmental problems associated with the use of PPPs are a matter of concern because PPPs may eventually end up in other parts of the environment, such as in soil or water. It is estimated that only 80% of the applied PPPs reach their targeted areas (Forster, 2004). One of the major concerns about the use of these organic compounds in agronomy and horticulture is their ability to leach into groundwater; especially, the highly mobile PPPs are a matter of current concern (Kalkhoff et al., 1998; Kolpin et al., 1998; Colella, 2007).

Several techniques have been developed for the removal of PPPs from water. Adsorption on activated carbon is the most widely used technology for purification of water contaminated by PPPs and other hazardous chemicals (De Wilde et al., 2009). However, there is an increasing interest in the use of natural materials (Bowman et al., 2000; Ranck et al., 2005).

Based on the results in Chapter 2, zeolites proved their applicability as adsorbent for treating waters contaminated with PPPs. Zeolites BEA and FAU obtained the best adsorption capacity, compared with other zeolites. These results are consistent with the higher values of the surface area, the Si/Al ratio and the pore limiting diameter of the zeolites. However, the mobility of the PPPs also has an influence of the adsorption rate. In general, non-ionic PPPs are relatively less mobile than ionic PPPs, and consequently their affinity for zeolites is higher (De Wilde et al., 2009). Therefore, not every zeolite is able to adsorb all PPPs.

When comparing the adsorption capacity of zeolites with other sorbents, such as activated carbon, polymeric resins and metal organic frameworks, various results were obtained. The zeolites and MOFs were able to adsorb the PPPs more rapidly in comparison with the other adsorbents, i.e. the activated carbon and a carbonaceous resin. However, in general, the latter showed generally the best adsorption capacities for all PPPs. Nevertheless, it should be noticed that the adsorption tests on MOFs were carried out when the usage and synthesis of MOFs was still very new at the lab. At this moment, new and more stable types of MOFs are known, which would result in a better adsorption of PPPs. For example, covalent porous crystalline polymers or COFs are new nanoporous materials. COFs are covalent porous crystalline polymers that enable the elaborate integration of organic building blocks into an ordered structure with atomic precision (Feng et al., 2012).

Zeolites are stable over a wide range of temperatures and acidic conditions (Anderson, 2000). Therefore, it is not expected to have loss of material during regeneration, which is the opposite for carbon. As a consequence, zeolites are not used only once and destroyed after saturation, as often is the case with activated carbon. However, it should be stressed that activated carbon is effective for adsorption of a broad range of solutes, while zeolites are selective. Due to their selectivity, zeolites should not be regarded as a replacement for activated carbon, but rather as an additional, dedicated adsorbent for a limited amount of target solutes. The activated carbon can remove the high influent concentrations, while the zeolite can remove the lower influent concentrations or concentrations that show poor removal with activated carbon (Lenntech, 2016). This has already been proposed for the adsorption of volatile organic compounds, but can also be applied for the removal of PPPs from water.

1.2.2 ANTIFUNGAL ACTIVITY OF ZEOLITES OR ADJUVANTS?

The results of Chapter 4 indicate some fungicidal effect of the zeolite formulations. However, further research confirmed that the adjuvants present in the formulations were responsible for the observed fungicidal effect. Insecticidal and fungicidal effects of adjuvants applied in high dosages can create a miscalculation on the activity of an active ingredient. Therefore, it is important to measure the possible impacts of adjuvants, i.e. toxicity, activity or synergistic effect.

Such selection tools are already widely used for active ingredients of PPPs and are called pesticide risk indicators (PRIs). These PRIs combine a range of data of the active ingredient (application rate data; physical, chemical and toxicological data; etc.) into a single or composite index for a comparative analysis of the risks posed by different PPPs (Labite et al., 2011).

Unfortunately, there is no legislation for adjuvants. The EU regulation (EC) N_o 1107/2009 states that it is required that the names and amounts of active ingredients, safeners and synergists be made public. Adjuvants do not fall into either one of these categories, and are thus exempt from public disclosure (Beck et al., 2012). This regulation, concerning the placing of PPPs on the market, has resulted in the withdrawal of many active ingredients. Since this is not the case for the adjuvants, some commercial products can also be toxic, regardless the active ingredient (Beck et al., 2012). These miscalculations on activity and toxicity of adjuvants have already been occurred.

For example, organosilicones may show insecticidal activity to several pests, however, the mechanism that causes the toxicity to insects and mites has not been adequately determined. The adjuvant Silwet L-77 has been reported to be effective against insects. Silwet L-77 alone was toxic to young *Diaphorina citri* (Hemiptera: Psyllidae) nymphs and increased the toxicity of imidacloprid and abamectin at lower-than-label rates. The adjuvant Kinetic also showed toxicity against *D. citri*, but less compared to Silwet L-77 (Srinivasan et al., 2008; Cocco and Hoy, 2008). Subsequently, naphthalene derivatives were found to have potent antibacterial and antifungal activity (Rokade and Sayyed, 2009).

Nevertheless, these insecticidal and fungicidal effects of the adjuvants are not the biggest concern. This in contrast with the possible toxic effects of some adjuvants. A well-known example is the commercial product Roundup. Glyphosate is still generally hypothesized to be the active ingredient for non-target side effects in Roundup. This may be related to adjuvants in formulation, which are more and more considered as responsible for glyphosate-based-herbicide toxicity (Mesnage et al., 2010; Williams et al., 2012). This is a general question that can arise for all PPPs. The detailed known composition of Roundup indicate that major adjuvants are ethoxylated, such as polyethoxylated alkylamines (POEAs) (Mesnage et al., 2013). In conclusion, PPP formulations should be studied as mixtures for toxic effects.

1.2.3 THE EFFECT OF THE INCREASE IN PHOTOSYNTHETIC ON FRUITS

It is known that zeolites are able to adsorb CO_2 molecules and release them slowly into the environment (Jaramillo and Chandross, 2004; Montanari and Busca, 2008). Based on the effect of kaolin, it was suggested that zeolites may also have beneficial effects on plants. These effects include a better light use efficiency, growth and leaf area production, as well as a higher photosynthesis rate. Furthermore, the water usage decreases because of a lower transpiration rate, which further accelerates leaf development.

The results of Chapter 6 illustrated that the zeolites were able to increase the photosynthesis of the apple trees. Despite no other benefits were observed, the increase in photosynthesis was an important finding. An increase in photosynthesis can also have beneficial effects on the fruits. This effect was not studied, but will be discussed in this section.

An increase in fruit yield and fruit size was observed after treating apple trees with a kaolin-based particle film (Glenn et al., 2002, 2005; Gindaba and Wand, 2015). Next to that, plant photosynthesis might affect the fruit taste. In general, the concentrations of sugars and vitamins in mature fruit are modulated by climate (temperature, sunlight, rainfall), whereas mineral contents are closely related to soil fertility. The availability and transport of carbohydrates from leaves to fruit determines the sugar content (Zhou et al., 2000). Environmental factors that improve the plant’s photosynthetic capacity, and therefore sugar metabolism, can generate sweeter fruit (Zhou et al., 2000).

Finally, sunburn of apples causes large economic losses to growers in several regions of the world. Fruits are more susceptible to sunburn compared to leaves, mainly because they are not endowed with efficient mechanisms of using and/or dissipating solar radiation (Jones, 1981; Blanke and Lenz, 1989). As a result, fruit surface temperature may rise to as high as 10 to 15°C higher than air temperature (Parchomchuk and Meheriuk, 1996). The positive effects of kaolin for controlling sunburn in various fruit have recently been well documented (Glenn et al., 2002; Le Grange et al., 2004; Melgarejo et al., 2004; Schupp et al., 2002; Wünsche et al., 2004). These positive effects for controlling sunburn were also observed during the ECO-ZEO project. Experiments were carried out on two sensitive apple varieties, i.e. a high susceptible variety (Granny Smith) and a medium susceptible variety (Golden). Zeolite 4A, as raw material (non-formulated powder spraying), showed some efficacy similar to kaolin clay.

2 POSSIBLE IMPACT OF ZEOLITES IN AGRICULTURE ON ECONOMIC, ENVIRONMENTAL AND SOCIAL LEVEL

2.1 ECONOMIC DIMENSION

Natural and synthetic zeolites are the key product segments of the zeolites market. Natural zeolites accounted for over 60% share of the total zeolite volume consumed in 2014. Synthetic zeolites are expected to be the fastest growing segment of zeolite market during the forecast period 2014-2020. This can be attributed to the rapidly growing demand for synthetic zeolite as a catalyst, adsorbent and detergent builder (Zion RC, 2015).

According to Zion RC (2015), the global zeolite market was valued at around USD 3.50 billion in 2014 and is expected to reach USD 4.50 billion in 2020, growing at a compound annual growth rate (CAGR) of around 3.5% between 2015 and 2020. In terms of volume, the global zeolite market stood at above 3 million tons in 2014 (**Figure 7-1**).

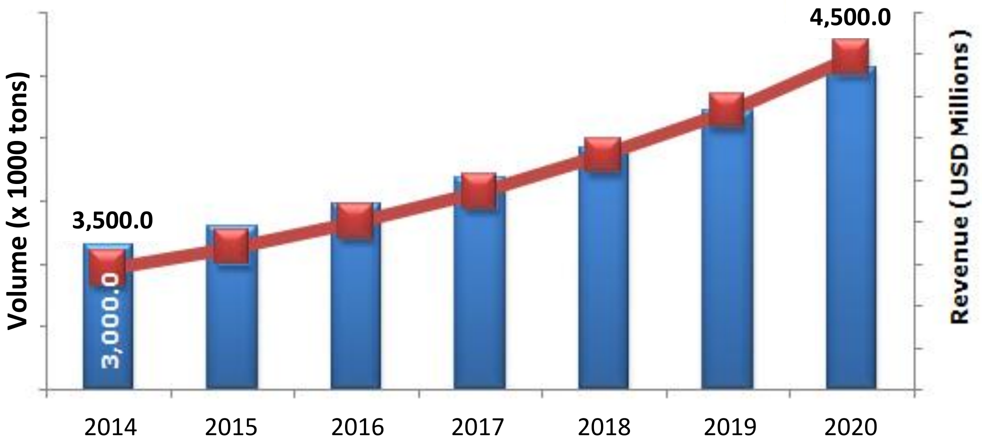


Figure 7-1. Global zeolite market from 2014-2020, indicating the consumption volume (🟦) and revenue (🟧) (Zion RC, 2015).

Prices for natural zeolites vary with zeolite content and processing. Prices listed in 'Industrial minerals and rocks' for industrial or agricultural applications were \$30 to \$70 per ton for granular products up to 400 µm and \$50 to \$120 per ton for ground material ranging from 400 µm to 44 µm. Quoted prices should be used only as a guideline because actual prices depend on the terms of the contract between seller and buyer (Virta, 2001).

Based on private communication with companies specialized in zeolite production and product formulation, the cost price of the zeolite formulations was calculated. The examples of formulation LTA 800 SC and LTA 850 WP are demonstrated in **Table 7-1** and **Table 7-2**. The cost price of the other WP formulations (BEA and FAU) is similar to that of LTA 850 WP. The calculated cost price of the zeolite formulations makes it possible to compare zeolites with a commercial PPP. **Table 7-3** illustrates a comparative performance of the cost price among the zeolite formulations and the commercially used PPPs in agriculture. These results demonstrated that the conventional PPPs are still cheaper to use. Of course, this depends on the used product. The zeolite formulation may be cheaper compared to some other products, e.g. kaolin and CpGV.

Given the limited economic advantages, it is questionable whether zeolites will substitute conventional PPPs. A small increase in PPP cost may be a clear burden for farmers to make the switch. However, visible residues of the zeolites on fruits or crops are often not acceptable and additional treatments are needed, e.g. water rinsing, which will increase the production cost. In some cases this treatment cannot be made without resulting in a depreciation of the fruit or crop and consequently a lower sale price. Higher cost production and lower sale price reduce the farmer's benefit.

Table 7-1. Cost price calculation of LTA Zeolite 800 SC.

LTA Zeolite 800 SC						
					For 1000 l	
Product	**€.kg^{-1}**	**Customs (€)**	**Shipping (€)**	**∑€**	**Material (kg)**	**Value (€)**
Zeolit 4A FMC Foret	**0.50**	0.02	0.001	0.53	800.00	420
Active substance						**420**
MORWET D425	**5.30**	0.25	0.011	5.57	30.00	167
SAG 1572	**13.00**	0.62	0.026	13.65	0.30	4
SUPRAGIL WP	**3.90**	0.19	0.008	4.10	10.00	41
REAKS 88 B	**5.00**	0.24	0.010	5.25	10.00	53
Rhodopol 23	**0.28**			0.28	20.00	6
Amebact C	-	-	-	-	-	-
Water	**0.01**	-	-	0.01	580.00	5
Additives						**275**
Scotch tape	0.57				2.0	1
box	0.35				83.34	29
Blank labels	0.01				83.3	0
Bottle HDPE	0.14				1 000.0	143
Cap bottle	0.03				1 000.0	34
Label 1l	0.02				1 000.0	16
Packaging material						**224**
Working cost		5.00			10.0	**50**
		Recap			**LTA Zeolite 800 SC**	
Active Substance					**1 L**	0.42
Additives						0.27
Packaging material						0.22
Complete Material Cost						**0.92**
Working cost						0.05
Complete Production Cost						**0.97**

Table 7-2. Cost price calculation of LTA Zeolite 850 WP.

LTA Zeolite 850 WP						
					For 1000 l	
Product	**€.kg^{-1}**	**Customs (€)**	**Shipping (€)**	**∑€**	**Material (kg)**	**Value (€)**
Zeolit 4A FMC Foret	**0.50**	0.02	0.001	0.53	850.00	446
Active substance						**446**
PVP K-30	**19.90**	0.96	0.040	20.90	50.00	1 045
Ultrasil VN 3	**1.66**			1.66	68.00	113
SOPROFOR	**2.60**	0.12	0.005	2.73	30.00	82
Additives						**1240**
Scotch tape	0.57				2.0	1
box	0.48				50.0	24
Blank labels	0.01				50.0	0
Foil	5.63				12.0	68
Packaging material						**93**
Working cost		5.0			10.0	**50**
		Recap			**LTA Zeolite 850 WP**	
Active Substance					**1 L**	0.45
Additives						1.24
Packaging material						0.09
Complete Material Cost						**1.78**
Working cost						0.05
Complete Production Cost						**1.83**

Table 7-3. Comparative performance of the total PPP cost among the zeolite formulations and the commercially used PPPs.

	Total PPP cost of the active ingredients used in this study[a]				
Active ingredient	Zeolite	Thiram	Chlorothalonil	Spinosad	Kaolin
Commercial product	Zeolite 800/850/920/950 SC/WP	Hermosan 80 WG	Daconil 50 SC	Conserve Pro SC	Surround WP
Formulation type	SC/WP	WG	SC	SC	WP
Active ingredient contents (%)	80/85/92/95	80	50	12	95
Application method	Spray	Spray	Spray	Spray	Spray
Application rate (kg product.ha^{-1})	50-60	2.2	1.5	0.8	25-40
N_o applications	4	4	2	3	4
Price (€.kg^{-1} or €.l^{-1})	1-2	11.7	22	120.81	3.16
PPP cost per application (€.ha^{-1})	50.00/100.00-60.00/120.00	25.74	33.00	96.65	79.00-126.40
Total PPP cost (€.ha^{-1})	**200.00/400.00-240.00/480.00**	**102.96**	**66.00**	**289.94**	**316.00-505.60**

	Total PPP cost of other commercially used active ingredients[a]					
Active ingredient	Metalaxyl	Mancozeb	Cymoxanil	Fenoxycarb	CpGV	Copper oxychloride
Commercial product	Xiluq plus	Mancopec 80	Curzate 60 WG	Insegar	Madex Top	Nucop 50 WP
Formulation type	WP	WP	WG	WG	EC	WP
Active ingredient contents (%)	25	80	60	25	10^{13} granuls.l^{-1}	50
Application method	Spray	Spray	Spray	Spray	Spray	Spray
Application rate (kg product.ha^{-1})	1.2	3.2	0.2	0.6	0.1	4
N_o applications	4	4	5	2	10	4
Price (€.kg^{-1} or €.l^{-1})	33.49	6.12	58.05	150.00	405.00	5.57
PPP cost per application (€.ha^{-1})	40.19	19.58	11.61	90.00	40.50	22.28
Total PPP cost (€.ha^{-1})	**160.75**	**78.34**	**58.05**	**180.00**	**405.00**	**89.12**

[a] The colours indicate (1) a lower cost price (🟩) to those of the zeolite formulations (🟨), (2) a cost price similar (🟧) to those of the zeolite formulations and (3) a higher cost price (🟥) to those of the zeolite formulations.

2.2 ENVIRONMENTAL DIMENSION

According to the EU directive 67/548/EEC and Regulation (EC) N_o 1272/2008, the used zeolites in this study are not considered to be dangerous for the environment. However, zeolites have a low solubility in water, what constitutes a transport pathway into the sediment. Additionally, inorganic compounds such as zeolites are not biodegradable. Subsequently, the application of these formulated products at concentrations around 60 kg.ha^{-1} to the target crop will have an impact on the soil levels of sodium, aluminium and silicate. Consequently, the use of zeolites can have an effect on the present soil microorganisms, predators, parasitoids and pollinators.

2.2.1 SOIL QUALITY

The total level of any nutrient or element in soil does not necessarily translate as plant availability and therefore, the conditions under which these elements may become plant available and potentially toxic or harmful to the plant should be considered.

Since zeolites are composed of silica and alumina, an increased level of these minerals in soil is expected after repeated applications. An increase of silica levels will add to the overall cation exchange capacity (CEC) of the soil in time. However, this will depend on the soil pH and its management, which can have a major effect on plant nutrient availability.

Whilst aluminium is not an essential element for either plants or animals, it can be translocated into the plant where it accumulates and, at certain levels, become toxic to plant growth. Aluminium is solubilized from silicates and oxides (non-toxic forms) to Al^{3+}, which is phytotoxic only under conditions of low pH. In extremely acid soils, only those species adapted to acid soils can tolerate high soil available aluminium level. In conclusion, the zeolites used in this study are likely to be used in managed orchards, nurseries and vineyards. Consequently, it is unlikely that very low pH conditions would be found, which would exacerbate any problem with increasing aluminium in the soil.

Extra-framework cations, like sodium, can also influence soil quality. Sodium is also not considered to be a plant essential element. However, excess of sodium under certain soil conditions may cause problems to crop health. Sodic soils tend to have poor structure with unfavourable physical properties, such as poor water infiltration and air exchange, which can reduce plant growth.

It is expected that serial applications of the zeolite LTA formulations, which contain more sodium compared to the other zeolites, will increase the level of sodium in the soil. Other zeolites that do not have a high level of sodium have been used successfully in soil remediation and as soil amendments and there is evidence that the contribution to the CEC in the soil is of benefit.

2.2.2 AGRO-BIODIVERSITY

Soil microorganisms are of great importance to the stability of the soil ecosystem and as such for the adequate growth of the crop. Therefore, soil microbial activity must be considered when assessing the environmental risks associated with new and existing agrochemicals.

Microorganisms are important to the fertility of soil as their degradation of organic matter and pollutants add to the pool of nutrients available to higher and lower plants. ECO-ZEO had identified two important soil nutrients, nitrogen and carbon, for eco-toxicological studies in soil within the context of EU directive 91/414/EEC. Based on the results, no statistical differences between the control and sample treatments were observed.

Additionally, among wild species that are important for the crop sustainability, predators, parasitoids and pollinators are the three groups that need to be preserved in order to maintain their ecosystem services. Predators and parasitoids of key crop pests are central elements in plant protection for avoiding pest populations to reach damaging levels, and therefore for reducing the amount of PPPs used in the crop. Pollinators are elements of general benefit for both crop and non-crop plants, and it is crucial for the good health of the agro-ecosystem to preserve their abundance and diversity. In order to evaluate the toxicity of zeolites to natural enemies and pollinators, ECO-ZEO performed some toxicity tests.

In apple orchards, the effect of the zeolites was evaluated on the parasitoid *Trichogramma evanescens* (Hymenoptera: Trichogrammatidae) and the predatory mite *Amblyseius swirskii* (Acari: Phytoseiidae). For the tomato crop, the effect of zeolites was evaluated on the predator *Macrolophus pygmaeus* (Heteroptera: Miridae) and the parasitoid *Diglyphus isaea* (Hymenoptera: Eulophidae). Tests on the pollinator *Bombus terrestris* (Hymenoptera: Apidae), are also required for product registration (Sterk et al., 2002). The results of the ECO-ZEO project indicated that the zeolites were not toxic to natural enemies (both predators and parasitoids) and pollinators, since it did not cause mortality to these beneficial insects.

2.3 SOCIAL DIMENSION

According to the European directive 2009/128/EC, that establishes a framework to achieve a sustainable use of PPPs, farmers must reduce the risks and impacts of PPPs use on the environment and human health.

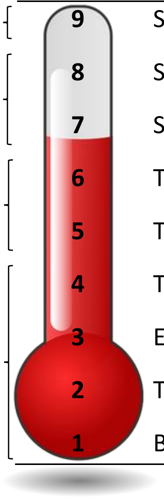
Since zeolites are not considered to be dangerous according to the EC Regulation 1272/2008 and its amendments, the substitution of conventional PPPs by the zeolite formulations will improve the working conditions of the farmers. Eye, skin and respiratory sensitization are also significantly lower than for conventional PPPs, what reduces the risks PPP operators, workers and bystanders.

3 CONVERSION OF THIS POOL OF KNOWLEDGE INTO LEGALIZED INDUSTRIAL PRACTICE

One of the most important key challenges for result oriented agricultural research is to convert science into legalized industrial practice. An overview of the different stages of innovation is given by Technology Readiness Levels (TRLs). It should be viewed as one of several tools that are needed to manage the progress of research and development activity within an organization and it illustrates how a technology is near to the market.

Technology Readiness Levels measure how ready equipment is for use now in an operating plant. The levels are a nine-point scale and based on a qualitative assessment of maturity (**Table 7-4**). Each level characterizes the progress in the development of a technology, from the idea (level 1) to the full deployment of the product in the marketplace (level 9). Of course, innovation does not stop there, but may continue into full deployment and market diffusion, as is clear from the dynamic theories of innovation briefly considered next (Ekins, 2009).

Table 7-4. Scientific research maturity road map to convert science into market competitive industrial practice.



	TRL	Technology Readiness Level
Industrial model for full production	9	System proven in operational environment with competitive manufacturing
Field demonstration	8	System complete and qualified, full industrial engineering
	7	System prototype demonstration in operational environment
Pilot plant demonstration	6	Technology demonstrated in industrially relevant environment
	5	Technology validated in industrially relevant environment
Basic scientific research	4	Technology validated in lab
	3	Experimental proof of concept
	2	Technology concept formulated
	1	Basic principles observed

The TRL9 is the only true valuable technical, economical and commercial proven demonstrated research maturity level to convert science into market competitive and legalized industrial practice. At this level, the elevated risks are truly identified, eliminated and/or minimized during the scale up process.

In general, the synthetic and natural zeolite types are rather well researched and have a large number of publications. However, innovative formulations and combinations would be of high interest in the agriculture, whereas end-users interest can only be raised if matured research at higher TRL levels is reached. At the end of this study, a TRL level around 4 was reached.

4 FUTURE RESEARCH

4.1 A GENERAL PURPOSE

Despite the possible adverse effects on soil quality, zeolites are environmentally friendly and sustainable products for agriculture and no negative effects were observed against natural enemies. The overwhelming advantage of zeolites is that its mode of action is unlikely to suffer resistance as the mechanism is physical rather than chemical. And although the control is by no means excellent, zeolites may well deserve a place as part of an integrated control system particularly in early stages of the fruit or crop development. In this way, the farmer has to use less chemical products, what makes the harvested products healthier for food consumption. The farmer will also have an additional protection against sunburn, resulting in fewer losses through damaged fruit.

Although the development of a PPP based on zeolites could be a big step forward in the view of a more sustainable agriculture, there are still many fiels for improvement and additional research is required.

First of all, the main difference between the use of zeolite formulations and other PPPs is the difference in application rate. Most PPPs are applied at rates between 1 and 5 kg.ha^{-1}, whereas zeolite formulations will be applied around 60 kg.ha^{-1}. This is a major disadvantage from the commercial point of view. The extra costs and inconvenience to the farmer in using a product with such high application rates are considerable and will make zeolites the least desirable of competing products. Ideally, a study should aim at reducing that high application rate. A possibility is to apply other compounds that generate some synergistic effects.

Another interesting topic for further investigation is the formulation of the zeolites. In this study, the used formulation type has both advantages and disadvantages. A first disadvantage is the fact that it is a ‘dust’, which makes it not the most appropriate formulation to work with. Secondly, the formulation is insoluble in water and it is difficult to keep the product in suspension. Notwithstanding, easy storage and transporting of a powder formulation is an advantage. Nevertheless, the used formulations in this study and in the ECO-ZEO project should first be adapted to the results obtained by the physico-chemical experiments.

One of the main challenges is to obtain a good coverage and equal distribution of the zeolites onto the plant leaves. Next, the zeolite formulations are not rainfast, which is also a disadvantage compared to chemical products. Despite the fact that the WP formulation obtained the best results compared to the other formulation types, all evaluated properties could and should be improved before using them in further experiments.

Additionally, the used zeolite formulations are rather contact products instead of systemic products. The benefit of this mode of action is that the product will not be present inside the fruit. However, the zeolite formulations will leave a visible deposit on the fruit and crops. Most consumers will interpret the deposit to be a chemical PPP and therefore poisonous. This misunderstanding will be very ingrained and will put off many people from buying these products. It is something unknown, something new, and people tend to follow their familiar habits. Consequently, the farmer has to wash the fruits, which will eventually lead to an increased cost. Further research should be dedicated to solve this visual problem after applying the zeolite formulations, e.g. develop a special kind of product for cleaning fruits before harvest.

The mode of action of the tested products only relied on the zeolite properties. The use of synthetic compounds that might induce a symbiotic or slow release effect was not considered. Combining of zeolites and synthetic compounds could allow low dose rates, which reduces the PPP's load.

Although there is a market for organic products, there is an even bigger one for synthetic compounds that work at low concentrations of a few kg.ha^{-1}. An EU-wide study could work out a screening using fungal and arthropod species and test different materials for instance natural zeolites, clays and bio-char with effective co-formulates. When it would be possible to develop a product with outstanding efficacy, the existing problems might be tolerated.

Finally, the use of zeolites as slow-release media of organic compounds, chemical PPPs, pheromones, etc. can be very interesting research topic. Next to that, the zeolites may also be chemically modified, which can be another research topic.

Nevertheless, it will be difficult to convince farmers to apply the zeolites. One application requires a high amount of product and the above described threats make it easier for the farmer to apply the well-known chemical products. It is not easy to compete with other farmers these days. The use and effect of zeolite products is still not known, which makes the farmers uncertain/reserved to use this product. A lot of marketing will be needed to convince the farmers.

4.2 A SPECIFIC PURPOSE

4.2.1 EFFECT OF ZEOLITES AGAINST LEAF SUCKING SOFT-BODIED INSECTS

Zeolites are based on interference with visual cues and physical interference, which keep insect pests from reaching their hosts by means of a barrier or trap (Boiteau et al., 2014). According to their mode of action, zeolites can be classified as contact poisons and repellents, what makes them able to control any insect, i.e. chewing insects as well as sucking insects (Reiley and Shry, 2002).

The modes of action of zeolites were derived from the known modes of action of kaolin, which is commercially used in agriculture as Surround WP. One of the first modes of action of particle films in general, and in particular Surround WP, is host camouflaging, i.e. making plants unrecognizable for their enemies. By camouflaging, Surround WP reduces pest oviposition and the overall pest population in the microclimate of the plant foliage (Glenn and Puterka, 2005). Additionally, the particle layer on the leaf surface interferes with the insect's feeding behaviour and affect egg laying by hindering their anchorage on the leaf surface. It was also found that the small particles attach to the insects, which irritates and repels them from the crop. This results in a lower egg laying of the surviving insects. Subsequently it was found that the body and wings of some adults became soiled, making the insects less mobile and preventing them from reaching the laying site (host location) on plants. The insects became less vital, because they lost much energy in order to try to remove the particles from their body and eventually starve (Glenn and Puterka, 2005).

In this PhD thesis the effect of zeolites was examined on eggs and larvae of hard-bodied chewing insects. However, the mode of action of kaolin is mainly focused on adults. That is why it is important that future research will investigate the effect of zeolites on adults *over* a prolonged *period* of *time.*

Subsequently, only hard-bodied insects were taken into account in this thesis. Despite the fact that the experiments were performed on larvae, which are soft-bodied, it will be important to take along soft-bodied insects in further testing. This will especially be important when applying adult insects. Because the exoskeleton of the soft-bodied insects is not as robust as with hard-bodied insects, it is thought that zeolites *would* tear microscopic holes in surfaces of soft-bodied insects, and would slice between the exoskeleton plates of hard-bodied insects. In hot, dry weather the injured insects rapidly dehydrate and die (McPartland et al., 2000). Soft-bodied insects that are easily to rear are aphids (Hemiptera: Aphidoidea). These species were also suppressed by the use of kaolin (Alavo and Abagli, 2011).

Finally, only chewing insects were taken into account in this thesis. It was thought that zeolites could have more effect on chewing insects, because of their possible abrasive effects on the insect's mandibles. However, future tests also have to take into account a leaf sucking insect in order to get a clear overview about the mode of action of the zeolites. For example, kaolin is very effective against the leaf sucking insect pear Psylla *Cacopsylla pyricola* (Hemiptera: Psyllidae). The psylla do not like to lay their eggs on sprayed trees, and the coating also irritates psylla nymphs. These nymphs secrete a sticky substance, known as honeydew, as they feed. The pear psylla nymphs pass through five instars, four of which are almost completely encased in honeydew (Bradley et al., 2009; Civolani, 2012). However, zeolites can have a desiccating effect on this substance, which can cause death.

4.2.2 EFFECT OF ZEOLITES ON SUNBURN

A very interesting topic for further investigation is the effect of zeolites on sunburn. While sunlight is essential for the health of all crops, excessive sunlight and heat can take a heavy toll on fruits, increasing stress on trees and reducing fruit quality and quantity (Omri, 2004).

The primary cause of solar injury is excessive heat damage. However, research in other crops suggests that ultraviolet radiation (UV) may also contribute to the solar injury. Ultraviolet radiation is required for synthesis of anthocyanin in apple, however, excessive environmental UV may be harmful to the plant (Glenn et al., 2002). The temperature of the sun-exposed fruit surface of apples is often 10 to 18°C higher than the maximum shaded air temperature. As a general guide, sunburn of apples can occur when shaded air temperatures are above 30°C and fruit surface temperatures are above 45°C (Schrader et al., 2003).

As described in Chapter 1, kaolin provides a proven solution to these environmental factors that affect apple orchards, which results in a higher-quality and more-marketable fruits. **Figure 7-2** gives an example of thermal infrared images of apples with and without a reflective kaolin particle film. It can be seen that the treated apple was 4°C cooler than the control at the hot spot (39°C versus 43°C) (Volker, 2014). Based on this information, it can be deduced that zeolites are also able to reduce heat stress and sunburn on several crops. However, further research is needed in order to verify this statement and whether the existing method can be optimized.

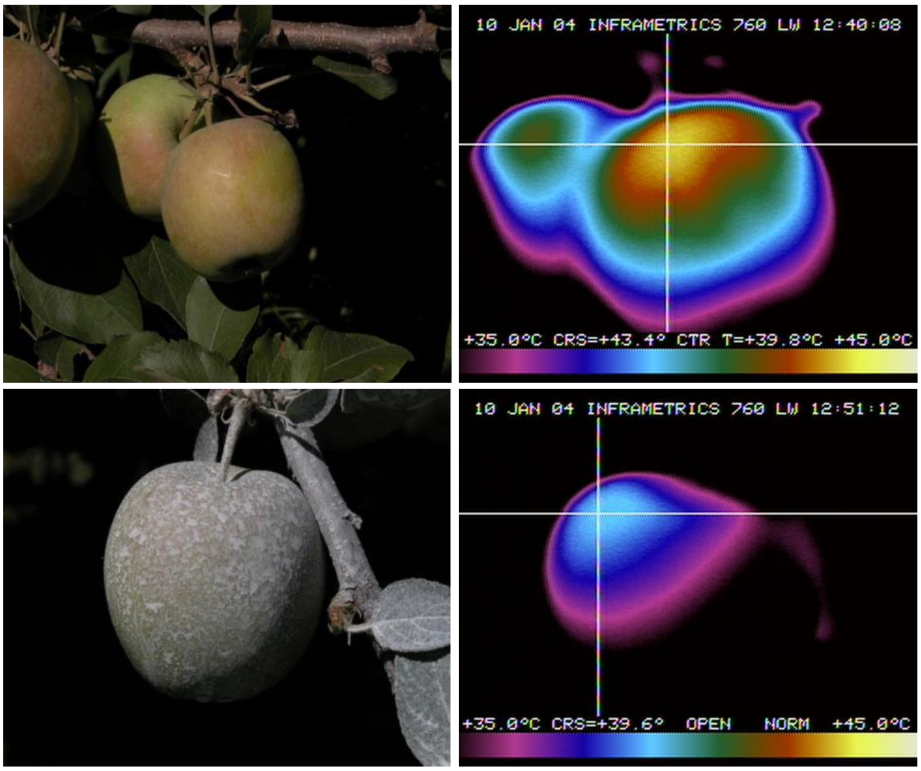


Figure 7-2. Thermal infrared images of apples with and without a reflective kaolin particle film (Volker, 2014).

4.2.3 EFFECT OF ZEOLITES ON ULTRAVIOLET SENSITIVE PLANT PROTECTION PRODUCTS

It is known that sunlight may cause UV degradation of PPPs. Many investigators have focused on this effect, in order to use UV radiations as a photochemical treatment to break down these PPP molecules (Kundua et al., 2005). However, sunlight photodegradation is one of the most destructive pathways for PPPs after their release into the environment.

In function of the efficacy of PPPs, it is important that they do not photodegrade during the time they are exercising their biocidal activity. On the other hand, for environmental considerations it is important that they can eventually be converted to innocuous, and preferable mineral, photoproducts (Burrows et al., 2002).

As mentioned in this PhD thesis, zeolites may be able to protect plants against these UV radiations in function of heat stress and sunburn. Therefore, these zeolites may also be able to protect PPPs applied on crops against early photodegradation. This effect will have some important benefits. Firstly, an increase in photostability of PPPs on crops will reduce the application frequencies or application rates of PPPs. Secondly, less photoproducts will be formed that can accumulate on the crops and can cause potential harm (Burrows et al., 2002; Andreu and Pico, 2004; Virag et al., 2007; Eyheraguibel et al., 2009).

Indicative studies were performed with zeolite FAU and bentazon, which is subject to a fast photodegradation (Eyheraguibel et al., 2009). After 1 hour exposure to UV radiation, 99% of the technical grade bentazon dissolved in water was decomposed, while adsorbed on zeolites the decomposition of bentazone was about 9%. Despite some effects of zeolites on photostability were already noticed by other researchers, the obtained result is very interesting in view of further research topics (Sopkova and Janokova, 1998; Kvachantiradze et al., 1999).

4.2.4 EFFECT OF ZEOLITES AS SLOW RELEASE AGENT

Based on the results of the adsorption tests, described in Part C of Chapter 2, it was noticed that zeolites were not able to compete with activated carbon for the removal of PPPs from contaminated water. Nevertheless, the zeolites obtained good adsorption results. For this reason, the adsorption of PPPs on zeolites may perhaps be applied in a different way, i.e. as slow release carriers.

Zeolites as slow release carrier for pesticides can have benefits, such as the extension of PPP residual activity and the reduction of (1) PPP application rates, (2) PPP levels in the environment and (3) PPP toxicity (Dasgupta and Roberts, 1991).

As mentioned in Chapter 1, zeolites are already used as slow release carriers. However, regardless to the use of zeolites as slow release fertilizers, zeolites are not widely used as slow release PPPs in agriculture. A lot of research is still needed before this can be changed.

It would be very useful to investigate the use of zeolites as carrier for biopesticides, i.e. pheromones, plant extracts and biological products in particular. Additionally, taken into account the beneficial effects of zeolites as plant protection agent, the use of zeolites as carrier might also induce some synergistic effects, which makes this topic incredibly provocative.

4.2.5 THE USE OF METAL-ORGANIC FRAMEWORKS

Twinty years have passed since the term “metal-organic framework” (MOF) first appeared in the literature (Yaghi and Li, 1995). The nanoporosity and incredibly high surface area of this material (> 3000 $m^2.g^{-1}$) initiated a new field of supramolecular chemistry. MOFs possess unique properties among the various classes of microporous and mesoporous materials, creating interest for an unprecedented range of applications. A small number of MOFs and related materials, such as zeolite imidazolate frameworks (ZIFs), are now available commercially, although only as specialty chemicals (Meek et al., 2011).

It has to be said that MOFs are a rapidly growing class of nanoporous materials. In 2005, another family of highly accessible porous materials, based on the effective assembly of different specific builders, has emerged. These solids are formed by the covalent combination of rigid and stable organic builders to construct novel robust porous covalent organic frameworks (COFs) (Côté et al., 2005). COFs are covalent porous crystalline polymers that enable the elaborate integration of organic building blocks into an ordered structure with atomic precision.

Within this important group of organic materials, those based on exclusively aromatic builder units, named as porous aromatic frameworks (PAFs), are especially remarkable and are still more robust and stable than conventional COFs or even MOFs, maintaining intrinsic electron rich structure, capability to be functionalized and high porosity (Diaz and Corma, 2016).

These COFs and, in particular, their sub-group based on PAFs are an interesting and emergent materials family with many possibilities in different application fields, such as those more conventional for porous materials (adsorption, separation or catalysis) and those related with sensor, photo-luminescence and optoelectronic, where advanced electron donor-acceptor structures are necessary (Diaz and Corma, 2016). For these reasons it can be very interesting to do further research on this topic. Their unique features, such as highly flexible molecular design, permanent porosity, and controllable pore size, and the diversity of available building blocks promise that this field, although currently in its infant stages, will grow into a rich and broad area of great importance (Feng et al., 2012). Unfortunately, the production time and costs of these materials remain a major drawback to use them on a large scale. This is an important difference with respect to the production of activated carbon and zeolites.

However, in function of this PhD thesis MOFs were used in order to adsorb PPPs. Based on the results, the selected MOFs were probably not the most appropriate materials to adsorb the PPPs. At this moment, new and more stable types of MOFs are present, which would result in a better adsorption of PPPs. For example, Basolite Z1200, a zinc based MOF, is found to be very efficient to adsorb PPP Mecroprop from aqueous solution (Bansal et al., 2013). Subsequently, zirconium based MOFs of UiO-67 have also been successfully developed as novel adsorbents for the efficient removal of organophosphorus PPPs from aqueous solution (Zhu et al., 2015). These observations indicate that it is important to do research on MOFs, because existing MOFs can also be improved and new MOFs can also be developed in function of PPP adsorption.

5 FINAL CONCLUSION

Fruits and vegetables are produced in numerous countries and regions with contrasting climates. Due to the current climate change, which is already very noticeable, farmers will be eventually forced to either change their crops or apply new practices. Therefore, it becomes very important to anticipate this evolution in research programs.

The goal of this research, together with the ECO-ZEO project, was to develop a new green crop protection product. However, based on the obtained results, it is not possible to develop one final product that suits all purposes. Despite some beneficial effects that were obtained in terms of PPP adsorption, fungicidal growth-inhibtion and photosynthesis, these results were not comparable with the effects of the already used commercial PPPs. Additionally, the limited economic advantages together with some disadvantages for the farmer when using these zeolites, i.e. high application rates, visible deposits on fruits and crops and possible toxic effects on soil quality, will deter the farmers to use zeolites as a PPP.

Nevertheless, the favourable environmental performance of the zeolites and the potential for upscaling in EU-countries and beyond, would make zeolites a product which is worth to be further explored.

CURRICULUM VITAE

PERSONAL DATA

Name	Caroline De Smedt
Date and place of birth	1 February 1987, Lokeren
Nationality	Belgian
Address	Hellestraat 193, 9190 Stekene, Belgium
Mail	caroline.desmedt@ugent.be

PROFESSIONAL CAREER

2012 - present	PhD student Department of Crop Protection Chemistry, Ghent University, Belgium

Research subject: the development of a pool of novel and eco-efficient applications of zeolites as a plant protection product within the framework of the ECO-ZEO project (grant agreement n$_{o}$. 282865)

EDUCATION

2010 - 2012	Master in Bioscience Engineering, option Environmental Technology Faculty of Bioscience Engineering, Ghent University, Belgium
2009 - 2010	Master in Industrial Engineering, option Environmental Technology Faculty of Industrial Engineering, Ghent University College, Belgium
2005 - 2009	Bachelor in Industrial Engineering, option Biochemistry Faculty of Industrial Engineering, Ghent University College, Belgium
1999 - 2005	Secondary school, option Humanities Sint-Jozef-Klein-Seminarie, Sint-Niklaas, Belgium

PUBLICATIONS

De Smedt C, Someus E and Spanoghe P, Potential and actual uses of zeolites in crop protection. Pest Manag Sci, **71**: 1355-1367 (2015).

De Smedt C, Ferrer F, Leus K and Spanoghe P, Removal of pesticides from aqueous solutions by adsorption on zeolites as solid adsorbents. Adsorpt Sci Techol, **33**: 457-485 (2015).

De Smedt C, Spanoghe P, Biswas S, Leus K and Van Der Voort P, Comparison of different solid adsorbents for the removal of mobile pesticides from aqueous solutions. Adsorption, **21**: 243-254 (2015).

De Smedt C, Höfte M, Dardenne F and Spanoghe P, Fungicide effect of zeolites and adjuvants on *Venturia inaequalis* and *Botrytis cinerea*. Submitted to Plos One.

De Smedt C, Van Damme V, De Clercq P and Spanoghe P, Insecticide effect of zeolites on the tomato leaf miner *Tuta absoluta* (Lepidoptera: Gelechiidae). Submitted to Insects.

De Smedt C, Steppe K and Spanoghe P, Beneficial effect of zeolites on plant photosynthesis. Submitted to Acta Hortic.

SCIENTIFIC ACTIVITIES

ORAL PRESENTATIONS

2012	Adsorption of pesticides on metal organic frameworks for water treatment. *64th International Symposium on Crop Protection (Ghent, Belgium)*
2015	Potential of zeolites as crop protection agent *1th Department Seminar (Ghent, Belgium)*

POSTER PRESENTATIONS

2013	Formulation and application of zeolites in the framework of a sustainable crop protection *65th International Symposium on Crop Protection (Ghent, Belgium)*
	Formulation and application of zeolites in the framework of a sustainable crop protection *Course on Theory and Methods of Research in Applied Biological Sciences (Ghent, Belgium)*
2014	Adsorption of pesticides on zeolites *66th International Symposium on Crop Protection (Ghent, Belgium)*
	Adsorption of pesticides on zeolites *13th IUPAC International congress of pesticide chemistry (California, USA)*

APPENDIX A

ZEOLITE STRUCTURE

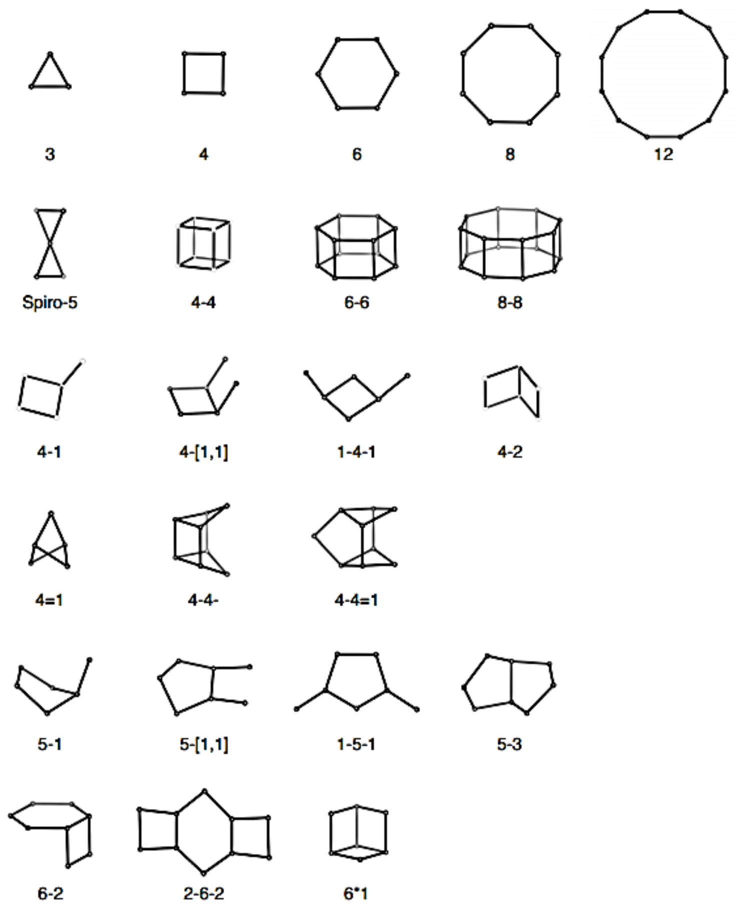


Figure A-1. Secondary building units and their symbols (Baerlocher et al., 2007).

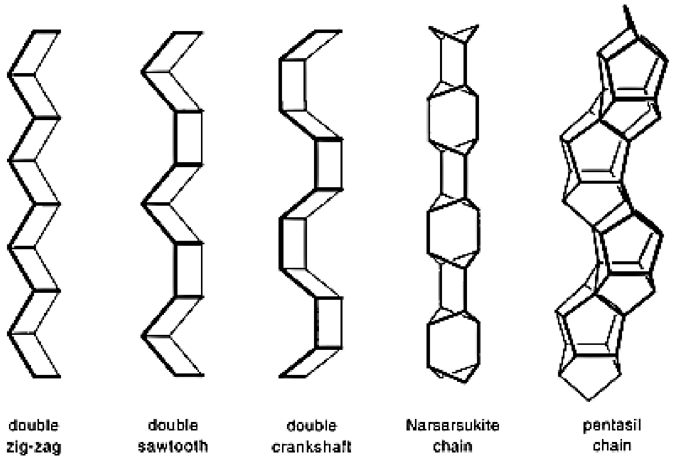
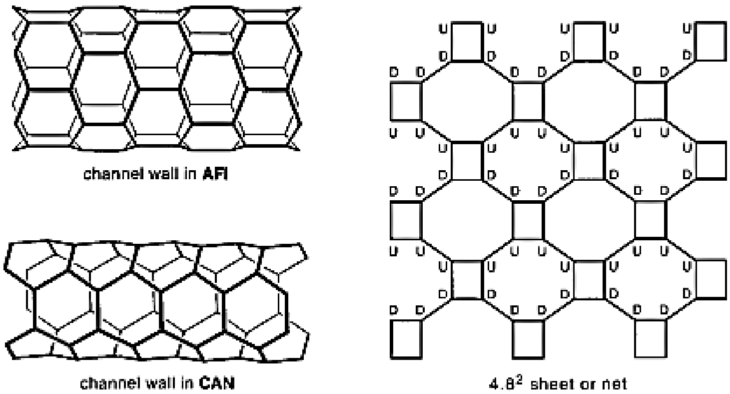


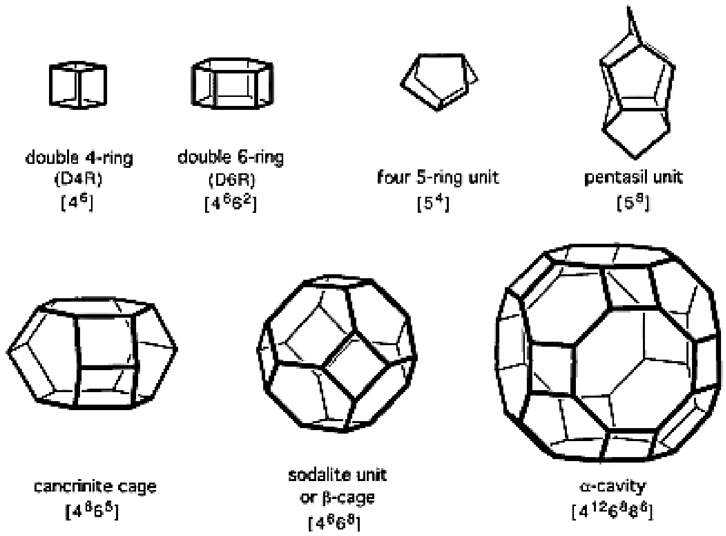
Figure A-2. Some chains that recur in several framework types (McCusker and Baerlocher, 2005).


channel wall in **AFI**

channel wall in **CAN**

4.8^2 sheet or net

Figure A-3. Two types of channel walls composed of 6-rings (left) and the GIS 4.8^2 sheet (right) (McCusker and Baerlocher, 2005).


double 4-ring (D4R) [4^6]

double 6-ring (D6R) [$4^6 6^2$]

four 5-ring unit [5^4]

pentasil unit [5^8]

cancrinite cage [$4^6 6^5$]

sodalite unit or β-cage [$4^6 6^8$]

α-cavity [$4^{12} 6^8 8^6$]

Figure A-4. Some subunits and cages/cavities that recur in several framework types (McCusker and Baerlocher, 2005).

APPENDIX B

THEORETICAL SELECTION OF ZEOLITES

Table B-1. Natural (green) and synthetic (black) zeolite framework types (FTC).

	FTC	Material name	Year
1	ABW	Li-A(BW)	1978
2	ACO	ACP-1	1998
3	AEI	AlPO-18	1992
4	AEL	AlPO-11	1987
5	AEN	AlPO-EN3	1999
6	AET	AlPO-8	1992
7	AFG	Afghanite	1978
8	AFI	AlPO-5	1987
9	AFN	AlPO-14	1998
10	AFO	AlPO-41	1992
11	AFR	SAPO-40	1992
12	AFS	MAPSO-46	1987
13	AFT	AlPO-52	1992
14	AFV	ZnAlPO-57	2014
15	AFX	SAPO-56	1994
16	AFY	CoAPO-50	1987
17	AHT	AlPO-H2	1994
18	ANA	Analcime	1978
19	APC	AlPO-C	1987
20	APD	AlPO-D	1987
21	AST	AlPO-16	1987
22	ASV	ASU-7	2000
23	ATN	MAPO-39	1992
24	ATO	AlPO-31	1992
25	ATS	MAPO-36	1992
26	ATT	AlPO-12-TAMU	1987
27	ATV	AlPO-25	1992
28	AVL	ZnAlPO-59	2014
29	AWO	AlPO-21	1998
30	AWW	AlPO-22	1992
31	BCT	Mg-BCCT	2001
32	BEA	Beta	1992
33	BEC	FOS-5 (Beta polymorph C)	2001
34	BIK	Bikitaite	1978
35	BOF	UCSB-15	2008
36	BOG	Boggsite	1992
37	BOZ	Be-10	2012
38	BPH	Beryllophosphate-H	1992
39	BRE	Brewsterite	1978
40	BSV	UCSB-7	2008
41	CAN	Cancrinite	1978
42	CAS	Cesium Aluminosilicate (Araki)	1992
43	CDO	CDS-1	2004
44	CFI	CIT-5	1998
45	CGF	Cobalt-Galiium-Phosphate-5	1996

Table B-1. Natural (green) and synthetic (black) zeolite framework types (FTC) (continued).

	FTC	Material name	Year		FTC	Material name	Year
46	CGS	Cobalt-Gallium-Phosphate-6	1997	**76**	GIU	Giuseppettite	2004
47	CHA	Chabazite	1978	**77**	GME	Gmelinite	1978
48	-CHI	Chiavennite	1987	**78**	GON	GUS-1	2000
49	-CLO	Cloverite	1992	**79**	GOO	Goosecreekite	1987
50	CON	CIT-1	1994	**80**	HEU	Heulandite	1978
51	CSV	CIT-7	2015	**81**	IFO	ITQ-51	2013
52	CZP	Chiral Zincophosphate	1996	**82**	IFR	ITQ-4	1997
53	DAC	Dachiardite	1978	**83**	-IFU	ITQ-54	2015
54	DDR	Deca-Dodecasil-3R	1987	**84**	IFW	ITQ-52	2014
55	DFO	DAF-1	1993	**85**	IFY	ITQ-50	2014
56	DFT	DAF-2	1998	**86**	IHW	ITQ-32	2006
57	DOH	Dodecasil-1H	1987	**87**	IMF	IM-5	2007
58	DON	UTD-1F	1999	**88**	IRN	ITQ-49	2014
59	EAB	TMA-E(AB)	1978	**89**	IRR	ITQ-44	2011
60	EDI	Edingtonite	1978	**90**	-IRY	ITQ-40	2014
61	EEI	SSZ-45	2014	**91**	ISV	ITQ-7	1999
62	EMT	EMC-2	1992	**92**	ITE	ITQ-3	1997
63	EON	ECR-1	2005	**93**	ITG	ITQ-38	2014
64	EPI	Epistilbite	1978	**94**	ITH	ITQ-13	2003
65	ERI	Erionite	1978	**95**	-ITN	ITQ-39	2014
66	ESV	ERS-7	1998	**96**	ITR	ITQ-34	2009
67	ETR	ECR-34	2004	**97**	ITT	ITQ-33	2013
68	EUO	EU-1	1987	**98**	-ITV	ITQ-37	2011
69	-EWT	EMM-23	2015	**99**	ITW	ITQ-12	2003
70	EZT	EMM-3	2006	**100**	IWR	ITQ-24	2004
71	FAR	Farneseite	2006	**101**	IVW	ITQ-27	2006
72	FAU	Faujasite	1978	**102**	IWS	ITQ-26	2008
73	FER	Ferrierite	1978	**103**	IWW	ITQ-22	2003
74	FRA	Franzinite	2000	**104**	JBW	Na-J (Barrer & White)	1992
75	GIS	Gismondine	1978				

Table B-1. Natural (green) and synthetic (black) zeolite framework types (FTC) (continued).

	FTC	Material name	Year		FTC	Material name	Year
105	JNT	JU-92	2015	**136**	MSE	MCM-68	2006
106	JOZ	LSJ-10	2012	**137**	MSO	MCM-61	1999
107	JRY	CoAPO-CJ40	2009	**138**	MTF	MCM-35	1999
108	JSN	CoAPO-CJ69	2012	**139**	MTN	ZSM-39	1987
109	JSR	JU-64	2013	**140**	MTT	ZSM-23	1987
110	JST	GaGeO-CJ63	2011	**141**	MTW	ZSM-12	1987
111	JSW	CoAPO-CJ62	2012	**142**	MVY	MCM-70	2010
112	KFI	ZK-5	1978	**143**	MWF	PST-6	2015
113	LAU	Laumontite	1978	**144**	MWW	MCM-22	1997
114	LEV	Levyne	1978	**145**	NAB	Nabesite	2003
115	LIO	Liottite	1978	**146**	NAT	Natrolite	1978
116	-LIT	Lithosite	2005	**147**	NES	NU-87	1992
117	LOS	Losod	1978	**148**	NON	Nonasil	1987
118	LOV	Lovdarite	1987	**149**	NPO	Oxonitridophosphate-1	2004
119	LTA	Linde Type A	1978	**150**	NPT	Oxonitridophosphate-2	2011
120	LTF	LZ-135	2009	**151**	NSI	Nu-6(2)	2005
121	LTJ	Linde Type J	2011	**152**	OBW	OSB-2	2004
122	LTL	Linde Type L	1978	**153**	OFF	Offretite	1978
123	LTN	Linde Type N	1987	**154**	OKO	COK-14	2013
124	MAR	Marinellite	2004	**155**	OSI	UiO-6	1996
125	MAZ	Mazzite	1978	**156**	OSO	OSB-1	2000
126	MEI	ZSM-18	1992	**157**	-PAR	Partheite	1987
127	MEL	ZSM-11	1978	**158**	PAU	Paulingite	1978
128	MEP	Melanophlogite	1987	**159**	PCR	IPC-4	2012
129	MER	Merlinoite	1978	**160**	PHI	Phillipsite	1978
130	MFI	ZSM-5	1978	**161**	PON	IST-1	2004
131	MFS	ZSM-57	1992	**162**	POS	PKU-16	2014
132	MON	Montesommaite	1992	**163**	PSI	PST-6	2015
133	MOR	Mordenite	1978	**164**	PUN	PKU-9	2010
134	MOZ	ZSM-10	2006	**165**	RHO	Rho	1978
135	MRE	ZSM-48	2008	**166**	-RON	Roggianite	1992

Table B-1. Natural (green) and synthetic (black) zeolite framework types (FTC) (continued).

	FTC	Material name	Year		FTC	Material name	Year
167	RRO	RUB-41	2004	**198**	SSF	SSZ-65	2008
168	RSN	RUB-17	1994	**199**	-SSO	SSZ-61	2014
169	RTE	RUB-3	1994	**200**	SSY	SSZ-60	2004
170	RTH	RUB-13	1994	**201**	STF	SSZ-35	1998
171	RUT	RUB-10	1994	**202**	STI	Stilbite	1978
172	RWR	RUB-24	2004	**203**	STO	SSZ-31 (polymorph I)	2008
173	RWY	UCR-20	2003	**204**	STT	SSZ-23	1998
174	SAF	STA-15	2010	**205**	STW	SU-32	2008
175	SAO	STA-1	1996	**206**	-SVR	SSZ-74	2008
176	SAS	STA-6	2000	**207**	SVV	SSZ-77	2013
177	SAT	STA-2	1997	**208**	SZR	SUZ-4	2006
178	SAV	STA-7	2000	**209**	TER	Terranovaite	1996
179	SBE	UCSB-8Co	1998	**210**	THO	Thomsonite	1978
180	SBS	UCSB-6GaCo	1998	**211**	TOL	Tounkite-like mineral	2007
181	SBN	UCSB-9	2008	**212**	TON	Theta-1	1987
182	SBT	UCSB-10GaZn	1998	**213**	TSC	Tschörtnerite	1998
183	SEW	SSZ-82	2013	**214**	TUN	TNU-9	2006
184	SFE	SSZ-48	2000	**215**	UEI	Mu-18	2002
185	SFF	SSZ-44	1998	**216**	UFI	UZM-5	2003
186	SFG	SSZ-58	2003	**217**	UOS	IM-16	2009
187	SFH	SSZ-53	2003	**218**	UOV	IM-17	2014
188	SFN	SSZ-59	2003	**219**	UOZ	IM-10	2004
189	SFO	SSZ-51	2004	**220**	USI	IM-6	2004
190	SFS	SSZ-56	2009	**221**	UTL	IM-12	2004
191	SFV	SSZ-57	2011	**222**	UWE	UiO-28	2005
192	SFW	SSZ-52	2013	**223**	UWY	IM-20	2011
193	SGT	Sigma-2	1987	**224**	VET	VPI-8	1995
194	SIV	SIV-7	2006	**225**	VFI	VFI	1992
195	SOD	Sodalite	1978	**226**	VNI	VNI	1995
196	SOF	SU-15	2008	**227**	VSV	VSV	1994
197	SOS	SU-16	2004	**228**	WEI	WEI	1993

Table B-1. Natural (green) and synthetic (black) zeolite framework types (FTC) (continued).

	FTC	Material name	Year		FTC	Material name	Year
229	-WEN	-WEN	1987				
230	YUG	YUG	1978				
231	ZON	ZON	1995				

Table B-2. Composition of natural zeolites.

FTC	**Material name**	**Composition**	**%**		**%**	
ANA	Analcime	Sodium	10.44	Na	14.08	Na_2O
		Aluminium	12.26	Al	23.16	Al_2O_3
		Silicon	25.51	Si	54.58	SiO_2
		Hydrogen	0.92	H	8.18	H_2O
		Oxygen	50.87	O		
	Ammonioleucite	Potassium	4.83	K	5.82	K_2O
		Aluminium	13.33	Al	25.18	Al_2O_3
		Silicon	27.75	Si	59.36	SiO_2
		Hydrogen	1.49	H		
		Nitrogen	5.19	N	9.65	$(NH_4)_2O$
		Oxygen	47.42	O		
	Hsianghualite	**Lithium**	**2.92**	**Li**	**6.29**	Li_2O
		Calcium	**25.29**	**Ca**	**35.39**	CaO
		Beryllium	**5.69**	**Be**	**15.78**	BeO
		Silicon	**17.72**	**Si**	**37.92**	SiO_2
		Oxygen	**40.39**	**O**		
		Fluorine	**7.99**	**F**		
	Leucite	Potassium	17.91	K	21.58	K_2O
		Aluminium	12.36	Al	23.36	Al_2O_3
		Silicon	25.74	Si	55.06	SiO_2
		Oxygen	43.99	O		
	Pollucite	**Cesium**	**27.98**	**Cs**	**29.66**	Cs_2O
		Rubidium	**1.20**	**Rb**	**1.31**	Rb_2O
		Sodium	**1.61**	**Na**	**2.17**	Na_2O
		Aluminium	**8.52**	**Al**	**16.10**	Al_2O_3
		Silicon	**20.69**	**Si**	**44.27**	SiO_2
		Hydrogen	**0.71**	**H**	**6.32**	H_2O
		Oxygen	**39.29**	**O**		
	Wairakite	Calcium	9.23	Ca	12.91	CaO
		Aluminium	12.42	Al	23.47	Al_2O_3
		Silicon	25.86	Si	55.33	SiO_2
		Hydrogen	0.93	H	8.29	H_2O
		Oxygen	51.56	O		
	Kirchhoffite	**Cesium**	**44.92**	**Cs**	**48.16**	Cs_2O
		Boron	**3.65**	**B**	**12.27**	B_2O_3
		Silicon	**19.98**	**Si**	**40.47**	SiO_2
		Oxygen	**32.44**	**O**		

Table B-2. Composition of natural zeolites (continued).

FTC	Material name	Composition	%		%	
BEA	Tschernichite	Sodium	0.87	Na	1.18	Na_2O
		Calcium	4.57	Ca	6.39	CaO
		Aluminium	8.20	Al	15.49	Al_2O_3
		Silicon	25.59	Si	54.76	SiO_2
		Hydrogen	2.45	H	21.89	H_2O
		Oxygen	58.32	O		
BIK	**Bikitaite**	**Lithium**	**3.40**	**Li**	**7.32**	**Li_2O**
		Aluminium	**13.22**	**Al**	**24.98**	**Al_2O_3**
		Silicon	**27.52**	**Si**	**58.88**	**SiO_2**
		Hydrogen	**0.99**	**H**	**8.83**	**H_2O**
		Oxygen	**54.87**	**O**		
BOG	Boggsite	Sodium	1.25	Na	1.68	Na_2O
		Calcium	4.34	Ca	6.08	CaO
		Aluminium	7.31	Al	13.81	Al_2O_3
		Silicon	28.91	Si	61.84	SiO_2
		Hydrogen	1.86	H	16.59	H_2O
		Oxygen	56.34	O		
BRE	**Brewsterite**	**Potassium**	**0.06**	**K**	**0.07**	**K_2O**
		Barium	**4.96**	**Ba**	**5.53**	**BaO**
		Strontium	**9.35**	**Sr**	**11.06**	**SrO**
		Aluminium	**8.36**	**Al**	**15.79**	**Al_2O_3**
		Silicon	**25.23**	**Si**	**53.98**	**SiO_2**
		Hydrogen	**1.52**	**H**	**13.54**	**H_2O**
		Oxygen	**50.52**	**O**		
		Or	Or		Or	
		Barium	**14.85**	**Ba**	**16.58**	**BaO**
		Strontium	**3.16**	**Sr**	**3.74**	**SrO**
		Aluminium	**7.78**	**Al**	**14.70**	**Al_2O_3**
		Silicon	**24.30**	**Si**	**51.99**	**SiO_2**
		Hydrogen	**1.45**	**H**	**12.99**	**H_2O**
		Oxygen	**48.45**	**O**		

Table B-2. Composition of natural zeolites (continued).

FTC	**Material name**	**Composition**	**%**		**%**	
CHA	Chabazite	Potassium	2.04	K	2.50	K_2O
		Sodium	0.24	Na		
		Calcium	2.09	Ca	2.80	CaO
		Magnesium	1.78	Mg	2.85	MgO
		Aluminium	8.46	Al	16.90	Al_2O_3
		Silicon	26.42	Si	56.22	SiO_2
		Hydrogen	2.11	H	18.30	H_2O
		Oxygen	56.86	O		
		Or	Or		Or	
		Potassium	**2.19**	**K**	**2.64**	K_2O
		Strontium	**8.20**	**Sr**	**9.69**	**SrO**
		Sodium	**0.86**	**Na**	**1.16**	Na_2O
		Calcium	**3.75**	**Ca**	**5.25**	**CaO**
		Aluminium	**11.61**	**Al**	**21.94**	Al_2O_3
		Silicon	**18.92**	**Si**	**40.47**	SiO_2
		Hydrogen	**2.07**	**H**	**18.54**	H_2O
		Oxygen	**52.39**	**O**		
		Or	Or		Or	
		Potassium	4.65	K	5.60	K_2O
		Sodium	0.46	Na	0.61	Na_2O
		Calcium	2.38	Ca	3.33	CaO
		Magnesium	0.10	Mg	0.16	MgO
		Aluminium	10.69	Al	20.20	Al_2O_3
		Silicon	22.26	Si	47.62	SiO_2
		Hydrogen	2.40	H	21.42	H_2O
		Oxygen	57.07	O		
		Or	Or		Or	

Table B-2. Composition of natural zeolites (continued).

FTC	**Material name**	**Composition**	**%**		**%**	
CHA	Chabazite	Potassium	3.92	K	4.73	K_2O
		Sodium	6.83	Na	9.21	Na_2O
		Strontium	0.42	Sr	0.50	SrO
		Calcium	0.73	Ca	1.02	CaO
		Magnesium	0.05	Mg	0.08	MgO
		Aluminium	11.68	Al	22.07	Al_2O_3
		Iron	0.05	Fe	0.07	FeO
		Silicon	19.86	Si	42.50	SiO_2
		Hydrogen	2.21	H	19.75	H_2O
		Oxygen	54.24	O		
		Or	Or		Or	
		Potassium	0.75	K	0.91	K_2O
		Sodium	0.07	Na	0.09	Na_2O
		Strontium	0.25	Sr	0.30	SrO
		Calcium	7.17	Ca	10.04	CaO
		Magnesium	0.05	Mg	0.08	MgO
		Aluminium	21.70	Si	19.33	Al_2O_3
		Silicon	2.55	H	46.43	SiO_2
		Hydrogen	57.22	O	22.82	H_2O
		Oxygen				
	Willhendersonite	Potassium	7.43	K	8.95	K_2O
		Calcium	7.61	Ca	10.65	CaO
		Aluminium	15.38	Al	29.05	Al_2O_3
		Silicon	16.00	Si	34.24	SiO_2
		Hydrogen	1.91	H	17.11	H_2O
		Oxygen	51.67	O		
CHI	**Chiavennite**	**Calcium**	**7.54**	**Ca**	**10.55**	**CaO**
		Manganese	**10.34**	**Mn**	**13.35**	**MnO**
		Beryllium	**3.39**	**Be**	**9.41**	**BeO**
		Silicon	**26.42**	**Si**	**56.52**	SiO_2
		Hydrogen	**1.14**	**H**	**10.17**	H_2O
		Oxygen	**51.17**	**O**	**51.17**	**O**

Table B-2. Composition of natural zeolites (continued).

FTC	Material name	Composition	%		%	
DAC	Dachiardite	Cesium	0.82	Cs	0.86	Cs_2O
		Potassium	2.01	K	2.42	K_2O
		Barium	0.08	Ba	0.09	BaO
		Sodium	0.54	Na	0.73	Na_2O
		Strontium	0.59	Sr	0.69	SrO
		Calcium	3.44	Ca	4.82	CaO
		Aluminium	7.31	Al	13.82	Al_2O_3
		Iron	0.06	Fe	0.09	Fe_2O_3
		Silicon	29.70	Si	63.54	SiO_2
		Hydrogen	1.41	H	12.62	H_2O
		Oxygen	54.04	O		
		Or	Or		Or	
		Potassium	1.53	K	1.85	K_2O
		Barium	0.08	Ba	0.08	BaO
		Sodium	3.29	Na	4.44	Na_2O
		Calcium	1.17	Ca	1.64	CaO
		Magnesium	0.05	Mg	0.09	MgO
		Aluminium	7.25	Al	13.70	Al_2O_3
		Iron	0.34	Fe	0.49	Fe_2O_3
		Silicon	30.45	Si	65.14	SiO_2
		Hydrogen	1.50	H	13.38	H_2O
		Oxygen	54.34	O		
EAB	**Bellbergite**	**Potassium**	**1.58**	**K**	**1.90**	K_2O
		Barium	**1.28**	**Ba**	**1.43**	**BaO**
		Sodium	**0.50**	**Na**	**0.67**	Na_2O
		Strontium	**6.54**	**Sr**	**7.74**	**SrO**
		Calcium	**6.61**	**Ca**	**9.25**	**CaO**
		Aluminium	**15.11**	**Al**	**28.55**	Al_2O_3
		Silicon	**15.73**	**Si**	**33.65**	SiO_2
		Hydrogen	**1.88**	**H**	**16.81**	H_2O
		Oxygen	**50.77**	**O**	**50.77**	**O**
EDI	**Edingtonite**	**Barium**	**27.05**	**Ba**	**30.21**	**BaO**
		Aluminium	**10.63**	**Al**	**20.09**	Al_2O_3
		Silicon	**16.60**	**Si**	**35.51**	SiO_2
		Hydrogen	**1.59**	**H**	**14.20**	H_2O
		Oxygen	**44.13**	**O**		

Table B-2. Composition of natural zeolites (continued).

FTC	Material name	Composition	%		%	
EON	Direnzoite	Potassium	4.02	K	4.85	K_2O
		Barium	0.03	Ba	0.03	BaO
		Sodium	0.56	Na	0.75	Na_2O
		Strontium	0.06	Sr	0.07	SrO
		Calcium	1.89	Ca	2.65	CaO
		Magnesium	0.82	Mg	1.37	MgO
		Aluminium	7.86	Al	14.86	Al_2O_3
		Iron	0.18	Fe	0.23	FeO
		Silicon	28.70	Si	61.40	SiO_2
		Hydrogen	1.58	H	14.10	H_2O
		Oxygen	54.29	O		
		Or	Or		Or	
		Barium	**14.85**	**Ba**	**16.58**	**BaO**
		Strontium	**3.16**	**Sr**	**3.74**	**SrO**
		Aluminium	**7.78**	**Al**	**14.70**	**Al_2O_3**
		Silicon	**24.30**	**Si**	**51.99**	**SiO_2**
		Hydrogen	**1.45**	**H**	**12.99**	**H_2O**
		Oxygen	**48.45**	**O**	**48.45**	**O**
EPI	Epistilbite	Calcium	6.59	Ca	9.21	CaO
		Aluminium	8.87	Al	16.75	Al_2O_3
		Silicon	27.69	Si	59.23	SiO_2
		Hydrogen	1.66	H	14.80	H_2O
		Oxygen	55.20	O		
ERI	Erionite	Potassium	2.07	K	2.50	K_2O
		Sodium	0.75	Na	1.01	Na_2O
		Calcium	3.15	Ca	4.40	CaO
		Magnesium	0.72	Mg	1.19	MgO
		Aluminium	8.20	Al	15.50	Al_2O_3
		Silicon	26.01	Si	55.64	SiO_2
		Hydrogen	2.18	H	19.44	H_2O
		Oxygen	56.92	O		
		Or	Or		Or	

Table B-2. Composition of natural zeolites (continued).

FTC	Material name	Composition	%		%	
ERI	Erionite	Potassium	2.78	K	3.35	K_2O
		Sodium	4.57	Na	6.16	Na_2O
		Calcium	0.16	Ca	0.22	CaO
		Magnesium	0.16	Mg	0.26	MgO
		Aluminium	7.27	Al	13.73	Al_2O_3
		Iron	0.04	Fe	0.05	FeO
		Silicon	28.26	Si	60.45	SiO_2
		Hydrogen	1.76	H	15.77	H_2O
		Oxygen	55.00	O		
		Or	Or		Or	
		Potassium	4.39	K	5.28	K_2O
		Barium	0.09	Ba	0.10	BaO
		Sodium	1.79	Na	2.42	Na_2O
		Calcium	1.34	Ca	1.88	CaO
		Magnesium	0.05	Mg	0.08	MgO
		Aluminium	7.34	Al	13.87	Al_2O_3
		Hydrogen	26.59	Si	56.88	SiO_2
		Oxygen	2.18	H	19.48	H_2O
			56.23	O		
FAU	Faujasite	Sodium	2.29	Na	3.08	Na_2O
		Calcium	1.99	Ca	2.79	CaO
		Magnesium	0.40	Mg	0.67	MgO
		Aluminium	8.95	Al	16.92	Al_2O_3
		Silicon	22.63	Si	48.42	SiO_2
		Hydrogen	3.06	H	27.33	H_2O
		Oxygen	60.67	O		
		Or	Or		Or	
		Sodium	1.13	Na	1.53	Na_2O
		Calcium	3.96	Ca	5.54	CaO
		Magnesium	0.40	Mg	0.66	MgO
		Aluminium	8.88	Al	16.77	Al_2O_3
		Silicon	22.44	Si	48.01	SiO_2
		Hydrogen	3.03	H	27.10	H_2O
		Oxygen	60.16	O		
		Or	Or		Or	

Table B-2. Composition of natural zeolites (continued).

FTC	Material name	Composition	%		%	
FAU	Faujasite	Sodium	1.15	Na	1.55	Na_2O
		Calcium	0.67	Ca	0.93	CaO
		Magnesium	2.43	Mg	4.03	MgO
		Aluminium	8.99	Al	16.99	Al_2O_3
		Silicon	22.74	Si	48.64	SiO_2
		Hydrogen	3.07	H	27.45	H_2O
		Oxygen	60.95	O		
FER	Ferrierite	Potassium	1.78	K	2.15	K_2O
		Barium	0.11	Ba	0.12	BaO
		Sodium	0.49	Na	0.66	Na_2O
		Strontium	0.50	Sr	0.59	SrO
		Calcium	0.49	Ca	0.69	CaO
		Magnesium	1.88	Mg	3.12	MgO
		Aluminium	7.12	Al	13.45	Al_2O_3
		Silicon	31.22	Si	66.79	SiO_2
		Hydrogen	1.38	H	12.32	H_2O
		Oxygen	55.03	O		
		Or	Or		Or	
		Potassium	3.07	K	3.70	K_2O
		Sodium	1.00	Na	1.35	Na_2O
		Calcium	0.21	Ca	0.30	CaO
		Magnesium	0.69	Mg	1.14	MgO
		Aluminium	5.16	Al	9.76	Al_2O_3
		Silicon	33.34	Si	71.33	SiO_2
		Hydrogen	1.39	H	12.41	H_2O
		Oxygen	55.13	O		
		Or	Or		Or	
		Potassium	1.45	K	1.75	K_2O
		Barium	0.11	Ba	0.12	BaO
		Sodium	2.70	Na	3.64	Na_2O
		Strontium	0.10	Sr	0.12	SrO
		Calcium	0.08	Ca	0.11	CaO
		Magnesium	0.35	Mg	0.59	MgO
		Aluminium	5.18	Al	9.78	Al_2O_3
		Silicon	33.40	Si	71.46	SiO_2
		Hydrogen	1.39	H	12.44	H_2O
		Oxygen	55.24	O		

Table B-2. Composition of natural zeolites (continued).

FTC	Material name	Composition	%		%	
GIS	Gismondine	Calcium	11.16	Ca	15.61	CaO
		Aluminium	15.02	Al	28.38	Al_2O_3
		Silicon	15.63	Si	33.45	SiO_2
		Hydrogen	2.52	H	22.56	H_2O
		Oxygen	55.67	O		
	Amicite	Potassium	11.32	K	13.64	K_2O
		Sodium	6.66	Na	8.98	Na_2O
		Aluminium	15.63	Al	29.53	Al_2O_3
		Silicon	16.27	Si	34.81	SiO_2
		Hydrogen	1.46	H	13.04	H_2O
		Oxygen	48.66	O		
	Gobbinsite	Potassium	6.13	K	7.38	K_2O
		Sodium	4.29	Na	5.78	Na_2O
		Calcium	1.79	Ca	2.51	CaO
		Aluminium	12.48	Al	23.59	Al_2O_3
		Silicon	20.96	Si	44.84	SiO_2
		Hydrogen	1.81	H	16.13	H_2O
		Oxygen	52.54	O		
	Garronite	Sodium	1.74	Na	2.35	Na_2O
		Calcium	7.58	Ca	10.61	CaO
		Aluminium	12.25	Al	23.15	Al_2O_3
		Silicon	21.26	Si	45.48	SiO_2
		Hydrogen	2.06	H	18.41	H_2O
		Oxygen	55.10	O		
GME	Gmelinite	**Potassium**	**0.21**	**K**	**0.25**	**K_2O**
		Sodium	**0.86**	**Na**	**1.16**	**Na_2O**
		Strontium	**5.68**	**Sr**	**6.71**	**SrO**
		Calcium	**3.96**	**Ca**	**5.54**	**CaO**
		Aluminium	**10.13**	**Al**	**19.13**	**Al_2O_3**
		Silicon	**22.23**	**Si**	**47.55**	**SiO_2**
		Hydrogen	**2.25**	**H**	**20.08**	**H_2O**
		Oxygen	**54.69**	**O**		
		Or	Or		Or	

Table B-2. Composition of natural zeolites (continued).

FTC	Material name	Composition	%		%	
GME	Gmelinite	Potassium	6.25	K	7.53	K_2O
		Sodium	1.96	Na	2.64	Na_2O
		Calcium	1.12	Ca	1.57	CaO
		Aluminium	9.81	Al	18.53	Al_2O_3
		Silicon	23.62	Si	50.54	SiO_2
		Hydrogen	2.15	H	19.19	H_2O
		Oxygen	55.09	O		
		Or	Or		Or	
		Potassium	0.31	K	0.38	K_2O
		Sodium	8.74	Na	11.79	Na_2O
		Calcium	0.06	Ca	0.08	CaO
		Aluminium	9.99	Al	18.88	Al_2O_3
		Silicon	23.15	Si	49.52	SiO_2
		Hydrogen	2.17	H	19.36	H_2O
		Oxygen	55.58	O		
GON	Gonnardite	Sodium	5.69	Na	7.67	Na_2O
		Calcium	4.96	Ca	6.94	CaO
		Aluminium	13.35	Al	25.22	Al_2O_3
		Silicon	20.84	Si	44.58	SiO_2
		Hydrogen	1.75	H	15.60	H_2O
		Oxygen	53.42	O		
GOO	Goosecreekite	Calcium	6.59	Ca	9.21	CaO
		Aluminium	8.87	Al	16.75	Al_2O_3
		Silicon	27.69	Si	59.23	SiO_2
		Hydrogen	1.66	H	14.80	H_2O
		Oxygen	55.20	O		
HEU	Heulandite	Potassium	0.60	K	0.72	K_2O
		Barium	0.29	Ba	0.33	BaO
		Sodium	1.03	Na	1.38	Na_2O
		Strontium	0.16	Sr	0.18	SrO
		Calcium	5.06	Ca	7.09	CaO
		Magnesium	0.01	Mg	0.01	MgO
		Aluminium	8.95	Al	16.91	Al_2O_3
		Silicon	26.54	Si	56.78	SiO_2
		Hydrogen	1.86	H	16.59	H_2O
		Oxygen	55.51	O		
		Or	Or		Or	

Table B-2. Composition of natural zeolites (continued).

FTC	Material name	Composition	%		%	
HEU	Heulandite	Potassium	0.79	K	0.95	K_2O
		Sodium	3.36	Na	4.53	Na_2O
		Calcium	2.60	Ca	3.64	CaO
		Aluminium	7.76	Al	14.66	Al_2O_3
		Silicon	28.85	Si	61.73	SiO_2
		Hydrogen	1.61	H	14.37	H_2O
		Oxygen	55.03	O		
		Or	Or		Or	
		Potassium	**3.24**	**K**	**3.90**	K_2O
		Barium	**0.57**	**Ba**	**0.63**	**BaO**
		Sodium	**0.76**	**Na**	**1.03**	Na_2O
		Strontium	**1.69**	**Sr**	**2.00**	**SrO**
		Calcium	**2.27**	**Ca**	**3.17**	**CaO**
		Magnesium	**0.54**	**Mg**	**0.89**	**MgO**
		Aluminium	**8.45**	**Al**	**15.96**	Al_2O_3
		Iron	**1.08**	**Fe**	**1.54**	Fe_2O_3
		Silicon	**25.64**	**Si**	**54.86**	SiO_2
		Hydrogen	**1.80**	**H**	**16.05**	H_2O
		Oxygen	**53.98**	**O**		
		Or	Or		Or	
		Potassium	**0.30**	**K**	**0.36**	K_2O
		Barium	**0.67**	**Ba**	**0.75**	**BaO**
		Sodium	**0.32**	**Na**	**0.43**	Na_2O
		Strontium	**6.39**	**Sr**	**7.55**	**SrO**
		Calcium	**2.45**	**Ca**	**3.43**	**CaO**
		Magnesium	**0.02**	**Mg**	**0.03**	**MgO**
		Aluminium	**8.61**	**Al**	**16.26**	Al_2O_3
		Silicon	**26.26**	**Si**	**56.19**	SiO_2
		Hydrogen	**1.68**	**H**	**15.01**	H_2O
		Oxygen	**53.31**	**O**		
		Or	Or		Or	

Table B-2. Composition of natural zeolites (continued).

FTC	Material name	Composition	%		%	
HEU	Heulandite	**Potassium**	**0.48**	**K**	**0.58**	**K_2O**
		Barium	**11.43**	**Ba**	**12.77**	**BaO**
		Sodium	**0.25**	**Na**	**0.34**	**Na_2O**
		Strontium	**0.88**	**Sr**	**1.04**	**SrO**
		Calcium	**1.89**	**Ca**	**2.64**	**CaO**
		Aluminium	**8.08**	**Al**	**15.27**	**Al_2O_3**
		Silicon	**25.36**	**Si**	**54.25**	**SiO_2**
		Hydrogen	**1.47**	**H**	**13.10**	**H_2O**
		Oxygen	**50.15**	**O**		
	Clinoptilolite	**Potassium**	**6.52**	**K**	**7.85**	**K_2O**
		Sodium	**0.69**	**Na**	**0.93**	**Na_2O**
		Strontium	**1.15**	**Sr**	**1.35**	**SrO**
		Calcium	**0.06**	**Ca**	**0.08**	**CaO**
		Magnesium	**0.16**	**Mg**	**0.27**	**MgO**
		Manganese	**0.02**	**Mn**	**0.03**	**MnO**
		Aluminium	**6.22**	**Al**	**11.74**	**Al_2O_3**
		Iron	**0.06**	**Fe**	**0.08**	**FeO**
		Silicon	**29.15**	**Si**	**62.37**	**SiO_2**
		Hydrogen	**1.71**	**H**	**15.28**	**H_2O**
		Oxygen	**54.27**	**O**		
		Or	Or		Or	
		Potassium	1.89	K	2.28	K_2O
		Barium	0.46	Ba	0.51	BaO
		Sodium	3.21	Na	4.33	Na_2O
		Calcium	0.90	Ca	1.27	CaO
		Manganese	0.02	Mn	0.03	MnO
		Aluminium	6.60	Al	12.46	Al_2O_3
		Iron	0.39	Fe	0.08	FeO
					0.47	Fe_2O_3
		Silicon	30.32	Si	64.87	SiO_2
		Hydrogen	1.52	H	13.59	H_2O
		Oxygen	54.68	O		
		Or	Or		Or	

Table B-2. Composition of natural zeolites (continued).

FTC	Material name	Composition	%		%	
HEU	Clinoptilolite	Potassium	1.50	K	1.80	K_2O
		Sodium	1.48	Na	1.99	Na_2O
		Calcium	2.78	Ca	3.89	CaO
		Magnesium	0.15	Mg	0.25	MgO
		Aluminium	6.61	Al	12.49	Al_2O_3
		Silicon	29.91	Si	63.98	SiO_2
		Hydrogen	1.74	H	15.57	H_2O
		Oxygen	55.84	O		
LAU	Laumontite	Calcium	8.52	Ca	11.92	CaO
		Aluminium	11.47	Al	21.67	Al_2O_3
		Silicon	23.88	Si	51.09	SiO_2
		Hydrogen	1.71	H	15.32	H_2O
		Oxygen	54.42	O		
LEV	Levyne	Potassium	0.52	K	0.63	K_2O
		Sodium	0.99	Na	1.34	Na_2O
		Calcium	7.26	Ca	10.16	CaO
		Aluminium	11.30	Al	21.35	Al_2O_3
		Silicon	21.79	Si	46.61	SiO_2
		Hydrogen	2.23	H	19.92	H_2O
		Oxygen	55.91	O		
		Or	Or		Or	
		Potassium	0.96	K	1.16	K_2O
		Sodium	5.73	Na	7.72	Na_2O
		Calcium	2.32	Ca	3.24	CaO
		Magnesium	0.13	Mg	0.21	MgO
		Aluminium	11.09	Al	20.95	Al_2O_3
		Silicon	21.35	Si	45.67	SiO_2
		Hydrogen	2.36	H	21.05	H_2O
		Oxygen	56.08	O		

Table B-2. Composition of natural zeolites (continued).

FTC	Material name	Composition	%		%	
LOV	**Lovdarite**	**Potassium**	**5.28**	**K**	**6.36**	**K_2O**
		Barium	**0.19**	**Ba**	**0.21**	**BaO**
		Sodium	**11.27**	**Na**	**15.19**	**Na_2O**
		Calcium	**0.36**	**Ca**	**0.50**	**CaO**
		Magnesium	**0.03**	**Mg**	**0.05**	**MgO**
		Titanium	**0.07**	**Ti**	**0.11**	**TiO_2**
		Beryllium	**2.53**	**Be**	**7.01**	**BeO**
		Aluminium	**0.96**	**Al**	**1.81**	**Al_2O_3**
		Iron	**0.08**	**Fe**	**0.11**	**Fe_2O_3**
		Silicon	**26.67**	**Si**	**57.06**	**SiO_2**
		Phosphorus	**0.04**	**P**	**0.10**	**P_2O5**
		Hydrogen	**1.28**	**H**	**11.46**	**H_2O**
		Oxygen	**51.26**	**O**		
LTL	**Perlialite**	**Potassium**	**10.33**	**K**	**12.45**	**K_2O**
		Aluminium	**10.70**	**Al**	**20.21**	**Al_2O_3**
		Thallium	**6.75**	**Tl**	**7.54**	**Tl_2O_3**
		Silicon	**22.27**	**Si**	**47.63**	**SiO_2**
		Hydrogen	**1.33**	**H**	**11.90**	**H_2O**
		Oxygen	**48.62**	**O**		
MAR	**Maricopaite**	**Calcium**	**1.73**	**Ca**	**2.42**	**CaO**
		Aluminium	**6.15**	**Al**	**11.62**	**Al_2O_3**
		Silicon	**20.09**	**Si**	**42.98**	**SiO_2**
		Hydrogen	**1.26**	**H**	**11.26**	**H_2O**
		Lead	**29.32**	**Pb**	**31.58**	**PbO**
		Oxygen	**41.44**	**O**		
MAZ	Mazzite	Potassium	2.65	K	3.19	K_2O
		Calcium	2.00	Ca	2.80	CaO
		Magnesium	1.73	Mg	2.87	MgO
		Aluminium	9.42	Al	17.79	Al_2O_3
		Silicon	26.50	Si	56.70	SiO_2
		Hydrogen	1.87	H	16.68	H_2O
		Oxygen	55.84	O		
		Or	Or		Or	

Table B-2. Composition of natural zeolites (continued).

FTC	Material name	Composition	%		%	
MAZ	Mazzite	Potassium	0.03	K	0.03	K_2O
		Barium	0.14	Ba	0.16	BaO
		Sodium	5.99	Na	8.07	Na_2O
		Calcium	0.12	Ca	0.17	CaO
		Magnesium	0.13	Mg	0.22	MgO
		Aluminium	7.59	Al	14.35	Al_2O_3
		Iron	0.46	Fe	0.60	FeO
		Silicon	26.94	Si	57.64	SiO_2
		Hydrogen	2.09	H	18.70	H_2O
		Oxygen	56.49	O		
MER	**Merlinoite**	**Potassium**	**6.25**	**K**	**7.53**	**K_2O**
		Barium	**2.09**	**Ba**	**2.34**	**BaO**
		Sodium	**0.53**	**Na**	**0.71**	**Na_2O**
		Calcium	**2.29**	**Ca**	**3.20**	**CaO**
		Aluminium	**9.56**	**Al**	**18.06**	**Al_2O_3**
		Iron	**0.43**	**Fe**	**0.55**	**FeO**
		Silicon	**24.28**	**Si**	**51.95**	**SiO_2**
		Hydrogen	**1.74**	**H**	**15.58**	**H_2O**
		Oxygen	**52.83**	**O**		
MFI	Mutinaite	Sodium	0.98	Na	1.32	Na_2O
		Calcium	2.27	Ca	3.17	CaO
		Aluminium	4.20	Al	7.94	Al_2O_3
		Silicon	33.78	Si	72.28	SiO_2
		Hydrogen	1.71	H	15.30	H_2O
		Oxygen	57.06	O		
MON	Montesommaite	Potassium	13.82	K	16.65	K_2O
		Sodium	0.19	Na	0.25	Na_2O
		Aluminium	10.42	Al	19.69	Al_2O_3
		Silicon	26.09	Si	55.80	SiO_2
		Hydrogen	0.83	H	7.40	H_2O
		Oxygen	48.66	O		
MOR	Mordenite	Potassium	0.45	K	0.54	K_2O
		Sodium	2.89	Na	3.90	Na_2O
		Calcium	2.29	Ca	3.21	CaO
		Aluminium	6.79	Al	12.83	Al_2O_3
		Silicon	31.49	Si	67.36	SiO_2
		Hydrogen	1.36	H	12.16	H_2O
		Oxygen	54.73	O		

Table B-2. Composition of natural zeolites (continued).

FTC	Material name	Composition	%		%	
NAB	**Nabesite**	**Sodium**	**10.27**	**Na**	**13.84**	**Na_2O**
		Beryllium	**2.30**	**Be**	**6.38**	**BeO**
		Silicon	**28.67**	**Si**	**61.34**	**SiO_2**
		Hydrogen	**2.01**	**H**	**17.93**	**H_2O**
		Oxygen	**56.76**	**O**		
NAT	Natrolite	Sodium	12.09	Na	16.30	Na_2O
		Aluminium	14.19	Al	26.82	Al_2O_3
		Silicon	22.16	Si	47.41	SiO_2
		Hydrogen	1.06	H	9.48	H_2O
		Oxygen	50.49	O		
	Mesolite	Sodium	3.95	Na	5.32	Na_2O
		Calcium	6.88	Ca	9.63	CaO
		Aluminium	13.90	Al	26.26	Al_2O_3
		Silicon	21.70	Si	46.42	SiO_2
		Hydrogen	1.38	H	12.37	H_2O
		Oxygen	52.19	O		
	Scolecite	Calcium	10.22	Ca	14.29	CaO
		Aluminium	13.75	Al	25.99	Al_2O_3
		Silicon	21.48	Si	45.94	SiO_2
		Hydrogen	1.54	H	13.78	H_2O
		Oxygen	53.01	O		
NES	Gottardiite	Sodium	0.68	Na	0.91	Na_2O
		Calcium	1.97	Ca	2.76	CaO
		Magnesium	0.72	Mg	1.19	MgO
		Aluminium	5.04	Al	9.53	Al_2O_3
		Silicon	32.32	Si	69.14	SiO_2
		Hydrogen	1.84	H	16.48	H_2O
		Oxygen	57.43	O		
OFF	Offretite	Potassium	2.96	K	3.56	K_2O
		Calcium	3.03	Ca	4.24	CaO
		Magnesium	1.17	Mg	1.94	MgO
		Aluminium	9.65	Al	18.24	Al_2O_3
		Silicon	24.73	Si	52.90	SiO_2
		Hydrogen	2.11	H	18.84	H_2O
		Oxygen	56.35	O		
PAR	Partheite	Calcium	12.40	Ca	17.35	CaO
		Aluminium	16.69	Al	31.54	Al_2O_3
		Silicon	17.38	Si	37.18	SiO_2
		Hydrogen	1.56	H	13.93	H_2O
		Oxygen	51.97	O		

Table B-2. Composition of natural zeolites (continued).

FTC	Material name	Composition	%	%
PAU	Paulingite	Potassium	3.06 K	3.69 K_2O
		Barium	0.40 Ba	0.45 BaO
		Sodium	0.58 Na	0.78 Na_2O
		Calcium	4.35 Ca	6.08 CaO
		Aluminium	8.53 Al	16.12 Al_2O_3
		Silicon	25.70 Si	54.99 SiO_2
		Hydrogen	2.01 H	17.96 H_2O
		Oxygen	55.36 O	
		Or	Or	Or
		Potassium	4.82 K	5.81 K_2O
		Barium	0.69 Ba	0.77 BaO
		Sodium	0.61 Na	0.82 Na_2O
		Calcium	2.09 Ca	2.93 CaO
		Aluminium	7.36 Al	13.90 Al_2O_3
		Silicon	25.12 Si	53.73 SiO_2
		Hydrogen	2.46 H	22.01 H_2O
		Oxygen	56.86 O	
PHI	Phillipsite	Potassium	3.61 K	4.35 K_2O
		Sodium	3.18 Na	4.29 Na_2O
		Calcium	3.08 Ca	4.31 CaO
		Aluminium	11.63 Al	21.97 Al_2O_3
		Silicon	22.47 Si	48.08 SiO_2
		Hydrogen	1.86 H	16.63 H_2O
		Oxygen	54.16 O	
		Or	Or	Or
		Potassium	4.73 K	5.70 K_2O
		Sodium	2.43 Na	3.28 Na_2O
		Calcium	4.24 Ca	5.94 CaO
		Aluminium	11.43 Al	21.59 Al_2O_3
		Silicon	22.09 Si	47.26 SiO_2
		Hydrogen	1.83 H	16.35 H_2O
		Oxygen	53.24 O	
		Or	Or	Or

Table B-2. Composition of natural zeolites (continued).

FTC	Material name	Composition	%		%	
PHI	Phillipsite	Potassium	3.56	K	4.29	K_2O
		Sodium	1.40	Na	1.88	Na_2O
		Calcium	6.09	Ca	8.52	CaO
		Aluminium	11.47	Al	21.68	Al_2O_3
		Silicon	22.18	Si	47.45	SiO_2
		Hydrogen	1.84	H	16.42	H_2O
		Oxygen	53.46	O		
	Harmotome	**Potassium**	**0.55**	**K**	**0.67**	K_2O
		Barium	**15.58**	**Ba**	**17.40**	**BaO**
		Sodium	**0.65**	**Na**	**0.88**	Na_2O
		Aluminium	**7.66**	**Al**	**14.46**	Al_2O_3
		Silicon	**23.91**	**Si**	**51.14**	SiO_2
		Hydrogen	**1.72**	**H**	**15.33**	H_2O
		Oxygen	**49.93**	**O**		
	Florkeite	Potassium	8.44	K	10.17	K_2O
		Sodium	1.65	Na	2.23	Na_2O
		Calcium	5.77	Ca	8.07	CaO
		Aluminium	15.54	Al	29.36	Al_2O_3
		Silicon	16.17	Si	34.60	SiO_2
		Hydrogen	1.74	H	15.56	H_2O
		Oxygen	50.68	O		
RHO	**Pahasapaite**	**Potassium**	**1.33**	**K**	**1.60**	K_2O
		Sodium	**0.13**	**Na**	**0.18**	Na_2O
		Lithium	**2.28**	**Li**	**4.91**	Li_2O
		Calcium	**6.24**	**Ca**	**8.73**	**CaO**
		Beryllium	**6.12**	**Be**	**16.99**	**BeO**
		Phosphorus	**21.04**	**P**	**48.22**	P_2O_5
		Hydrogen	**2.17**	**H**	**19.38**	H_2O
		Oxygen	**60.69**	**O**		
-RON	**Roggianite**	**Calcium**	**14.82**	**Ca**	**20.74**	**CaO**
		Beryllium	**1.67**	**Be**	**4.63**	**BeO**
		Aluminium	**9.98**	**Al**	**18.86**	Al_2O_3
		Silicon	**20.78**	**Si**	**44.45**	SiO_2
		Hydrogen	**1.27**	**H**	**11.33**	H_2O
		Oxygen	**51.49**	**O**		
STI	Stellerite	Calcium	5.69	Ca	7.96	CaO
		Aluminium	7.66	Al	14.47	Al_2O_3
		Silicon	27.90	Si	59.68	SiO_2
		Hydrogen	2.00	H	17.89	H_2O
		Oxygen	56.76	O		

Table B-2. Composition of natural zeolites (continued).

FTC	Material name	Composition	%		%	
STI	Stilbite	Sodium	0.80	Na	1.08	Na_2O
		Calcium	5.57	Ca	7.79	CaO
		Aluminium	7.50	Al	14.17	Al_2O_3
		Silicon	27.32	Si	58.46	SiO_2
		Hydrogen	2.10	H	18.78	H_2O
		Oxygen	56.70	O		
		Or	Or		Or	
		Sodium	2.39	Na	3.22	Na_2O
		Calcium	4.17	Ca	5.83	CaO
		Aluminium	7.48	Al	14.14	Al_2O_3
		Silicon	27.27	Si	58.34	SiO_2
		Hydrogen	2.10	H	18.74	H_2O
		Oxygen	56.59	O		
	Barrerite	Potassium	1.55	K	1.86	K_2O
		Sodium	4.67	Na	6.30	Na_2O
		Calcium	1.26	Ca	1.76	CaO
		Magnesium	0.15	Mg	0.26	MgO
		Aluminium	8.24	Al	15.58	Al_2O_3
		Iron	0.02	Fe	0.03	FeO
		Silicon	23.80	Si	50.93	SiO_2
		Hydrogen	1.94	H	17.33	H_2O
		Oxygen	58.36	O		
TER	Terranovaite	Potassium	0.14	K	0.17	K_2O
		Sodium	1.73	Na	2.34	Na_2O
		Calcium	2.66	Ca	3.72	CaO
		Magnesium	0.09	Mg	0.14	MgO
		Aluminium	5.95	Al	11.25	Al_2O_3
		Silicon	34.12	Si	72.99	SiO_2
		Hydrogen	1.05	H	9.37	H_2O
		Oxygen	54.26	O		
THO	Thomsonite	Sodium	2.85	Na	3.84	Na_2O
		Calcium	9.94	Ca	13.91	CaO
		Aluminium	16.73	Al	31.60	Al_2O_3
		Silicon	17.41	Si	37.25	SiO_2
		Hydrogen	1.50	H	13.40	H_2O
		Oxygen	51.58	O		
		Or	Or		Or	

Table B-2. Composition of natural zeolites (continued).

FTC	Material name	Composition	%		%	
THO	Thomsonite	**Sodium**	**2.57**	**Na**	**3.47**	**Na_2O**
		Strontium	**13.74**	**Sr**	**16.25**	**SrO**
		Calcium	**2.69**	**Ca**	**3.77**	**CaO**
		Aluminium	**15.11**	**Al**	**28.55**	**Al_2O_3**
		Silicon	**15.73**	**Si**	**33.64**	**SiO_2**
		Hydrogen	**1.60**	**H**	**14.32**	**H_2O**
		Oxygen	**48.56**	**O**		
TSC	**Tschortnerite**	**Potassium**	**0.96**	**K**	**1.16**	**K_2O**
		Barium	**1.68**	**Ba**	**1.88**	**BaO**
		Strontium	**4.30**	**Sr**	**5.08**	**SrO**
		Calcium	**8.03**	**Ca**	**11.23**	**CaO**
		Aluminium	**13.24**	**Al**	**25.01**	**Al_2O_3**
		Copper	**7.79**	**Cu**	**8.78**	**Cu_2O**
		Silicon	**13.78**	**Si**	**29.48**	**SiO_2**
		Hydrogen	**1.81**	**H**	**16.20**	**H_2O**
		Oxygen	**48.41**	**O**		
VSV	**Gaultite**	**Sodium**	**11.53**	**Na**	**15.55**	**Na_2O**
		Zinc	**16.40**	**Zn**	**20.41**	**ZnO**
		Silicon	**24.65**	**Si**	**52.74**	**SiO_2**
		Hydrogen	**1.26**	**H**	**11.30**	**H_2O**
		Oxygen	**46.15**	**O**		
WEI	**Weinebeneite**	**Calcium**	**11.04**	**Ca**	**15.44**	**CaO**
		Beryllium	**7.45**	**Be**	**20.66**	**BeO**
		Phosphorus	**17.06**	**P**	**39.09**	**P_2O_5**
		Hydrogen	**2.78**	**H**	**24.81**	**H_2O**
		Oxygen	**61.68**	**O**		
YUG	Yugawaralite	Calcium	6.79	Ca	9.49	CaO
		Aluminium	9.14	Al	17.26	Al_2O_3
		Silicon	28.53	Si	61.04	SiO_2
		Hydrogen	1.37	H	12.20	H_2O
		Oxygen	54.18	O		
Unassigned	**Alflarsenite**	**Sodium**	**4.57**	**Na**	**6.15**	**Na_2O**
		Calcium	**15.92**	**Ca**	**22.27**	**CaO**
		Beryllium	**5.37**	**Be**	**14.90**	**BeO**
		Silicon	**22.31**	**Si**	**47.73**	**SiO_2**
		Hydrogen	**1.00**	**H**	**8.94**	**H_2O**
		Oxygen	**50.84**	**O**		

Table B-2. Composition of natural zeolites (continued).

FTC	Material name	Composition	%		%	
Un-assigned	Cowlesite	Calcium	8.98	Ca	12.56	CaO
		Aluminium	12.09	Al	22.84	Al_2O_3
		Silicon	18.88	Si	40.38	SiO_2
		Hydrogen	2.71	H	24.21	H_2O
		Oxygen	57.35	O		
Un-assigned	**Kalborsite**	**Potassium**	**23.70**	**K**	**28.55**	K_2O
		Aluminium	**10.91**	**Al**	**20.61**	Al_2O_3
		Silicon	**17.03**	**Si**	**36.43**	SiO_2
		Boron	**1.53**	**B**	**4.92**	B_2O_3
		Hydrogen	**0.41**	**H**	**3.64**	H_2O
		Chlorine	**3.58**	**Cl**		
		Oxygen	**42.84**	**O**		
Un-assigned	Tvedalite	Potassium	17.91	K	21.58	K_2O
		Aluminium	12.36	Al	23.36	Al_2O_3
		Silicon	25.74	Si	55.06	SiO_2
		Oxygen	43.99	O		

Table B-3. Internal pore space of the selected natural zeolite framework types (FTC).

	FTC	**Material name**	**Chemical formula**	**Internal pore space (Å)**
1	**ANA**	**Analcime**	**$\vert Na(H_2O)\vert\ [AlSi_2O_6]$**	**4.21**
		Leucite	**$K\ [AlSi_2O_6]$**	
		Wairakite	**$\vert Ca(H_2O)_2\vert\ [Al_2Si_4O_{12}]$**	
2	BEA	Tschernichite	$\vert (Ca,Mg,Na_{0.5})(\ H_2O)_8\ \vert\ [Al_2Si_6O_{16}]$	6.68
3	BOG	Boggsite	$\vert (Ca,Na_{0.5},K_{0.5})_9\ (H_2O)_{70}\vert\ [Al_{18}Si_{78}O_{192}]$	8.05
4	CHA	Chabazite	$\vert (Ca_{0.5},K,Na)_x(H_2O)_{12}\vert\ [Al_x\ Si_{12-x}\ O_{24}]$	7.37
		Willhendersonite	$\vert K_2Ca_2\ (H_2O)_{10}\vert\ [Al_6Si_6O_{24}]$	
5	**DAC**	**Dachiardite**	**$\vert (\ Ca_{0.5},Na,K)_5(H_2O)_{13}\vert [Al_5\ Si_{19}\ O_{48}]$**	**5.28**
6	EON	Direnzoite	$\vert NaK_6MgCa_2(H_2O)_{36}\vert\ [Al_{13}Si_{47}O_{120}]$	7.83
7	**EPI**	**Epistilbite**	**$\vert (Ca,Na_2)_3(H_2O)_{16}\vert\ [Al_6Si_{18}O_{48}]$**	**5.47**
8	ERI	Erionite	$\vert K_2(K,Na,Ca_{0.5})_7(H_2O)_{30}\vert\ [Al_9Si_{27}O_{72}]$	7.04
9	FAU	Faujasite	$\vert (Na,Ca_{0.5},Mg_{0.5},K)_x(H_2O)_{16}\vert [Al_xSi_{12-x}O_{24}]$	11.24
10	FER	Ferrierite	$\vert Mg_{0.5}K,\ Na,\ Ca_{0.5})_6(H_2O)_{20}\vert\ [Al_6Si_{30}O_{72}]$	6.31
11	**GIS**	**Gismondine**	**$\vert Ca_4\ (H_2O)_{18}\vert\ [Al_8Si_8O_{32}]$**	**4.97**
		Amicite	**$\vert K_4Na_4\ (H_2O)_{10}\vert\ [Al_8Si_8O_{32}]$**	
		Gobbinsite	**$\vert (Na,Ca_{0.5})_6\ (H_2O)_{12}\vert\ [Al_6Si_{10}O_{32}]$**	
		Garronite	**$\vert (Ca_{0.5},Na)_6\ (H_2O)_{14}\vert\ [Al_6Si_{10}O_{32}]$**	
12	GME	Gmelinite	$\vert (Ca_{0.5},Sr,K,Na)_8(H_2O)_{22}\vert [Al_8Si_{16}O_{48}]$	7.76
13	GON	Gonnardite	$\vert (Na,Ca_{0.5})_{8-10}(H_2O)_{12}\vert\ [Al_{8+x}Si_{12-x}O_{40}]$	6.32
14	**GOO**	**Goosecreekite**	**$\vert Ca_2(H_2O)_{10}\vert\ [Al_4Si_{12}O_{32}]$**	**4.54**
15	HEU	Heulandite	$\vert (Ca_{0.5},Sr_{0.5},Ba_{0.5},Mg_{0.5},Na,K)_9(H_2O)_{24}\vert [Al_9\ Si_{27}\ O_{72}]$	5.97
		Clinoptilolite	$\vert (K,Na,Ca_{0.5},Sr_{0.5},Ba_{0.5},Mg_{0.5})_6(H_2O)_{20}\vert [Al_6\ Si_{30}\ O_{72}]$	
16	LAU	Laumontite	$\vert Ca_4(H_2O)_{18}\vert\ [Al_8Si_{16}O_{48}]$	6.04
17	LEV	Levyne	$\vert (Ca_{0.5},Sr,K,Na)_6(H_2O)_{17}\vert\ [Al_6Si_{12}O_{36}]$	7.10
18	MAZ	Mazzite	$\vert Na_4(H_2O)_{30}\vert\ [Al_{10}Si_{26}O_{72}]$	8.09
19	MFI	Mutinaite	$\vert Na_3Ca_4\ (H_2O)_{60}\vert\ [Al_{11}Si_{85}O_{192}]$	6.36
20	**MON**	**Montesommaite**	**$\vert K_9(H_2O)_{10}\vert\ [Al_9Si_{23}O_{64}]$**	**4.24**
21	MOR	Mordenite	$\vert Na_2,Ca,K_2)4(H_2O)_{28}\vert\ [Al_8Si_{40}O_{96}]$	6.70
22	**NAT**	**Natrolite**	**$\vert Na_2(H_2O)_2\vert\ [Al_2Si_3O_{10}]$**	**4.52**
		Mesolite	**$\vert Na_2Ca_2(H_2O)_8\vert\ [Al_6Si_9O_{30}]$**	
		Scolecite	**$\vert Ca(H_2O)_3\vert\ [Al_2Si_3O_{10}]$**	
23	NES	Gottardiite	$\vert (Na,K)_3\ Mg_3Ca_5(H_2O)_{93}\vert\ [Al_{19}Si_{117}O_{272}]$	7.04

Table B-3. Internal pore space of the selected natural zeolite framework types (FTC) (continued).

	FTC	Material name	Chemical formula	Internal pore space (Å)
24	OFF	Offretite	$\vert(CaKMg)(H_2O)_{16}\vert[Al_5Si_{13}O_{36}]$	7.00
25	**PAR**	**Partheite**	**$\vert(Ca_2(H_2O)_4\vert\ [Al_4Si_4O_{15}(OH)_2]$**	**4.21**
26	PAU	Paulingite	$\vert(Ca_{0.5},K,Na,Ba_{0.5})_{10}\ (H_2O)_{27\text{-}34}\vert[Al_{10}Si_{32}O_{84}]$	10.48
27	**PHI**	**Phillipsite**	**$\vert(Na,K,Ca_{0.5})_x(H_2O)_{12}\vert\ [Al_xSi_{16\text{-}x}O_{32}]$**	**5.40**
		Florkeite	**$\vert(K_3Ca_2Na\ (H_2O)_{12}\vert\ [Al_8Si_8O_{32}]$**	
28	STI	Stellerite	$\vert(Ca)_4(H_2O)_{28}\vert\ [Al_8Si_{28}O_{72}]$	6.29
		Stilbite	$\vert(Na,Ca_{0.5},\ K)_9(H_2O)_{26}\vert[Al_9\ Si_{27}\ O_{72}]$	
		Barrerite	$\vert(Na,K,Ca_{0.5})_8(H_2O)_{26}\vert\ [Al_8Si_{28}O_{72}]$	
29	TER	Terranovaite	$\vert NaCa\ (H_2O)_{13}\vert\ [Al_3Si_{17}O_{40}]$	7.00
30	**THO**	**Thomsonite**	**$\vert((Sr,Ca)_2Na)(H_2O)_6\vert\ [Al_5Si_5O_{20}]$**	**5.15**
31	**YUG**	**Yugawaralite**	**$\vert Ca_2\ (H_2O)_8\vert\ [Al_4Si_{12}O_{32}]$**	**4.49**
32	Unassigned	Cowlesite	$\vert Ca(H_2O)_{5.3}\vert\ [Al_2Si_3O_{10}]$	/
33	Unassigned	Tvedalite	$\vert(Ca,Mn)_4\ (H_2O)_3\vert\ [Be_3Si_6O_{17}(OH)_4]$	/

Table B-4. Si/Al ratio of the selected natural zeolite framework types (FTC).

	FTC	**Material name**	**Si/Al-ratio**
1	BEA	Tschernichite	3.54
2	BOG	Boggsite	4.48
3	CHA	Chabazite	1.93-3.33
		Willhendersonite	**1.18**
4	EON	Direnzoite	4.13
5	ERI	Erionite	3.59-4.40
6	FAU	Faujasite	2.86
7	FER	Ferrierite	4.97-7.31
8	GME	Gmelinite	2.62-2.73
9	**GON**	**Gonnardite**	**1.77**
10	HEU	Heulandite	3.36-4.21
		Clinoptilolite	5.12-5.20
11	LAU	Laumontite	2.36
12	LEV	Levyne	2.18
13	MAZ	Mazzite	3.19-4.02
14	MFI	Mutinaite	9.10
15	MOR	Mordenite	5.25
16	NES	Gottardiite	7.25
17	OFF	Offretite	2.90
18	PAU	Paulingite	3.41-3.87
19	STI	Stellerite	4.12
		Stilbite	4.13
		Barrerite	3.27
20	TER	Terranovaite	6.49
21	**Unassigned**	**Cowlesite**	**1.77**
22	Unassigned	Tvedalite	2.36

Table B-5. Crystal and channel system together with other properties of the selected natural zeolite framework types (FTC).

FTC	Material name	Crystal system	Channel system	Other properties
FAU	Faujasite	cubic	3D	rare
PAU	**Paulingite**	**cubic**	**3D**	**rare**
ERI	**Erionite**	**hexagonal**	**3D**	**carcinogenic**
GME	**Gmelinite**	**hexagonal**	**3D**	**rare**
LEV	**Levyne**	**hexagonal**	**2D**	**rare**
MAZ	**Mazzite**	**hexagonal**	**1D**	**rare**
OFF	**Offretite**	**hexagonal**	**1D**	**carcinogenic**
HEU	**Heulandite**	**monoclinic**	**2D**	**widely found**
HEU	Clinoptilolite	monoclinic	2D	widely found
LAU	**Laumontite**	**monoclinic**	**1D**	**rare**
NES	**Gottardiite**	**monoclinic**	**2D**	**rare**
STI	**Stellerite**	**monoclinic**	**2D**	**rare**
STI	**Stilbite**	**monoclinic**	**2D**	**widely found**
STI	**Barrerite**	**monoclinic**	**2D**	**rare**
BOG	**Boggsite**	**orthorhombic**	**2D**	**rare**
EON	**Direnzoite**	**orthorhombic**	**2D**	**new**
FER	**Ferrierite**	**orthorhombic**	**2D**	**rare**
MFI	Mutinaite	orthorhombic	3D	rare
MOR	Mordenite	orthorhombic	1D	widely found
TER	**Terranovaite**	**orthorhombic**	**2D**	**rare**
Unassigned	**Tvedalite**	**orthorhombic**	**/**	**Unknown structure**
CHA	Chabazite	rhombohedral	3D	widely found
BEA	Tschernichite	tetragonal	3D	rare

APPENDIX C

NITROGEN ADSORPTION/DESORPTION ISOTHERMS

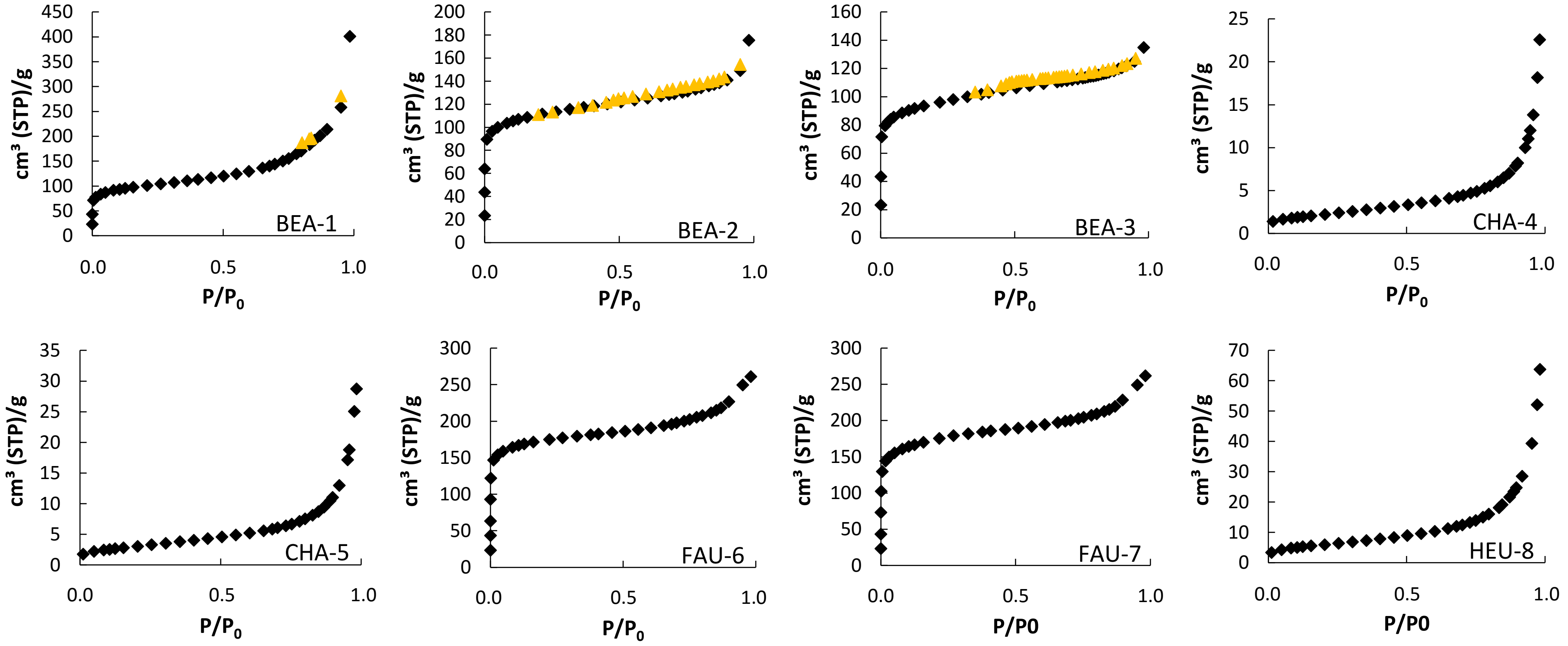


Figure C-1. Nitrogen adsorption/desorption isotherms of the different zeolites tested in Part B of Chapter 2 (**Table 2-8**).

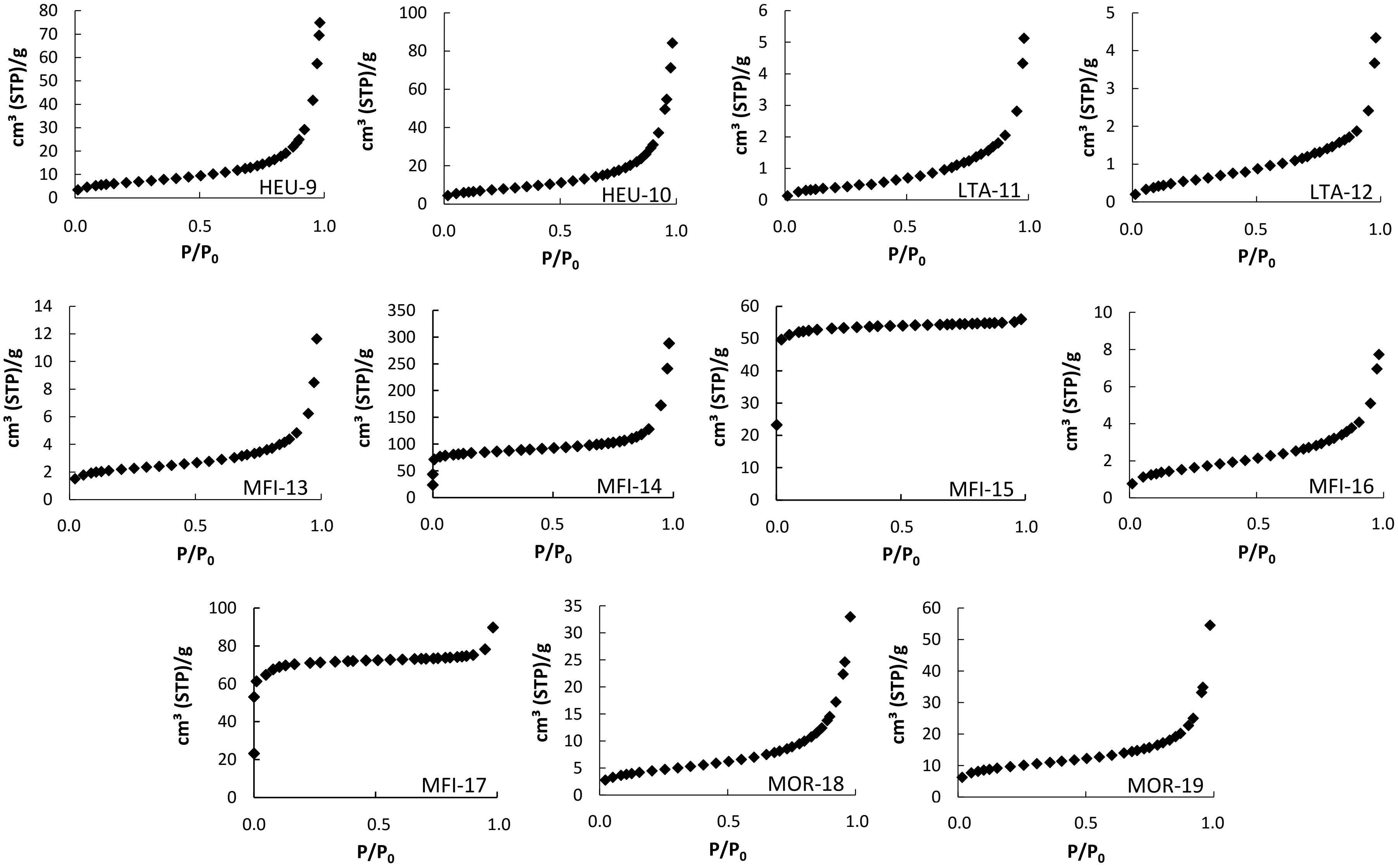


Figure C-1. Nitrogen adsorption/desorption isotherms of the different zeolites tested in Part B of Chapter 2 (**Table 2-8**) (continued).

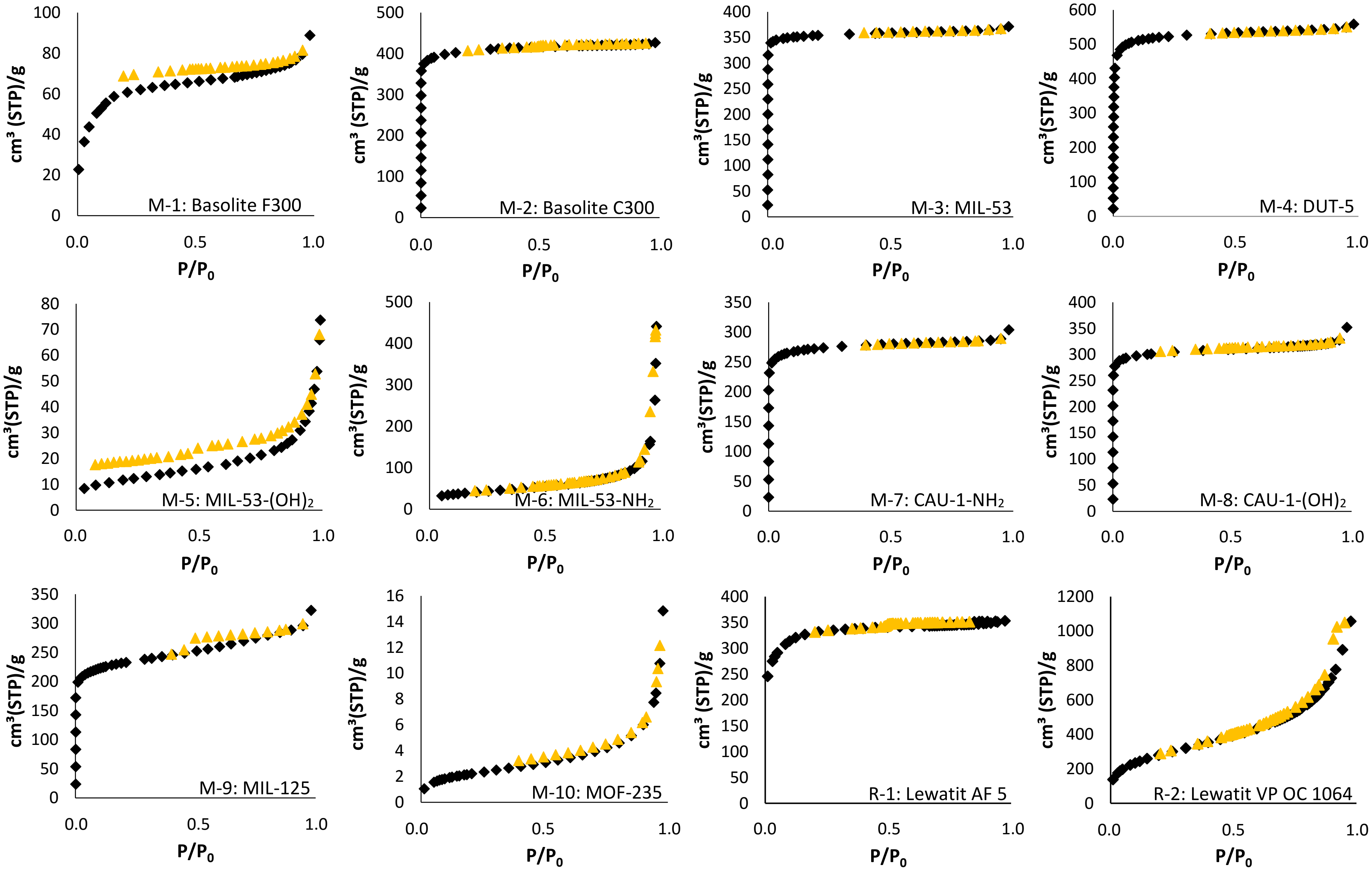


Figure C-2. Nitrogen adsorption/desorption isotherms of the different adsorbents tested in Part C of Chapter 2 (**Table 2-22**).

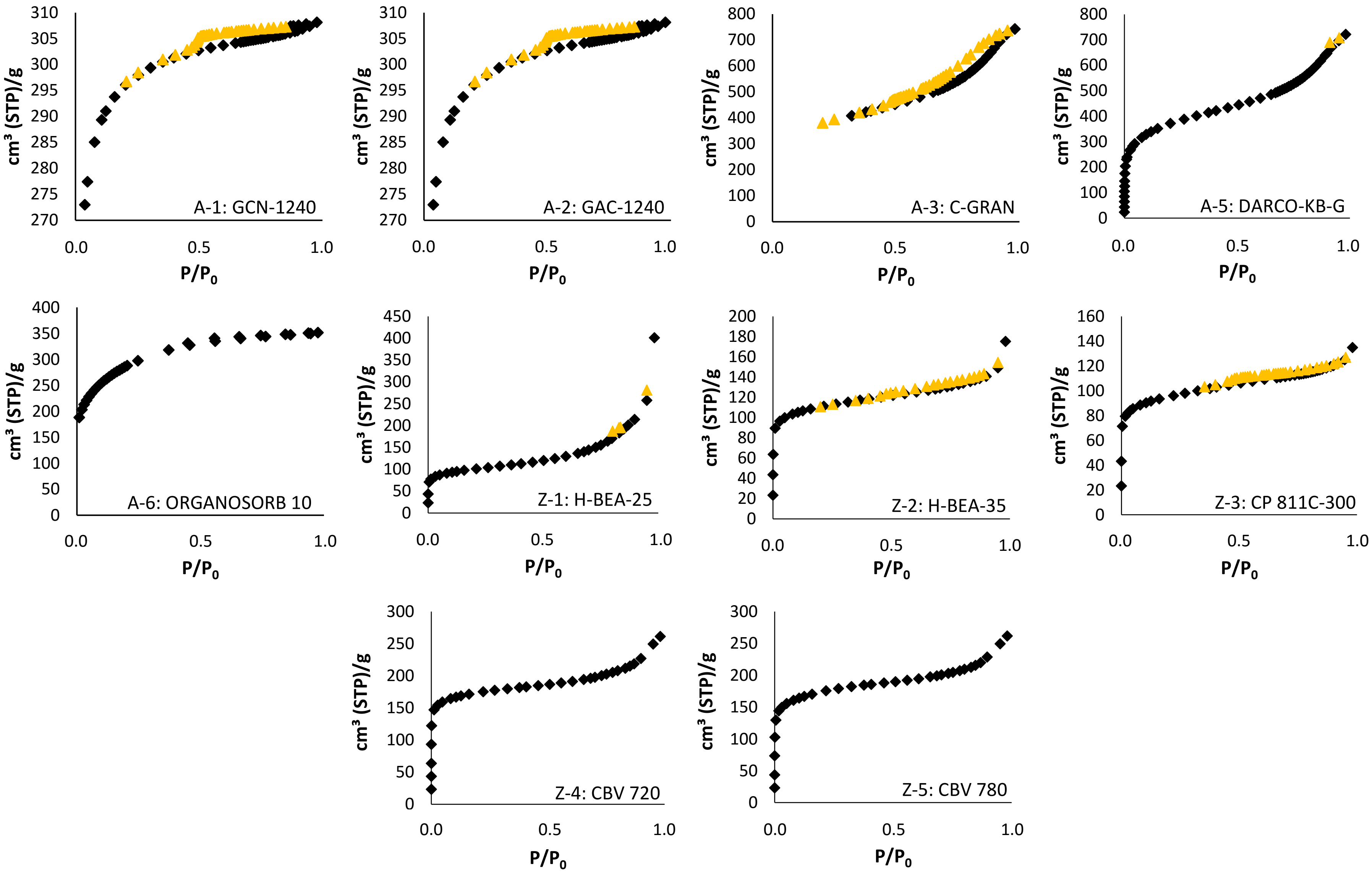


Figure C-2. Nitrogen adsorption/desorption isotherms of the different adsorbents tested in Part C of Chapter 2 (**Table 2-22**) (continued).

APPENDIX D

WATER STABILITY OF MOFs

For some MOFs information on water stability was found in literature. These references were added to the figure. For the other MOFs, XRPD patterns were measured after 2 days in water. When there is a large difference between the XRPD pattern before and after 2 days in water, it can be assumed that this structure does not remain stable. Based on the XRPD patterns M-3, M-6 and M-7 retain their structural integrity and crystallinity. M-4 and M-10 were slightly decomposed, what can be defined as not stable.

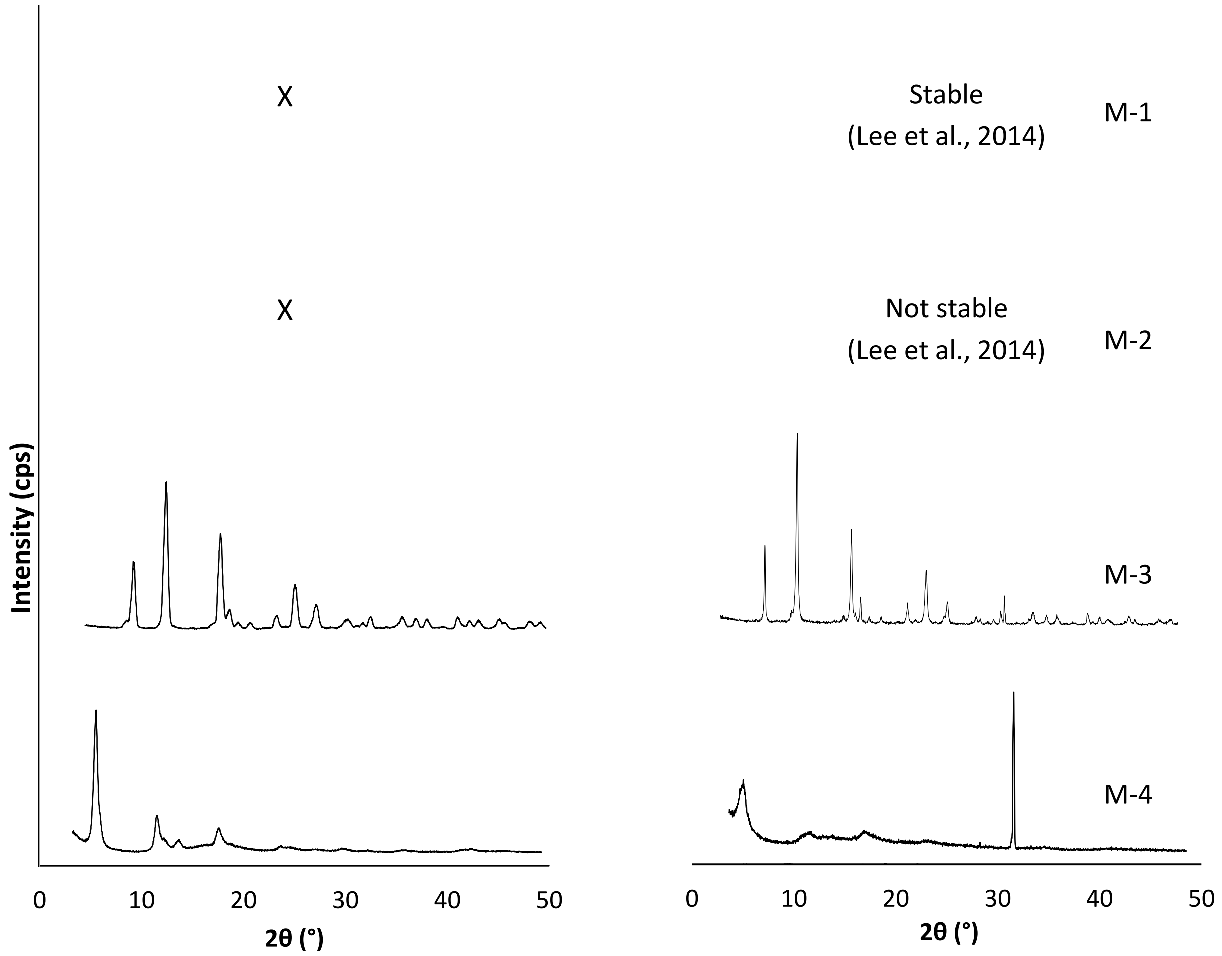


Figure D-1. Water stability of the tested MOFs in Part C of Chapter 2 (**Table 2-22**).

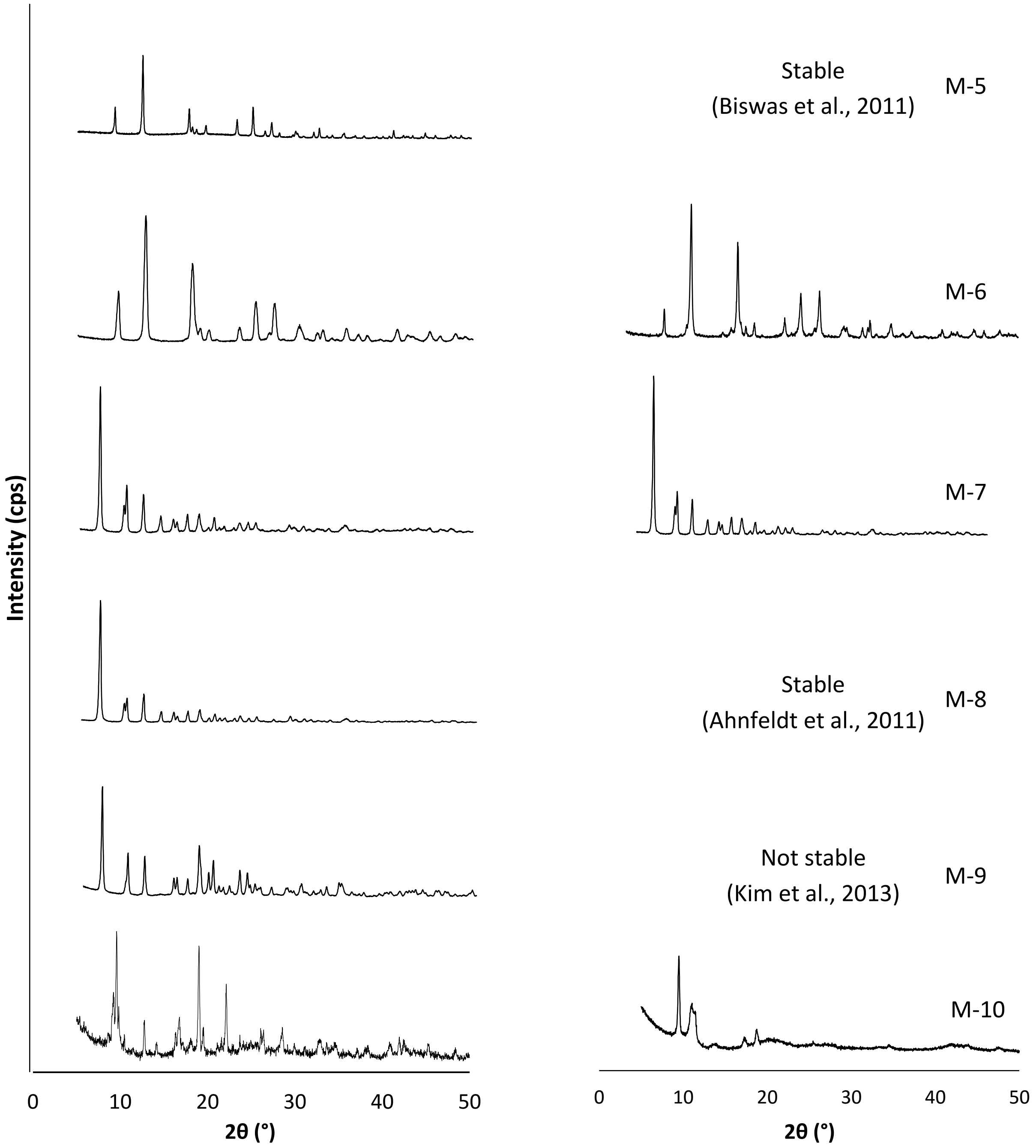


Figure D-1. Water stability of the tested MOFs in Part C of Chapter 2 (**Table 2-22**) (continued).

APPENDIX E

ANTIFUNGAL ACTIVITY OF ZEOLITES AND ADJUVANTS

Table E-1. The measured colony radius (+ SE) and calculated growth inhibition (+ SE) of the fungicide materials presented in Part A of Chapter 4 (**Table 4-3**) against *Venturia inaequalis* compared to the control (n=8).

Zeolite	**Concentration (ppm)**	**Colony radius (cm)**	**Growth relative to control (%)**	**Inhibition (%)**
BEA	0	2.26 + 0.02	100.00	0.00 + 1.08
	400	2.20 + 0.01	97.49	2.51 + 0.97
	4000	2.10 + 0.02[a]	93.08	6.92 + 1.23
	20000	2.08 + 0.02[a]	92.20	**7.80 + 1.08**
BEA 850 WP	0	2.26 + 0.02	100.00	0.00 + 1.08
	400	2.17 + 0.02	96.21	3.79 + 0.99
	4000	2.09 + 0.03[a]	92.39	7.61 + 1.55
	20000	1.62 + 0.01[a]	71.81	**28.19 + 0.71**
BEA 950 WP	0	2.26 + 0.02	100.00	0.00 + 1.08
	400	2.08 + 0.04[a]	92.00	8.00 + 1.99
	4000	1.84 + 0.01[a]	81.61	18.39 + 0.64
	20000	1.44 + 0.02[a]	63.69	**36.31 + 0.99**
FAU	0	2.26 + 0.02	100.00	0.00 + 1.08
	400	2.17 + 0.01	95.98	4.02 + 0.88
	4000	1.79 + 0.04[a]	79.26	20.74 + 1.73
	20000	1.63 + 0.02[a]	72.22	**27.78 + 1.00**
FAU 850 WP	0	2.26 + 0.02	100.00	0.00 + 1.08
	400	1.95 + 0.03[a]	86.32	13.68 + 1.57
	4000	1.62 + 0.01[a]	71.76	28.33 + 0.68
	20000	1.37 + 0.02[a]	60.70	**39.30 + 1.06**

[a] Significant different from the control treatment ($p < 0.05$).

Table E-1. The measured colony radius (± SE) and calculated growth inhibition (± SE) of the fungicide materials presented in Part A of Chapter 4 (**Table 4-3**) against *Venturia inaequalis* compared to the control (n=8) (continued).

Zeolite	Concentration (ppm)	Colony radius (cm)	Growth relative to control (%)	Inhibition (%)
FAU 920 WP	0	2.26 ± 0.02	100.00	0.00 ± 1.08
	400	1.94 ± 0.03[a]	85.96	14.04 ± 1.36
	4000	1.62 ± 0.04[a]	71.65	28.35 ± 1.81
	20000	1.14 ± 0.02[a]	50.56	**49.44 ± 0.93**
LTA	0	2.26 ± 0.02	100.00	0.00 ± 1.08
	400	1.58 ± 0.01[a]	69.88	30.12 ± 0.58
	4000	0.00 ± 0.00[a]	0.00	100.00 ± 0.00
	20000	0.00 ± 0.00[a]	0.00	**100.00 ± 0.00**
LTA 800 SC	0	2.26 ± 0.02	100.00	0.00 ± 1.08
	400	1.29 ± 0.01[a]	57.04	42.96 ± 0.60
	4000	0.00 ± 0.00[a]	0.00	100.00 ± 0.00
	20000	0.00 ± 0.00[a]	0.00	**100.00 ± 0.00**
LTA 850 WP	0	2.26 ± 0.02	100.00	0.00 ± 1.08
	400	1.22 ± 0.01[a]	54.23	45.77 ± 0.60
	4000	0.00 ± 0.00[a]	0.00	100.00 ± 0.00
	20000	0.00 ± 0.00[a]	0.00	**100.00 ± 0.00**
Hermosan 80	0	2.26 ± 0.02	100.00	0.00 ± 1.08
	400	0.00 ± 0.00[a]	0.00	100.00 ± 0.00
	4000	0.00 ± 0.00[a]	0.00	100.00 ± 0.00
	20000	0.00 ± 0.00[a]	0.00	**100.00 ± 0.00**

[a] Significant different from the control treatment ($p < 0.05$).

Table E-2. The measured colony radius (± SE) and calculated growth inhibition (± SE) of the fungicide materials presented in Part A of Chapter 4 (**Table 4-3**) against *Botrytis cinerea* compared to the control (n=8).

Zeolite	Concentration (ppm)	Colony radius (cm)	Growth relative to control (%)	Inhibition (%)
BEA	0	3.67 ± 0.04	100.00	0.00 ± 1.45
	400	3.50 ± 0.07	95.32	4.68 ± 2.25
	4000	3.35 ± 0.14	91.17	8.83 ± 3.80
	20000	2.22 ± 0.09[a]	60.37	**39.63 ± 2.60**
BEA 850 WP	0	3.67 ± 0.04	100.00	0.00 ± 1.45
	400	3.47 ± 0.15	94.56	5.44 ± 4.32
	4000	1.08 ± 0.05[a]	29.32	70.68 ± 1.49
	20000	1.00 ± 0.10[a]	27.33	**72.67 ± 2.78**
BEA 950 WP	0	3.67 ± 0.04	100.00	0.00 ± 1.45
	400	2.41 ± 0.01[a]	65.54	34.46 ± 0.71
	4000	1.68 ± 0.05[a]	45.76	54.24 ± 1.39
	20000	0.96 ± 0.04[a]	26.22	**73.78 ± 1.00**
FAU	0	3.67 ± 0.04	100.00	0.00 ± 1.45
	400	3.49 ± 0.08	95.04	4.96 ± 2.30
	4000	3.48 ± 0.10	94.70	5.30 ± 2.98
	20000	2.87 ± 0.08[a]	78.22	**21.78 ± 2.22**
FAU 850 WP	0	3.67 ± 0.04	100.00	0.00 ± 1.45
	400	3.06 ± 0.15[a]	83.44	16.56 ± 4.06
	4000	2.35 ± 0.01[a]	63.96	36.04 ± 0.72
	20000	1.43 ± 0.06[a]	38.95	**61.05 ± 1.79**
FAU 920 WP	0	3.67 ± 0.04	100.00	0.00 ± 1.45
	400	2.86 ± 0.05[a]	78.03	21.97 ± 1.70
	4000	2.53 ± 0.05[a]	68.82	31.18 ± 1.55
	20000	0.94 ± 0.05[a]	25.55	**74.45 ± 1.31**

[a] Significant different from the control treatment ($p < 0.05$).

Table E-2. The measured colony radius (+ SE) and calculated growth inhibition (+ SE) of the fungicide materials presented in Part A of Chapter 4 (**Table 4-3**) against *Botrytis cinerea* compared to the control (n=8) (continued).

Zeolite	**Concentration (ppm)**	**Colony radius (cm)**	**Growth relative to control (%)**	**Inhibition (%)**
LTA	0	3.67 + 0.04	100.00	0.00 + 1.45
	400	3.56 + 0.15	96.86	3.14 + 4.15
	4000	0.33 + 0.02 [a]	8.88	91.12 + 0.49
	20000	0.00 + 0.00 [a]	0.00	**100.00 + 0.00**
LTA 800 SC	0	3.67 + 0.04	100.00	0.00 + 1.45
	400	2.58 + 0.13 [a]	70.37	29.63 + 3.58
	4000	0.40 + 0.07 [a]	10.91	89.09 + 1.99
	20000	0.00 + 0.00 [a]	0.00	**100.00 + 0.00**
LTA 850 WP	0	3.67 + 0.04	100.00	0.00 + 1.45
	400	2.52 + 0.06 [a]	68.71	31.29 + 1.84
	4000	0.44 + 0.02 [a]	11.94	88.06 + 0.67
	20000	0.00 + 0.00 [a]	0.00	**100.00 + 0.00**
Daconil 50	0	3.67 + 0.04	100.00	0.00 + 1.45
	400	0.35 + 0.04 [a]	9.47	90.53 + 1.03
	4000	0.23 + 0.04 [a]	6.23	93.77 + 1.04
	20000	0.20 + 0.01 [a]	5.41	**94.59 + 0.35**

[a] Significant different from the control treatment ($p < 0.05$).

Table E-3. The measured colony radius (± SE) and calculated growth inhibition (± SE) of the adjuvants presented in Part B of Chapter 4 (**Table 4-7**) against *Venturia inaequalis* compared to the control (n=8).

	Zeolite	**Colony radius (cm)**	**Growth relative to control (%)**	**Inhibition (%)**
	Control	2.27 ± 0.01	100.00	0.00 ± 0.37
a	Agrilan 789 DRY	1.76 + 0.01[a]	77.62	22.38 ± 0.20
b	Morwet EFW Powder	0.55 + 0.01[a]	24.32	75.68 ± 0.32
c	Rhodorsil EP Antim EP	1.28 + 0.01[a]	56.51	43.49 ± 0.17
d	PVP K-30	1.90 + 0.01[a]	83.91	16.09 ± 0.27
e	Ultrasil VN 3	1.91 + 0.01[a]	84.26	15.74 ± 0.28
f	Morwet D425	1.02 ± 0.02[a]	44.92	55.08 ± 0.78
g	SAG 1572	1.98 ± 0.01[a]	87.39	12.61 ± 0.32
h	Supragil WP	1.50 ± 0.01[a]	66.16	33.84 ± 0.27
i	Reax 88B	2.09 ± 0.03[a]	91.97	8.03 ±1.29
j	Rhodopol 23	1.78 + 0.00[a]	78.31	21.69 ± 0.14
k	Amebact C	2.20 + 0.00[a]	97.16	2.84 ± 0.18
l	Soprophor FL	1.92 + 0.02[a]	84.79	15.21 ± 0.41

[a] Significant different from the control treatment ($p < 0.05$).

Table E-4. The measured colony radius (± SE) and calculated growth inhibition (± SE) of the adjuvants presented in Part B of Chapter 4 (**Table 4-7**) against *Botrytis cinerea* compared to the control (n=8).

	Zeolite	**Colony radius (cm)**	**Growth relative to control (%)**	**Inhibition (%)**
	Control	3.60 ± 0.01	100.00	0.00 ± 0.18
a	Agrilan 789 DRY	3.53 ± 0.01	97.99	2.01 ± 0.23
b	Morwet EFW Powder	0.97 ± 0.01[a]	26.85	73.15 ± 0.23
c	Rhodorsil EP Antim EP	2.50 ± 0.02[a]	69.41	30.59 ± 0.69
d	PVP K-30	3.30 ± 0.03[a]	91.66	8.34 ± 0.93
e	Ultrasil VN 3	3.26 ± 0.02[a]	90.73	9.27 ± 0.69
f	Morwet D425	0.71 ± 0.01[a]	19.67	80.33 ± 0.20
g	SAG 1572	2.68 ± 0.05[a]	74.53	25.47 ± 1.37
h	Supragil WP	1.73 ± 0.02[a]	48.10	51.90 ± 0.54
i	Reax 88B	3.15 ± 0.04[a]	87.62	12.38 ± 1.01
j	Rhodopol 23	3.57 ± 0.07	99.25	0.75 ± 1.84
k	Amebact C	3.50 ± 0.03	97.28	2.72 ± 0.93
l	Soprophor FL	2.65 ± 0.05[a]	73.53	26.47 ± 1.41

[a] Significant different from the control treatment ($p < 0.05$).

APPENDIX F

INSECTICIDAL ACTIVITY OF ZEOLITES

Table F-1. Percentage corrected mortality (± SE) of *Tuta absoluta* eggs in topical and residual bioassays, using the insecticide materials presented in Part A of Chapter 5 (**Table 5-3**) (n=8).

Treatment	**Egg mortality (%)**					
	Mortality			Corrected mortality		
	400 mg.l^{-1}	4000 mg.l^{-1}	20000 mg.l^{-1}	400 mg.l^{-1}	4000 mg.l^{-1}	20000 mg.l^{-1}
			Topical bioassay			
Blanco	7.14 ± 2.66	7.14 ± 2.66	7.14 ± 2.66			
Z-1	7.50 ± 3.65	10.00 ± 3.78	10.00 ± 3.78	0.38 ± 4.87	3.08 ± 4.98	3.08 ± 4.98
Z-2	15.00 ± 3.27	20.00 ± 5.35	30.00 ± 5.35	8.46 ± 4.55	13.85 ± 6.44	24.62 ± 6.47[a]
Z-3	15.00 ± 3.27	12.50 ± 5.26	25.00 ± 3.27	8.46 ± 4.55	5.77 ± 6.35	19.23 ± 4.57
Z-4	17.50 ± 2.50	22.00 ± 5.54	20.00 ± 4.71	11.15 ± 3.94	16.00 ± 6.63	13.85 ± 5.84
Z-5	31.11 ± 3.51	26.00 ± 4.27	30.00 ± 6.15	25.81 ± 4.80	20.31 ± 5.45	24.62 ± 7.25
Z-6	25.00 ± 5.00	18.00 ± 4.67	22.50 ± 4.53	19.23 ± 6.12	11.69 ± 5.79	16.54 ± 5.68
Z-7	10.00 ± 3.78	7.50 ± 5.26	7.50 ± 3.66	3.08 ± 4.98	0.38 ± 6.35	0.38 ± 4.87
Z-8	15.00 ± 5.00	16.67 ± 4.14	27.50 ± 5.26	8.46 ± 6.10	10.26 ± 5.31	21.92 ± 6.38[a]
Z-9	17.50 ± 5.90	17.50 ± 4.53	22.50 ± 5.90	11.15 ± 6.98	11.15 ± 5.67	16.54 ± 6.99
C-1	95.00 ± 3.27	92.50 ± 3.66	95.00 ± 3.27	94.62 ± 5.29	91.92 ± 5.54	94.62 ± 5.29
B-1	4.29 ± 2.28	4.71 ± 2.12	11.43 ± 4.04	0.00 ± 0.00	0.00 ± 0.00	4.62 ± 5.21
B-2	2.86 ± 2.86	8.57 ± 4.04	12.50 ± 5.26	0.00 ± 0.00	1.54 ± 5.21	5.77 ± 6.35

[a] Significant different from its non-formulated zeolite product ($P < 0.05$).

Table F-1. Percentage corrected mortality (± SE) of *Tuta absoluta* eggs in topical and residual bioassays, using the insecticide materials presented in Part A of Chapter 5 (**Table 5-3**) (n=8) (continued).

Treatment	**Egg mortality (%)**					
	Mortality			Corrected mortality		
	400 mg.l^{-1}	4000 mg.l^{-1}	20000 mg.l^{-1}	400 mg.l^{-1}	4000 mg.l^{-1}	20000 mg.l^{-1}
	Residual bioassay					
Blanco	4.29 ± 2.28	4.29 ± 2.28	4.29 ± 2.28			
Z-1	32.50 ± 3.66	32.50 ± 6.48	35.00 ± 7.32	29.48 ± 4.56	29.48 ± 7.21	32.09 ± 8.04
Z-2	17.50 ± 5.90	27.50 ± 5.26	25.00 ± 5.00	13.81 ± 6.62	24.25 ± 6.02	21.64 ± 5.76
Z-3	22.50 ± 7.01	32.50 ± 5.26	30.00 ± 6.55	19.03 ± 7.71	29.48 ± 6.03	26.87 ± 7.27
Z-4	15.00 ± 5.00	30.00 ± 6.55	32.50 ± 6.48	11.19 ± 5.75	26.87 ± 7.27	29.48 ± 7.21
Z-5	12.50 ± 3.66	35.00 ± 5.00	40.00 ± 5.35	8.58 ± 4.51	32.09 ± 5.79	37.31 ± 6.13
Z-6	22.50 ± 5.90	42.50 ± 4.53	47.50 ± 6.48	19.03 ± 6.62	39.93 ± 5.38	45.15 ± 7.25
Z-7	20.00 ± 5.35	22.50 ± 5.90	25.00 ± 5.00	16.42 ± 6.08	19.03 ± 6.62	21.64 ± 5.76
Z-8	17.50 ± 4.53	30.00 ± 3.78	27.50 ± 3.66	13.81 ± 5.31	26.87 ± 4.65	24.25 ± 4.54
Z-9	20.00 ± 5.35	37.50 ± 7.96	30.00 ± 5.35	16.42 ± 6.08	34.70 ± 8.69	26.87 ± 6.10
C-1	25.00 ± 6.27	25.00 ± 5.00	32.50 ± 3.66	21.64 ± 6.99	21.64 ± 5.76	29.48 ± 4.56
B-1	4.00 ± 1.84	8.00 ± 3.04	10.00 ± 2.41	0.00 ± 0.00	3.88 ± 3.97	5.97 ± 3.46
B-2	9.09 ± 4.15	10.91 ± 3.15	18.18 ± 4.23	5.02 ± 4.94	6.92 ± 4.06	14.52 ± 5.03

Table F-2. Percentage corrected mortality (± SE) of *Tuta absoluta* eggs and larvae in topical and residual bioassays, using the insecticide materials presented in Part A of Chapter 5 (**Table 5-3**) (n=8).

Treatment	**Egg + larvae mortality (%)**					
	Mortality			Corrected mortality		
	400 mg.l^{-1}	4000 mg.l^{-1}	20000 mg.l^{-1}	400 mg.l^{-1}	4000 mg.l^{-1}	20000 mg.l^{-1}
			Topical bioassay			
Blanco	8.57 ± 3.45	8.57 ± 3.45	8.57 ± 3.45			
Z-1	32.50 ± 6.48	32.50 ± 5.26	40.00 ± 5.35	26.17 ± 8.09	26.17 ± 6.95	34.38 ± 7.08
Z-2	32.50 ± 3.66	37.50 ± 4.53	47.50 ± 3.66	26.17 ± 5.59	31.64 ± 6.35	42.58 ± 5.73
Z-3	35.00 ± 3.27	35.00 ± 3.27	42.50 ± 4.53	28.91 ± 5.32	28.91 ± 5.32	37.11 ± 6.39
Z-4	32.50 ± 6.48	46.00 ± 4.27	40.00 ± 5.77	26.17 ± 8.09	40.94 ± 6.20	34.38 ± 7.47
Z-5	40.00 ± 3.33	40.00 ± 2.98	42.00 ± 4.67	34.38 ± 5.41	34.38 ± 5.16	36.56 ± 6.50
Z-6	45.00 ± 3.27	36.00 ± 4.00	42.50 ± 4.53	39.84 ± 5.42	30.00 ± 5.89	37.11 ± 6.39
Z-7	22.50 ± 7.01	20.00 ± 7.56	22.50 ± 5.90	15.23 ± 8.56	12.50 ± 9.10	15.23 ± 7.50
Z-8	30.00 ± 6.55	40.00 ± 3.48	52.50 ± 5.26	23.44 ± 8.14	34.38 ± 5.52[a]	48.05 ± 7.12[a]
Z-9	40.00 ± 6.55	52.50 ± 3.66	52.50 ± 5.26	34.38 ± 8.20	48.05 ± 5.80[a]	48.05 ± 7.12[a]
C-1	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 5.34	100.00 ± 5.34	100.00 ± 5.34
B-1	10.00 ± 3.48	10.59 ± 2.50	18.57 ± 3.90	1.56 ± 5.36	2.21 ± 4.66	10.94 ± 5.71
B-2	2.86 ± 2.86	17.14 ± 2.86	20.00 ± 5.35	0.00 ± 0.00	9.38 ± 4.92	12.50 ± 6.98
			Residual bioassay			
Blanco	8.57 ± 4.04	8.57 ± 4.04	8.57 ± 4.04			
Z-1	62.50 ± 4.27	57.50 ± 5.56	60.00 ± 9.43	58.98 ± 6.94	53.52 ± 7.88	56.25 ± 11.49
Z-2	45.00 ± 6.90	57.50 ± 5.56	50.00 ± 5.04	39.84 ± 8.92	53.52 ± 7.88	45.31 ± 7.34
Z-3	55.00 ± 5.91	67.50 ± 6.11	67.50 ± 7.07	50.78 ± 8.15	64.45 ± 8.50	64.45 ± 9.35
Z-4	52.50 ± 10.65	60.00 ± 8.73	57.50 ± 9.72	48.05 ± 12.64	56.25 ± 10.81	53.52 ± 11.75
Z-5	40.00 ± 6.17	65.00 ± 3.09	67.50 ± 4.96	34.38 ± 8.21	61.72 ± 6.19	64.45 ± 7.55
Z-6	42.50 ± 6.61	80.00 ± 6.17	80.00 ± 3.56	37.11 ± 8.63	78.13 ± 8.78	78.13 ± 6.83
Z-7	40.00 ± 8.73	60.00 ± 6.17	57.50 ± 7.51	34.38 ± 10.63	56.25 ± 8.44	53.52 ± 9.62
Z-8	47.50 ± 9.39	62.50 ± 7.51	52.50 ± 4.96	42.58 ± 11.33	58.98 ± 9.68	48.05 ± 7.31
Z-9	57.50 ± 8.31	72.50 ± 11.23	67.50 ± 3.45	53.52 ± 10.38	69.92 ± 13.42	64.45 ± 6.47
C-1	97.50 ± 2.36	100.00 ± 0.00	100.00 ± 0.00	97.27 ± 6.68	100.00 ± 6.25	100.00 ± 6.25
B-1	14.00 ± 3.87	17.00 ± 3.33	19.17 ± 3.90	5.94 ± 6.12	9.22 ± 5.74	11.59 ± 6.16
B-2	10.91 ± 4.15	14.55 ± 2.82	20.00 ± 4.02	2.56 ± 6.33	6.53 ± 5.39	12.50 ± 6.26

[a] Significant different from its non-formulated zeolite product ($P < 0.05$).

LIST OF REFERENCES

Abasi CY, Abio AA and Igwe JC, Adsorption of iron (III), lead (II) and cadmium (II) ions by unmodified Raphia palm (Raphia hookeri) fruit endocarp. Environ Res J, **5**: 104-113 (2011).

Abbate PE, Dardanelli JL, Cantarero MG, Maturano M, Melchiori RJM and Suero EE, Climatic and water availability effects on water-use efficiency in wheat. Crop Sci, **44**: 474-483 (2004).

Abbott WS, A method of computing the effectiveness of an insecticide. J Econ Entomol, **18**: 265-267 (1925).

Abou-Khaled A, Hagan RM, Davenport DC, Effects of kaolinite as a reflective antitranspirant on leaf temperature, transpiration, photosynthesis, and water-use efficiency. Water Resour Res, **6**: 280-289 (1970).

Ackley MW, Rege SU and Saxena H, Application of natural zeolites in purification and separation of gases. Micropor Mesopor Mat, **61**: 25-42 (2003).

Acton QA, Sulfur compounds - advances in research and application. Scholarly Editions, Atlanta, Georgia, 726 p. (2013).

Adiyodi KG and Adiyodi RG, Morphology and cytology of the accessory sex glands in invertebrates. Int Rev Cytol, **43**: 353-398 (1976).

Agrios GN, Fruit and general diseases caused by Ascomycetes and Deuteromycetes (mytosporic fungi). In: Plant pathology, ed. by Agrios GN. Elsevier, Burlington, Vermont, pp. 501-521 (2005).

Ahnfeldt T, Moellmer J, Guillerm V, Staudt R, Serre C and Stock N, High-throughput and time-resolved energy-dispersive X-ray diffraction (EDXRD) study of the formation of CAU-1-$(OH)_2$: microwave and conventional heating. Chem Eur J, **17**: 6462-6468 (2011).

Ainsworth EA and Long SP, What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. New Phytol, **165**: 351-372 (2005).

Ainsworth EA and Rogers A, The response of photosynthesis and stomatal conductance to rising CO_2: mechanisms and environmental interactions. Plant Cell Environ, **30**: 258-270 (2007).

Al-Dosary MM, Al-Bekairi AM and Moursy EB, Morphology of the egg shell and the developing embryo of the Red Palm Weevil, *Rhynchophorus ferrugineus*. Saudi J Biol Sci, **17**: 177-183 (2010).

Al-Dwairi RA and Al-Rawajfeh AE, Recent patents of natural zeolites applications in environment, agriculture and pharmaceutical industry. Recent Pat Chem Eng, **5**: 20-27 (2012).

Ali MA, Brison B and Thomas WJ, Synthesis, characterization and catalytic activity of ZSM-5 zeolites having variable silicon-to-aluminum ratios. Appl Catal A, **252**: 149-162 (2003).

Allen EA, Hoch HC, Steadman JR and Stavely RG, Influence of leaf surface on spore deposition and the epiphytic growth of phytopathogenic fungi. In: Microbial ecology of leaves, ed. By Andrews JH and Hirano SS. Springer, New York, New York, pp. 87-110 (1991).

Allendorf MD, Bauer CA, Bhakta RK and Houk RJT, Luminescent metal-organic frameworks. Chem Soc Rev, **38**: 1330-1352 (2009).

Allendorf M, Bétard A and Fischer RA, Deposition of thin films for sensor applications. In: Metal-organic frameworks: applications from catalysis to gas storage, ed. by Farrusseng D. Wiley VCH, Weinheim, Germany, pp. 309-338 (2011).

Alavo TBV and Abagli AZ, Effect of kaolin particle film formulation against populations of the aphid Lipaphis erysimi Kalt. (Homoptera: Aphididae) in Cabbage. Open Entomol J, **5**: 49-53 (2011).

Alyokhin A, Colorado potato beetle management on potatoes: current challenges and future prospects. In: Fruit, vegetable and cereal science and biotechnology - Potato II, ed. by Tennant P and Benkeblia N. Global Science Books, Kagawa-ken, Japan, pp. 10-19 (2009).

An J, Geib SJ and Rosi NL, Cation-triggered drug release from a porous zinc-adeninate metal-organic framework. J Am Chem Soc, **131**: 8376-8377 (2009).

An J, Geib SJ and Rosi NL, High and selective CO_2 uptake in a cobalt adeninate metal-organic framework exhibiting pyrimidine- and amino-decorated pores. J Am Chem Soc, **132**: 38-39 (2010).

Anderson MA, Removal of MTBE and other organic contaminants from water by sorption to high silica zeolites. Environ Sci Technol, **34**: 725-727 (2000).

Andreu V and Pico Y, Determination of pesticides and their degradation products in soil: critical review and comparison methods. Trends Analyt Chem, **23**: 772-789 (2004).

Arnó J and Gabarra R, Controlling *Tuta absoluta*, a new invasive pest in Europe. Training in Integrated Pest Management - N_o 5, IRTA, Cabrils, Spain, 8 p. (2010).

Ashraf M and Bashir A, Relationship of photosynthetic capacity at the vegetative stage and during grain yield of two hexaploid wheat (*Triticum aestivum* l.) cultivars differing in yield. Eur J Agron, **19**: 277-287 (2003).

Bae YS, Farha OK, Hupp JT and Snurr RQ, Enhancement of CO_2/N_2 selectivity in a metal-organic framework by cavity modification. J Mater Chem, **19**: 2131-2134 (2009).

Baerlocher C, McCusker LB and Olsen DH, Atlas of zeolite framework types. Elsevier, Amsterdam, the Netherlands, 398 p. (2007).

Baerlocher C and McCusker LB, Database of zeolite structures (2010). Available at: http://www.iza-structure.org (Accessed: 29 April 2014).

Bagheri H and Mohammadi A, Pyrrole-based conductive polymer as the solid-phase extraction medium for the preconcentration of environmental pollutants in water samples followed by gas chromatography with flame ionization and mass spectrometry detection. J Chromatogr, **1015**: 23-30 (2003).

Bai Y, He GJ, Zhao YG, Duan CY, Dang DB and Meng QJ, Porous material for absorption and luminescent detection of aromatic molecules in water. Chem Commun, **106**: 1530-1532 (2006).

Balbyshev NF and Lorenzen JH, Hypersensitivity and egg drop, a novel mechanism of host-plant resistance to Colorado potato beetle (Coleoptera: Chrysomelidae). J Econ Entomol, **90**: 652-657 (1997).

Balsari P, Marucco P and Tamagnone M, Assessment of the incidence of adjuvants on the spray deposits in different vine cultivars. In: Proceedings of the 6th international symposium on adjuvants for agrochemicals, ed. by de Ruiter H. ISAA, Amsterdam, the Netherlands, pp. 94-100 (2001).

Bansal OP, Degradation of pestides. In: Pesticides: evaluation of environmental pollution, ed. by Rathore HS and Nollet LML. CRC Press, Boca Raton, Florida, pp. 47-78 (2012).

Bansal P, Bharadwaj LM, Deep A and Kaushik P, Zn based metal organic framework as adsorbent material for mecroprop. Res J Recent Sci, 2: 84-86 (2013).

Baran A and Tarnawski M, Phytotoxkit/Phytotestkit and Microtox® as tools for toxicity assessment of sediments. Ecotoxicol Environ Saf, **98**: 19-27 (2013).

Bardin M, Fargues J, Couston L and Nicot PC, Compatibility between biopesticides used to control grey mould, powdery mildew and whitefly on tomato. Biol Control **46**: 476-483 (2008).

Barrientos R, Apablaza J, Norero A and Estay P, Temperatura base y constante termica de desarrollo de la polilla del tomate, *Tuta absoluta* (Lepidoptera: Gelechiidae). Cienc Investig Agrar, **25**: 133-137 (1998).

BASF, GPS safety summary zeolite (2012). Available at: https://www.basf.com (Accessed: February 2016).

Baup S, Jaffre C, Wolbert D and Laplanche A, Adsorption of pesticides onto granular activated carbon: determination of surface diffusivities using simple batch experiments. Adsorption, **6**: 218-228 (2000).

Beament JWL, The role of cuticle and egg-shell membranes in the penetration of insecticides. Ann Appl Biol, **39**: 142-143 (1952).

Beck B, Steurbaut W and Spanoghe P, How to define green adjuvants. Pest Manag Sci, **68**: 1107-1110 (2012).

Beever RE and Weeds PL, Taxonomy and genetic variation of Botrytis and Botryotinia. In: Botrytis: biology, pathology and control, ed. by Elad Y, Williamson B, Tudzynski P and Delen N. Kluwer Academic Publishers, Dordrecht, the Netherlands, pp. 29-52 (2004).

Bellussi G, He M, Wright P and Wilson S, International Zeolite Association (IZA) (2012). Available at: http://www.iza-online.org (Accessed: January 2013).

Beltrami M, Rossi D and Baudo R, Phytotoxicity assessment of lake Orta sediments. Aquat Ecosyst Health, **2**: 391-401 (1999).

Belviso C, Cavalcante F, Lettino A and Fiore S, A and X-type zeolites synthesised from kaolinite at low temperature. Appl Clay Sci, **80-81**: 162-168 (2013).

Bengochea P, Saelices R, Amor F, Adan A, Budia F, del Estal P, Vinuela E and Medina P, Non-target effects of kaolin and coppers applied on olive trees for the predatory lacewing *Chrysoperla carnea*. Biocontrol Sci Technol, **24**: 625-640 (2014).

Bernard BK and Gordon EB, An evaluation of the common mechanism approach to the food quality protection act: captan and four related fungicides, a practical example. Int J Toxicol, 19: 43–61 (2000).

Bishop BA and Grafius E, Insecticide resistance in the Colorado potato beetle. In: Chrysomelidae biology, Vol. 1, ed. by Jolivet PHA and Cox ML. SBP, Amsterdam, the Netherlands, pp. 355-377 (1996).

Biswas S, Ahnfeldt T and Stock N, New functionalized flexible Al- MIL-53-X (X = -Cl, -Br, -CH_3, -NO_2, -(OH_2)) solids: syntheses, characterization, sorption, and breathing behavior. Inorg Chem, **50**: 9518-9526 (2011).

Blok C, Aguilera M and van Os E, Validation of a new phytotoxicity test (Phytotoxkit) against an established four-week growing test with pre-grown plant plugs. Acta Hortic **819**: 209-214 (2009).

Bo P and Baoshan X, Adsorption mechanisms of organic chemicals on carbon nanotubes. Environ Sci Technol, **42**: 9005-9013.

Bogdanov B, Georgiev D, Angelova K and Yaneva K, Natural zeolites: clinoptilolite review. International conference on economics and society development on the base of knowledge, Stara Zagora, Bulgaria (2009a).

Bogdanov B, Georgiev D, Angelova K and Yaneva K, Synthetic zeolites and their industrial and environmental applications review. International conference on economics and society development on the base of knowledge, Stara Zagora, Bulgaria (2009b).

Bognolo G, Surfactant applications in the context of structure-performance relationships. In: Surface active behaviour of performance surfactants, ed. by Karsa DR. Academic Press, Sheffield, UK, pp. 1-65 (2000).

Boiteau G and Blanc J-PRL, Colorado potato beetle: life Stages. Publication 1878/E, Agriculture Canada, Ottawa, Canada, 13p (1992).

Boiteau G and Noronha C, Topical, residual and ovicidal contact toxicity of three reduced-risk insecticides against the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Crambidae), on potato. Pest Manag Sci, **63**: 1230-1238 (2007).

Boiteau G, Lowery DT and Boisclair J, Insect pest management in organic cropping systems based on ecological principles. In: Managing energy, nutrients, and pests in organic field crops, ed. by Martin RC and MacRae R. CRC Press, Boca Raton, Florida, pp. 175-214 (2014).

Boivon A, Cherrier R and Schiavon MA, Comparison of five pesticides adsorption and desorption processes in thirteen contrasting field soils. Chemosphere, **61**: 668-676 (2005).

Bolis V and Busco C, Thermodynamic study of water adsorption in high-silica zeolites. J Phys Chem, **110**: 14849-14859 (2006).

Bonan G, Chapter 9: Leaves and plants. In: Ecologial climatology, ed. by Bonan G. Cambridge University Press, Cambridge, UK, pp. 1-49 (2002).

Bowen JK, Mesarich CH, Bus VGM, Beresford RM, Plummer KM and Templeton MD, *Venturia inaequalis*: the causal agent of apple scab. Mol Plant Pathol, **12**: 105-122 (2011).

Bradley FM, Ellis BW and Philips E, Rodale’s ultimate encyclopedia of organic gardening: the indispensable green resource for every gardener. Rodale, New York, New York, 720 p. (2009).

Brennan JK, bandosz TJ, Thomson kT and Gubbins KE, Water in porous carbons. Colloids Surf A Physicochem Eng Asp, **187-188**: 539-568 (2001).

Bringe K, Schumacher CFA, Schmitz-Eiberger M, Steiner U and Oerke EC, Ontogenetic variation in chemical and physical characteristics of adaxial apple leaf surfaces. Phytochemistry, **67**: 161-170 (2006).

Britt D, Furukawa H, Wang B, Glover TG and Yaghi OM, Highly efficient separation of carbon dioxide by a metal-organic framework replete with open metal sites. Proc Natl Acad Sci USA, **106**: 20637-20640 (2009).

Brust G, Insect pest management (2016). Available at: https://extension.umd.edu (Accessed: June 2016).

Bu XH and Feng PY, Crystalline microporous and open framework materials. In: The chemistry of nanostructured materials, ed. by Yang P. World Scientific Publishing, Hong Kong, pp. 1-38 (2003).

Bukovac MJ, Fader RG and Luque P, Effects of urea and ammonium nitrate on penetration of NAA through enzymatically isolated tomato fruit cuticular membranes. In: Pesticide formulations and application systems, ed. by Volgas G, Downer R and Lopez H. ASTM, West Conshohocken, Pennsylvania, pp. 15-31 (2003).

Bunce JA, Carbon dioxide effects on stomatal responses to the environment and water use by crops under field conditions. Oecol, **140**: 1-10 (2004).

Burrows HD, Canle ML, Santaballa JA and Steenken S, Reaction pathyways and mechanisms of photodegradation of pesticides. J Photoch Photobio B, **67**: 71-108 (2002).

Butler Ellis MC and Tuck CR, How adjuvants influence spray formation with different hydraulic nozzles. Crop Prot, **18**: 101-109 (1999).

Butler Ellis MC, Tuck CR and Miller PCH, How surface tension of surfactant solutions influences the characteristics of sprays produced by hydraulic nozzles used for pesticides application. Colloids Surf A, **180**: 267-276 (2001).

Campos MR, Silva TBM, Silva WM, Silva JE and Siqueira HAA, Spinosyn resistance in the tomato borer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). J Pest Sci, **88**: 405-412 (2015).

Cantore V, Pace B and Albrizio R, Kaolin-based particle film technology affects tomato physiology, yield and quality. Environ Exp Bot, **66**: 279-288 (2009).

Caputo D and Pepe F, Experiments and data processing of ion exchange equilibria involving Italian natural zeolites: a review. Micropor Mesopor Mat, **105**: 222-231 (2007).

Carberry JJ, Chemical and catalytic reaction engineering. General Publishing Company, Ontario, Canada, 642 p. (2001).

Carisse O and Bernier J, Effect of environmental factors on growth, pycnidial production and spore germination of Microsphaeropsis isolates with biocontrol potential against apple scab. Mycol Res, **106**: 1455-1462 (2002).

Carisse O and Jobin T, Apple scab: improving understanding for better management. Publication 10203/E, Agriculture and Agri-Food Canada, Ontario, Canada, 26 p. (2006).

Carlile WR and Coules A, Fungicides and biological control. In: Control of crop diseases, ed. by Carlile WR and Coules A. Cambridge University Press, Cambridge, UK, pp. 98-145 (2012).

Carvalho FP, Agriculture, pesticides, food security and food safety. Environ Sci Policy, **9**: 685-692 (2006).

Casagrande RA, The Colorado potato beetle: 125 years of mismanagement. Bull Entomol Soc Am, **33**: 142-150 (1987).

Cekova B, Kocev D, Kolcakovska E and Stojanova D, Zeolites as alcohol adsorbents from aqueous solutions. Acta Periodica Technologica, **37**: 83-87 (2006).

Celik FE, Kim TJ and Bell AT, Effect of zeolite framework type and Si/Al ratio on dimethoxymethane carbonylation. J Catal, **270**: 185-195 (2010).

Chavera GS, Martinze RS and Guardia RA, The alternative control of powdery mildew complex (*Leveillula taurica* and *Erysiphe* sp.) in tomato in the Azapa Valley, Chile. Crop Prot, **40**: 119-130 (2013).

Chaves MM, Osorio J and Pereira JS, Water use efficiency and photosynthesis. In: Water use efficiency in plant biology, ed. by Bacon M. CRC Press, Boca Raton, Florida, pp. 42-74 (2004).

Chefetza B, Bilkisb YI and Polubesovaa T, Sorption–desorption behavior of triazine and phenylurea herbicides in Kishon river sediments. Water Res, 38: 4383–4394 (2004).

Chen SK, Edwards CA and Subler S, Effects of the fungicides benomyl, captan and chlorothalonil on soil microbial activity and nitrogen dynamics in laboratory incubations. Soil Biol Biochem, **33**: 1971–1980 (2001).

Chevalier M, Lespinasse Y and Renaudin S, A microscopic study of the different classes of symptoms coded by the V_f gene in apple for resistance to scab (*Venturia inaequalis*). Plant Pathol, **40**: 249-256 (1991).

Chevillard A, Angellier-Coussy H, Guillard V, Gontard N and Gastaldi E, Controlling pesticide release via structuring agropolymer and nanoclays based materials. J Hazard Mater, **205**: 32-39 (2012).

Cicala G, Bruno G and Capezzuto P, Plasma deposition of microcrystalline silicon: the role of plasma-surface interaction on the microstructure. In: Handbook of surfaces and interfaces of materials, ed. by Nalwa HS. Academic Press, San Diego, California, pp. 509-545 (2001).

Civolani S, The past and present of pear protection against the pear Psylla, *Cacopsylla pyri* L. In: Insecticides - pest engineering, ed. by Perveen F. Intecht, Rijeka, Croatia, pp. 385-408 (2012).

Clariant, Advanced zeolite materials (2010). Available at: http://www.zeolites.clariant.com (Accessed: February 2014).

Cleland WW, Andrews TJ, Gutteridge S, Hartman FC and Lorimer GH, Mechanism of Rubisco: the carbamate as general base. Chem Rev, **98**: 549-561 (1998).

Cocco A and Hoy MA, Toxicity of organosilicone adjuvants and selected pesticides to the Asian citrus psyllid (Hemiptera: Psyllidae) and its parasitoid *Tamarixia radiate* (Hymenoptera: Eulophidae). Florida Entomol, **91**: 610-620 (2008).

Cocco A, Deliperi S and Delrio G, Control of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in greenhouse tomato crops using the mating disruption technique. J Appl Entomol, **137**: 16-28 (2012).

Cohen E, Rozen H, Joseph T, Braun S and Margulies *L. Photoprotection* of *Bacillus thuringiensis* kurstaki from ultraviolet-irradiation. J Invertebr Pathol, **57**: 343-351 (1991).

Colella C, Recent advances in natural zeolite applications based on external surface interaction with cations and molecules. In: Studies in surface science and catalysis: from zeolites to porous MOF materials - the 40th anniversary of international zeolite conference, ed. by Xu R, Gao Z, Chen J and Yan W. Elsevier, Amsterdam, the Netherlands, pp. 2063-2073 (2007).

Condon AG, Richards RA, Rebetzke GJ and Farquhar GD, Improving intrinsic water-use efficiency and crop yield. Crop Sci, **42**: 122-131 (2002).

Consoli FL, Botelho PSM and Parra JRP, Selectivity of insecticides to the egg parasitoid *Trichogramma galloi* Zucchi, 1988, (Hym., Trichogrammatidae). J Appl Entomol, **125**: 37-43 (2001).

Corma A, State of the art and future challenges of zeolites as catalysts. J Catal, **216**: 298-312 (2003).

Costerton JW, Stewart PS and Greenberg EP, Bacterial biofilms: a common cause of persistent infections. Science, **284**: 1318-1322 (1999).

Côté AP, Benin AI, Ockwig NW, O'Keeffe M, Matzger AJ and Yaghi OM, Porous, crystalline, covalent organic frameworks. Science, 310: 1166-1170 (2005).

Cox C and Surgan M, Unidentified inert ingredients in pesticides: implications for human and environmental health. Environ Health Perspect, **114**: 1803-1806 (2006).

Cubillas P and Anderson MW, Synthesis mechanism: crystal growth and nucleation. In: Zeolites and catalysis: synthesis, reactions and applications, ed. by Cejka J, Corma A and Zones S. Wiley VCH, Weinheim, Germany, pp. 1-56 (2010).

Cundy CS and Cox PA, The hydrothermal synthesis of zeolites: precursors, intermediates and reaction mechanism. Micropor Mesopor Mat, **82**: 1-78 (2005).

Cychosz KA and Matzger AJ, Water stability of microporous coordination polymers and the adsorption of pharmaceuticals from water. Langmuir, **26**: 17198-17202 (2010).

Cychosz KA, Ahmad R and Matzger AJ, Liquid phase separations by crystalline microporous coordination polymers. Chem Sci, **1**: 293-302 (2010).

Czerniwska-Kusza I, Ciesielczuk T, Kusza G and Cichon A, Comparison of the phytotoxkit microbiotest and chemical variables for toxicity evaluation of sediments. Environ Toxicol **21**: 367-372 (2006).

Czerniawska-Kusza I and Kusza G, The potential of the phytotoxkit microbiotest for hazard evaluation of sediments in eutrophic freshwater ecosystems. Environ Mont Assess, **179**: 113-121 (2011).

Dagli F and Bahsi SU, Topical and residual toxicity of six pesticides to Orius majusculus. Phytoparasitica, **37**: 399-405 (2009).

DalCorso G, Manara A, Piasentin S and Furini A, Nutrient metal elements in plants. Metallomics, **6**: 1770-1788 (2014).

Damour G, Simonneau T, Cochard H and Urban L, An overview of models of stomatal conductance at the leaf level. Plant Cell Environ, **33**: 1419-1438 (2010).

Dangl JL and Jones JDG, Plant pathogens and integrated defence responses to infection. Nature, **411**: 826-833 (2001).

Dardenne F, Nobels I, de Coen W and Blust R, Dose-response relationships and statistical performance of a battery of bacterial gene profiling assays. Appl Microbiol Biotechnol, **75**: 223-234 (2007).

Dasgupta TP and Roberts EV, Slow release formulation part 1 – encapsulation of pesticides by starch matrices. Jam J Sci Tech, **2**: 17-24 (1991).

Dawodu FA, Akpomie GK and Ogbu IC, Isotherm modeling on the equilibrium sorption of cadmium (II) from solution by Agbani clay. IJMCR, **3**: 9-45 (2012).

de Lange RSA, Keizer K and Burggraaf AJ, Analysis and theory of gas transport in microporous sol-gel derived ceramic membranes. J Membr Sci, **104**: 81-100 (1995).

De Wilde T, Mertens J, Spanoghe P, Ryckeboer J, Jaeken P and Springael D, Sorption kinetics and its effects on retention and leaching. Chemosphere, **72**: 509-516 (2008).

De Wilde T, Spanoghe P, Ryckeboer J, Jaeken P and Springael D, Sorption characteristics of pesticides on matrix substrates used in biopurification systems. Chemosphere, **75**: 100-108 (2009).

Dean R, Van Kan JAL, Pretorius ZA, Hammond-Kosack KE, Di Pietro A, Spanu PD, Rudd JJ, Dickman M, Kahmann R, Ellis J and Foster GD, The top 10 fungal pathogens in molecular plant pathology. Mol Plant Pathol, **13**: 414-430 (2012).

Deka RC, Acidity in zeolites and their characterization by different spectroscopic methods. Ind J Chem Technol, **5**: 109-123 (1998).

Decagon Devices, Water activity of dry and dehydrated products (2006). Available at: http://www.decagon.com (Accessed: 1 June 2015).

Decagon Devices, Vapor sorption analyzer (operator's manual) (2015). Available at: http://www.aqualab.com (Accessed: 1 June 2015).

Decognet V, Ravetti F, Martin C and Nicot PC, Improved leaf pruning reduces development of stem cankers caused by grey mould in greenhouse tomatoes. Agron Sustainable Dev, **30**: 465-472 (2010).

Deegan RD, Bakajin O, Dupont TF, Huber G, Nagel SR and Witten TA, Contact line deposits in an evaporating drop. Phys Rev E, **62**: 756-765 (2000).

Delcan J and Melgarejo P, Mating behaviour and vegetative compatibility in Spanish populations of *Botryotinia fuckeliana*. Eur J Plant Pathol, **108**: 391-400 (2002).

Delihas N and Forst S, MicF: an antisense RNA gene involved in response of *Escherichia coli* to global stress factors. J Mol Biol, **313**: 1-12 (2001).

Denayer JFM, De Vos D and Leflaive P, Separation of xylene isomers. In: Metal-organic frameworks: applications from catalysis to gas storage, ed. by Farrusseng D. Wiley VCH, Weinheim, Germany, pp. 171-190 (2011).

Desneux N, Wajnberg E, Wyckhuys KAG, Burgio G, Arpaia S, Narváez-Vasquez CA, González-Cabrera J, Ruescas DC, Tabone E, Frandon J, Pizzol J, Poncet C, Cabello T and Urbaneja A, Biological invasion of European tomato crops by *Tuta absoluta*: ecology, geographic expansion and prospects for biological control. J Pest Sci, **83**: 197-215 (2010).

Desneux N, Luna MG, Guillemaud T and Urbaneja A, The invasive South American tomato pinworm, *Tuta absoluta*, continues to spread in Afro-Eurasia and beyond: the new threat to tomato world production. J Pest Sci, **84**: 403-408 (2011).

Desotec, Activated carbon (2011). Available at: http://www.desotec.com (Accessed: May 2012).

Devesa-Rey R, Moldes AB, Diaz-Fierros F and Barral MT, Toxicity of Anllons river sediment extracts using Microtox and the Zucconi phytotoxicity test. Bull Environ Contam Toxicol, **80**: 225-230 (2008).

Dias MS, Phytotoxicity: an overview of the physiological responses of plants exposed to fungicides. J Bot, **2012**: 1-4 (2012).

Diaz U and Corma A, Ordered covalent organic frameworks, COFs and PAFs – from preparation to application. Coordin Chem Rev, **311**: 85-124 (2016).

Dieleman JA, Meinen E, Elings A, Uenk D, Uittien JJ, Broekhuijsen AGM, de Visser PHB and Marcelis LFM, Effecten van langdurig hoog CO_2 op groei en fotosynthese bij paprika. In: Eindrapport van het project ‘Efficiënt gebruik van CO_2’. Plant Research International, Wageningen, The Netherlands, 32 p. (2003).

Diez A, Gustavsson N and Nyström T, The universal stress protein A of *Escherichia coli* is required for resistance to DNA damaging agents and is regulated by a RecA/FtsK-dependent regulatory pathway. Mol Microbiol, **36**: 1494-1503 (2000).

Djamin A and Pathak MD, Role of silica in resistance to Asiatic rice borer, *Chilo suppressalis* (Walker), in rice varieties. J Econ Entomol, **60**: 347-351 (1967).

Dow AgroSciences, Conserve SC turf and ornamental (2015). Available at: http://www.dowagro.com (Accessed: April 2015).

Dubus IG, Barriuso E and Calvet R, Sorption of weak organic acids in soils: clofencet, 2,4-D and salicylic acid. Chemosphere, **45**:767–774 (2001).

Duca M, Water regime. In: Plant physiology, ed. by Duca M. Springer, Cham, Switzerland, pp. 39-64 (2015).

Dufour R, Insect IPM in apples - kaolin clay (2001). Available at: http://attra.ncat.org (Accessed: May 2014).

ECO-ZEO, Developing a pool of novel and eco-efficient applications of zeolite for the agriculture sector (2012-2016). Available at: http:// http://www.ecozeo.eu

ECO-ZEO, Deliverable 3.2: Raw data and conclusions of lab assays. 58 p. (2016).

EFSA, Opinion of the scientific panel on additives and products or substances used in animal feed on the request from the Commission on the use of synthetic sodium aluminium silicate (zeolite) for the reduction of risk of milk fever in dairy cows. EFSA J, **160**: 1-11 (2004).

Ekins P, An economic perspective on technological transitions related to energy and climate change. International conference on environment and energy innovations in economic dynamics. Rome, Italy (2009).

Elad Y, Köhl J and Fokkema NJ, Control of infection and sporulation of *Botrytis cinerea* on bean and tomato by saprophytic yeasts. Phytopathology, **84**: 1193-1200 (1994).

Elad Y, Williamson B, Tudzynski P and Delen N, Botrytis: biology, pathology and control. Kluwer Academic Publishers, Dordrecht, the Netherlands, 428 p. (2004).

Elaiopoulos K, Uses of Natural Zeolites in Operations Involving Organic Gases and Vapors. In: Handbook of natural zeolites, ed. by Inglezakis VJ and Zorpas AA. Bentham Science Publishers, Oak Park, Illinois, pp. 238-287 (2012).

Ellsworth DS, CO_2 enrichment in a maturing pine forest: are CO_2 exchange and water status in the canopy affected? Plant Cell Environ, **22**: 461-472 (1999).

Emino ER and Warman PR, Biological assay for compost quality. Compost Sci Util, **12**: 342-348 (2004).

EPPO, Data sheets on quarantine pests: *Leptinotarsa decemlineata* (2004). Available at: http://www.eppo.int (Accessed: January 2015).

EPPO, Phytotoxicity assessment (2014). Available at: http://www.eppo.int (Accessed: May 2016).

Eriksson H, Controlled release of preservatives using dealuminated zeolite Y. J Biochem Biophys Methods, **70**: 1139-1144 (2008).

Ernesto PB, Have AT, Vant Klooster JW and Van Kan JAL, Fungal and plant gene expression during synchronized infection of tomato leaves by *B. cinerea*. Eur J Plant Pathol, **104**: 207-220 (1998).

Eyheraguibel B, ter Halle A and Richard C, Photodegradation of bentazon, clopyralid, and triclopyr on model leaves: importance of a systemic evaluation of pesticides photostability on crops. J Agric Food Chem, 57: 1960-1966 (2009).

Faers MA, Annulus spray deposit structures and enhanced a.i.-adjuvant association with adjuvanted flowables. In: Proceedings of the 8th international symposium on adjuvants for agrochemicals, ed. by Gaskin RE. ISAA, Columbus, Ohio (2007).

Farha OK and Hupp JT, Rational design, synthesis, purification, and activation of metal-organic framework materials. Acc Chem Res, **43**: 1166-1175 (2010).

Farrusseng D, Aguado S and Pinel C, Metal-organic frameworks: opportunities for catalysis. Angew Chem Int Ed, **48**: 7502-7513 (2009).

Feng X, Dinga X and Jiang D, Covalent organic frameworks. Chem Soc Rev, **41**: 6010–6022 (2012).

Ferey G, The long story and the brilliant future of crystallized porous solids. Struct Bonding, **132**: 87-134 (2009).

Fernandez S and Montagne A, Biologica del minador del tomate, *Scrobipalpula absoluta* (Meyick). Bol Entomol Venez (ns), **5**: 89-99 (1990).

Ferro DN, Logan JA, Voss RH and Elkinton JS, Colorado potato beetle (Coleoptera: Chrysomelidae) temperature-dependent growth and feeding rates. Environ Entomol, **14**: 343-348 (1985).

Filho MM, Vilela EF, Jhamb GN, Attygallec A, Svatos A and Meinwalde J, Initial studies of mating disruption of the tomato moth, *Tuta absoluta* (Lepidoptera: Gelechiidae) using synthetic sex pheromone. J Braz Chem Soc, **11**: 621-628 (2000).

Finsy V, Ma L, Alaerts L, De Vos DE, Baron GV and Denayer JFM, Separation of CO_2/CH_4 mixtures with the MIL-53(Al) metal-organic framework. Micropor Mesopor Mat, **120**: 221-227 (2009).

Fiola DB and Gill SA, Integrated pest management: scouting overview. In: Total crop management for greenhouse production-Bulletin 361, ed. by Kenney S. University of Maryland extension, Maryland, pp. 3-16 (2011).

First EL, Gounaris CE, Wei J and Floudas CA, Computational characterization of zeolite porous networks: an automated approach**.** Phys Chem Chem Phys, **13**: 17339-17358 (2014).

Fishel FM, Pesticide formulations. UF/IFAS EDIS publication PI-231 (2013). Available at: http://edis.ifas.ufl.edu (Accessed: January 2015).

Flanigen EM, Zeolites and molecular sieves - a historical perspective. In: Chemistry of zeolites and related porous materials-synthesis and structure, ed. by van Bekkum H, Flanigen EM, Jacobs PA and Jansen JC. Wiley VCH, Weinheim, Germany, pp. 11-36 (2001).

Flemming HC, Sorption sites in biofilms. Water Sci Technol, **32**: 27-33 (1995).

Fletcher AJ, Thomas KM and Rosseinsky MJ, Flexibility in metal-organic framework materials: impact on sorption properties. J Solid State Chem, **178**: 2491-2510 (2005).

Flexas J, Galmés J, Gallé A, Gulías J, Pou A, Ribas-Carbó M, Tomás M and Medrano H, Improving water use efficiency in grapevines: potential physiological targets for biotechnological improvement. Aust J Grape Wine Res, **161**: 106-121 (2010).

Flint ML, Integrated pest management for rice. University of California, ANR Communication Services, Publication 3280, Oakland, California, 100 p. (1993).

Fogel MN, Schneider MI, Desneux N, González B and Ronco AE, Impact of the neonicotinoid acetamiprid on immature stages of the predator *Eriopis connexa* (Coleoptera: Coccinellidae). Ecotoxicol, **22**: 1063-1071 (2013).

Fontana A and Campbell C, Water activity. In: Handbook of food analysis: physical characterization and nutrient analysis, ed. by Nollet LML. Marcel Dekker, New York, New York, pp. 39-54 (2004).

Foo KY and Hameed BH, Detoxification of pesticide waste via activated carbon adsorption process. J Hazard Mater, **175**: 1-11 (2010).

Foqué D and Nuyttens D, Effects of nozzle type and spray angle on spray deposition in ivy pot plants. Pest Manag Sci, **67**: 199-208 (2011).

Forgash AJ, Insecticide resistance in the Colorado potato beetle. In: Proceedings of the symposium on the Colorado potato beetle, 17th International Congress of Entomology, ed. by Ferro DN and Voss RH. Massachusetts Experiment Station, Amherst, Massachusetts, pp. 33-53 (1985).

Fries E, Spilocacea pomi. Fr Nov Fl Suec, **5**: 79 (1819).

Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Montgomery JA, Vreven T, Kudin KN, Burant JC, Millam JM, Iyengar SS, Tomasi J, Barone V, Mennucci B, Cossi M, Scalmani G, Rega N, Petersson GA, Nakatsuji H, Hada M, Ehara M, Toyota K, Fukuda R, Hasegawa J, Ishida M, Nakajima T, Honda Y, Kitao O, Nakai H, Klene M, Li X, Knox JE, Hratchian HP, Cross JB, Bakken V, Adamo C, Jaramillo J, Gomperts R, Stratmann RE, Yazyev O, Austin AJ, Cammi R, Pomelli C, Ochterski JW, Ayala PY, Morokuma K, Voth GA, Salvador P, Dannenberg JJ, Zakrzewski VG, Dapprich S, Daniels AD, Strain MC, Farkas O, Malick DK, Rabuck AD, Raghavachari K, Foresman JB, Ortiz JV, Cui Q, Baboul AG, Clifford S, Cioslowski J, Stefanov BB, Liu G, Liashenko A, Piskorz P, Komaromi I, Martin RL and Fox DJ, Gaussian 09 (Revision A.02) (2009). Available at: http://www.gaussian.com (Accessed: 1 June 2015).

Furneaux PJS and Mackay AL, The composition, structure, and formation of the chorion and the vitelline membrane of the insect egg-shell. In: The insect integument, ed. By Hepburn HR. Elsevier, Amsterdam, the Netherlands, pp. 157-176 (1976).

Galvan TL, Koch RL and Hutchison WD, Toxicity of commonly used insecticides in sweet corn and soybean to multicolored Asian lady beetle (Coleoptera: Coccinellidae). J Econ Entomol, **98**: 780-789 (2005).

Garcia GA, Sensitivity analysis of a leaf photosynthesis-stomatal resistance model. PhD thesis, University of Tennessee, Tennessee (2014).

Gaskin RE, Elliott G and Steele KD, Novel organosilicone adjuvants to reduce agrochemical spray volumes on row crops. N Z Plant Prot, **53**: 350-354 (2000).

Gaskin RE, Manktelow DW and Elliott GS, New adjuvant technology for pesticide use on wine grapes. N Z Plant Prot, **55**: 154-158 (2002).

Gaskin RE, Steele KD and Forster WA, Characterising plant surfaces for spray adhesion and retention. N Z Plant Prot, **58**: 179-183 (2005).

Georgiev D, Bogdanov B, Angelova K, Markovska I and Yancho H, Synthetic zeolites, structure, classification, current trends in zeolite synthesis - review. International conference on economics and society development on the base of knowledge, Stara Zagora, Bulgaria (2009).

Gerstl Z, Nasser A and Mingelgrin U, Controlled release of pesticides into soils from clay-polymer formulations. J Agric Food Chem, **46**: 3797-3802 (1998).

Gevao B and Jones KC, Pesticides and persistent organic pollutants. In: Agriculture, hydrology and water quality, ed. by Haygarth PM and Jarvis SC. Cromwell Press, Trowbridge, UK, pp. 83-106 (2002).

Ghaemi A, Torab-Mostaedi M and Ghannadi-Maragheh M, Characterizations of strontium (II) and barium (II) adsorption from aqueous solutions using dolomite powder. J Hazard Mater, **190**: 916-921 (2011).

Glazebrook J, Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu Rev Phytopathol, **43**: 205-227 (2005).

Glenn DM, Puterka GJ, Vanderzwet T, Byers RE and Feldhake C, Hydrophobic particle films: a new paradigm for suppression of arthropod pests and plant diseases. J Econ Entomol, **92**: 759-771 (1999).

Glenn DM, Puterka GJ, Drake SR, Unruh TR, Knight AL, Baherle P, Prado E and Baugher TA, Particle film application influences apple leaf physiology, fruit yield, and fruit quality. J Am Soc Hortic Sci, **126**: 175-181 (2001).

Glenn DM, Prado E, Erez A, McFerson J and Puterka GJ, A reflective, processed-kaolin particle film affects fruit temperature, radiation reflection, and solar injury in apple. J Amer Soc Hort Sci, 127: 188-193 (2002).

Glenn DM, Erez A, Puterka GJ and Gundrum P, Particle films affect carbon assimilation and yield in 'Empire' apple. J Am Soc Hortic Sci, **128**: 356-362 (2003).

Glenn DM and Puterka GJ, Particle films: a new technology for agriculture. In: Horticultural Reviews, ed. by Janick J. John Wiley and Sons, Hoboken, New Jersey, pp. 1-44 (2005).

Godfrey LD, Grafton-Cardwell EE, Kaya HK and Chaney WE, Microorganisms and their byproducts, nematodes, oils and particle films have important agricultural uses. Calif Agric, **59**: 35-40 (2005).

Gontijo PC, Picanço MC, Pereira EJG, Martins JC, Chediak M and Guedes RNC, Spatial and temporal variation in the control failure likelihood of the tomato leaf miner, *Tuta absoluta*. Ann Appl Biol, **162**: 50-59 (2013).

Grange M, Wand S and Theron K, Effect of kaolin applications on apple fruit quality and gas exchange of apple leaves. Acta Hortic, **636**: 545-550 (2004).

Greer DH, Photon flux density and temperature-dependent responses of photosynthesis and photosystem II performance of apple leaves grown in field conditions. Funct Plant Biol, **42**: 782-791 (2015).

Grimaldi D and Engel MS, Evolution of the Insects. Cambridge University Press, Cambridge, UK, 755 p. (2005).

Groisman L, Rav-Acha C, Gerstl Z and Mingelgrin U, Sorption of organic compounds of varying hydrophobicities from water and industrial wastewater by long- and short-chain organoclays. Appl Clay Sci, **24**: 159-166 (2004).

Guarneri AA, Lazzari C, Diotaiuti L and Lorenzo MG, The effect of relative humidity on the behaviour and development of *Triatoma brasiliensis*. Physiol Entomol, **27**: 142-147 (2002).

Guedes RNC and Picanço MC, The tomato borer *Tuta absoluta* in South America: pest status, management and insecticide resistance. EPPO Bull, **42**: 211-216 (2012).

Guha L, Seenivasagan T, Bandyopadhyay P, Iqbal ST, Sathe M, Sharma P, Parashar BD and Kaushik MP, Oviposition and flight orientation response of Aedes aeqypti to certain aromatic aryl hydrazono esters. Parasitol Res, **111**: 975-982 (2012).

Gullino ML, Leroux P and Smith CM, Uses and challenges of novel compounds for plant disease control. Crop Prot, 19: 1–11 (2000).

Gunderson CA, Sholtis JD, Wullshleger SD, Tissue DT, Hanson PJ and Norby RJ, Environmental and stomatal control of photosynthetic enhancement in the canopy of a sweetgum (*Liquidambar styraciflua* L.) plantation during 3 years of CO_2 enrichment. Plant Cell Environ, **25**: 379-393 (2002).

Gupta GK, Apple scab (*Venturia inaequalis* (Che.) Wint.). In: Plant diseases of international importance. Diseases of fruit crops, ed. by Kumar J, Chaube HS, Singh US and Mukhopadhyay AN. Prentice Hall, Englewood Cliffs, New Jersey, pp. 1-31 (1992).

Gustafson DI, Groundwater ubiquity score - a simple method for assessing pesticide leachability. Environ Toxicol Chem, **8**: 339-357 (1989).

Habermann G, Machado EC, Rodrigues JD and Medina CL, CO_2 assimilation, photosynthetic light response curves, and water relations of Pêra sweet orange plants infected with Xylela fastidiosa. Braz J Plant Physiol, **15**: 79-87 (2003).

Hahn M, The rising threat of fungicide resistance in plant pathogenic fungi: Botrytis as a case study. J Chem Biol, **7**: 133–141 (2014).

Halasz I, Kim S and Marcus B, Hydrophilic and hydrophobic adsorption on Y zeolites. Mol Phys, **100**: 3123-3132 (2002).

Hall FR, Chapple AC, Downer RA, Kirchner LM and Thacker JRM, Pesticide application as affected by spray modifiers. Pestic Sci, **38**: 123-133 (1993).

Hall FR, Downer RA, Cooper JA, Ebert TA and Ferree DC, Changes in spray retention by apple leaves during a growing season. HortScience, **32**: 858-860 (1997).

Hanan SA and Samya ZS, Effects of certain insecticides on eggs of *Spodoptera Littoralis*. Egypt J Agric Res, **92**: 875-883 (2014).

Hanssens J, Decision support for tomato growers based on plant responses, modelling and greenhouse energy consumption. PhD thesis, Ghent University, Belgium (2014).

Haque E, Jun JW and Jhung SH, Adsorptive removal of methyl orange and methylene blue from aqueous solution with a metal-organic framework material, iron terephthalate (MOF-235). J Hazard Mater, **185**: 507-511 (2011).

Hardi MD, Serre C, Frot T, Rozes L, Maurin G, Sanchez C and Ferey G, A new photoactive crystalline highly porous titanium (IV) dicarboxylate. J Am Chem Soc, **131**: 10857-10859 (2009).

Harvey CC and Lagaly G, Conventional applications. In: Handbook of clay science, ed. by Bergaya F, Theng BKG and Lagaly G. Elsevier, Amsterdam, the Netherlands, pp. 501-540 (2006).

Hasan Z and Jhung SH, Removal of hazardous organics from water using metal-organic frameworks (MOFs): Plausible mechanisms for selective adsorptions. J Hazard Mater, **283**: 329–339 (2015).

Hazen JL, Adjuvants - terminology, classification, and chemistry. Weed Technol, **14**: 773-784 (2000).

Henschel A, Senkovska I and Kaskel S, Liquid-phase adsorption on metal-organic frameworks. Adsorpt, **17**: 219-226 (2011).

HERA, Risk Assessment of Sodium Aluminum Silicate - Zeolite A (2004). Available at: http://www.heraproject.com (Accessed: May 2013).

Herrick JD, Maherali H and Thomas RB, Reduced stomatal conductance in sweetgum (Liquidambar styraciflua) sustained over long-term CO_2 enrichment. New Phytol, **162**: 387-396 (2004).

Herzfeld D and Sargent K, Chapter 4: pesticide formulations. In: Private pesticide applicator safety eduction manual, ed. by Goodman N. University of Minnesota, Minneapolis, Minnesota, pp. 85-108 (2011).

Heuskin S, Verheggen FJ, Haubruge E, Wathelet J-P and Lognay G, The use of semiochemical slow-release devices in integrated pest management strategies. Biotechnol Agron Soc Environ, **15**: 459-470 (2011).

Hilker M, Stein C, Schroeder R, Varama M and Mumm R, Insect egg deposition induces defence responses in *Pinus sylvestris*: characterisation of the elicitor. J Exp Biol, **20**: 1849-1854 (2005).

Hilker M and Meiners T, Early herbivore alert: insect eggs induce plant defense. J Chem Ecol, **32**: 1379-1397 (2006).

Ho YS, Porter JF and McKay G, Equilibrium isotherm studies for the sorption of divalent metal ions onto peat: copper, nickel and lead single component systems. Water Air Soil Pollut, **141**: 1-33 (2002).

Hoad SP, Jeffree CE and Grace J, Effects of wind and abrasion on cuticular integrity in *Fagus sylvatica* L. and consequences for transfer of pollutants through leaf surfaces. Agricult Ecosys Environ, **42**: 275-289 (1992).

Hoepfner V, Deringer VL and Dronskowski R, Hydrogen-bonding networks from first-principles: exploring the guanidine crystal. J Phys Chem A, 116: 4551–4559 (2012).

Hoffmann EJ, Middleton SM and Wise JC, Ovicidal activity of organophosphate, oxadiazine, neonicotinoid and insect growth regulator chemistries on northern strain plum curculio, *Conotrachelus nenupha.* J Insect Sci, **8**: 29 (2008).

Holding DR and Streich AM, Plant growth processes: transpiration, photosynthesis and respiration (2013). Available at: http://extension.unl.edu (Accessed: January 2016).

Holloway PJ, Surface factors affecting the wetting of leaves. Pestic Sci, **1**: 156-163 (1970).

Honghong Y, Hua D, Xiaolong T, Qiongfen Y, Xuan Z and Haiyan L, Adsorption equilibrium and kinetics for SO_2, NO, CO_2 on zeolites FAU and LTA. J Hazard Mater, **203-204**: 111-117 (2012).

Horcajada P, Serre C, Vallet-Regi M, Sebban M, Taulelle F and Ferey G, Metal-organic frameworks as efficient materials for drug delivery. Angew Chem Int Ed, **45**: 5974-5978 (2006).

Horn H and Morgenroth E, Transport of oxygen, sodium chloride, and sodium nitrate in biofilms. Chem Eng Sci, **61**: 1347-1356 (2006).

Hrenovic J and Tibljas D, Phosphorus removal from wastewater by bioaugmented activated sludge with different amounts of natural zeolite addition. In: Studies in surface science and catalysis: impact of zeolites and other porous materials on the new technologies at the beginning of the millenium - Proceedings of the 2nd international FEZA conference, ed. by Aiello R, Testa F and Giordano G. Elsevier, Amsterdam, the Netherlands, pp. 1743-1750 (2002).

Hsieh CT and Teng H, Langmuir and Dubinin-Radushkevich analyses on equilibrium adsorption of activated carbon fabrics in aqueous solutions. J Chem Technol Biotechnol, **75**: 1066-1072 (2000).

Hu YH and Zhang L, Hydrogen storage in metal-organic frameworks. Adv Mater, **22**: E117-E130 (2010).

Huang J, Walker ED, Giroux PY, Vulule J and Miller JR, Ovipositional site selection by Anopheles gambiae: influences of substrate moisture and texture. Med Vet Entomol, **19**: 442-450 (2005).

Ibrahim K, Mineralogy and chemistry of natrolite from Jordan. Clay Miner, **39**: 47-55 (2004).

Inagaki M and Feiyu K, Carbon materials science and engineering - from fundamentals to applications. Tsinghua University Press, Beijing, China, 610 p. (2006).

Ingram DM and Meister CW, Managing Botrytis gray mold in greenhouse tomatoes using traditional and bio-fungicides. Plant Health Progress, 50-62 (2006).

IRAC, IRAC MoA classification scheme (version 7.3.1) (2014). Available at: http://www.irac-online.org (Accessed: September 2015).

Isman MB, Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annu Rev Entomol, **51**: 45-66 (2006).

IUPAC, Reporting physisoprtion data for gas/solid systems with special reference to the determination of surface area and porosity. Pure Appl Chem, **57**: 603-19 (1985).

Jackson MA, Dunlap CA and Jaronski ST, Ecological considerations in producing and formulating fungal enomopathogens. In: The ecology of fungal entomopathogens, ed. by Roy HE, Vega FE, Chandler D, Goettel MS, Pell JK and Wajnberg E. Springer, Dordrecht, the Netherlands, pp. 129-146 (2010).

Jacques RL Jr, The potato beetles of Florida (Coleoptera: Chrysomelidae). Entomol Circ, **271**: 1-2 (1985).

Jagiello J and Thommes M, Comparison of DFT characterization methods based on N_2, Ar, CO_2, and H_2 adsorption applied to carbons with various pore size distributions. Carbon, **42**: 1227-1232 (2004).

Jaramillo E and Chandross M, Adsorption of small molecules in LTA zeolites. 1. NH_3, CO_2, and H_2O in zeolite 4A. J Phys Chem B, **108**: 20155-20159 (2004).

Jenks M and Ashworth E, Plant epicuticular waxes: function, production, and genetics. Hort Rev, 23: 1-68 (1999).

Jha G, Thakur K and Thakur P, The Venturia apple pathosystem: pathogenicity mechanisms and plant defense responses. J Biomed Biotechnol, **28**: 1-10 (2009).

Jhung SH, Lee JH, Yoon JW, Serre C, Ferey G and Chang JS, Microwave synthesis of chromium terephthalate MIL-101 and its benzene sorption ability. Adv Mater, **19**: 121-122 (2007).

Jifon JL and Syvertsen JP, Kaolin particle film applications can increase photosynthesis and water use efficiency of 'ruby red' grapefruit leaves. J Am Soc Hortic Sci, **128**: 107-112 (2003).

Jolivet P, The Colorado beetle menaces Asia (*Leptinotarsa decemlineata* Say 1824) (Col. Chrysomelidae). Entomologiste, **47**: 29-48 (1991).

Juang RS, Wu FC and Tseng RL, Adsorption isotherms of phenolic compounds from aqueous solutions onto activated carbon. J Chem Eng Data, **41**: 487-492 (1996).

Jung SJ, Kim MH, Kim HD, Kim YH and Kim SR, Synergy between microwave irradiation and promoter addition about synthesis of nanosized TPA-silicalite. In: Studies in surface science and catalysis: recent advances in the science and technology of zeolites and related materials - Proceedings of the 14th international zeolite conference, ed. by van Steen E, Claeys IM and Callanan LH. Elsevier, Amsterdam, the Netherlands, pp. 180-183 (2004).

Juniper BE, The leaf from the inside and the outside: a microbe's perspective. In: Microbial ecology of leaves, ed. by Andrews JH and Hirano SS. Springer, New York, New York, pp. 21-42 (1991).

Kadirvelu K and Namasivayam C, Agricultural by-products as metal adsorbents: sorption of lead (II) from aqueous solutions onto coirpith carbon. Environ Technol, **21**: 1091-1097 (2000).

Kaewchai S, Soytong K and Hyde KD, Mycofungicides and fungal biofertilizers. Fungal Divers, **38**: 25-50 (2009).

Kahler B, A synopsis of the polyvalent qualities of zeolite-clinoptilolite and the proposed uses within the Namibian medical, pharmaceutical, industrial and economical sectors: a proposal for an integrated research. ISTJN, **3**: 42-63 (2014).

Kahn BA and Damicone JP, Kaolin particle film product applications before harvest begins may not improve marketable yields of fresh tomatoes. HortTechnology, **18**: 144-147 (2008).

Kalkhoff SJ, Kolpin DW, Thurman EM, Ferrer I and Barcelo D, Degradation of chloroacetanilide herbicides: the prevalence of sulfonic and oxanilic acid metabolites in Iowa groundwaters and surface waters. Environ Sci Technol, **32**: 1738-1740 (1998).

Kaszab Z, Kaolin-based particle films for arthropod control. In: Encyclopedia of entomology, ed. by Capinera JL. Springer, Heidelberg, Germany, pp. 2075-2080 (2008).

Kawai T, Yanagihara T and Tsutsumi K, Adsorption characteristics of chloroform on modified zeolites from gaseous phase as well as its aqueous solution. Colloid Polym Sci, **272**: 1620-1626 (1994).

Keitt GW and Jones IK, Studies of the epidemiology and control of apple scab. Wis Agric Exp Stn Res Bull, **73**: 1-104 (1926).

Khanizadeh S, DeEll J and Hakam N, Use of chlorophyll fluorescence to evaluate chilling tolerance in strawberry plants. Acta Hortic, **538**: 453-456 (2000).

Kiliç T, First record of *Tuta absoluta* in Turkey. Phytoparasitica, **38**: 243-244 (2010).

Kimball BA, Influence of elevated CO_2 on crop yield. In: Carbon dioxide enrichment of greenhouse crops, ed. by Enoch HZ and Kimball BA. CRC Press, Boca Raton, Florida, pp. 105-115 (1986).

Klassen W, World food security up to 2010 and the global pesticide situation. In: Proceedings of the 8th international congress on pesticide chemistry, ed. by Ragsdale NN, Kearny PC and Plimmer JR. American Chemical Society, Washington DC, Washington DC, pp. 1-32 (1995).

Knight AL, Unruh TR, Christianson BA, Puterka GJ and Glenn DM, Effects of a kaolin-based particle film on obliquebanded leafroller (Lepidoptera: Tortricidae). J Econ Entomol, **93**: 744-749 (2000).

Knowles A, Recent developments of safer formulations of agrochemicals. Environmentalist, **28**: 35-44 (2008).

Kobayashi H, Yokoyama H, Feng B and Fukuoka A, Dehydration of sorbitol to isosorbide over H-beta zeolites with high Si/Al ratios. Green Chem, **17**: 2732-2735 (2015).

Kolasinski, KW, Surface science: foundations of catalysis and nanoscience. Wiley VCH, Weinheim, Germany, 574 p. (2012).

Köller W, Chemical approaches to managing plant pathogens. In: Handbook of pest management, ed. by Ruberson JR. Marcel Dekker, New York, New York, pp. 337-376 (1999).

Kolpin DW, Barbash JE and Gilliom RJ, Occurrence of pesticides in shallow groundwater of the United States: initial results from the national water-quality assessment program. Environ Sci Technol, **32**: 558-566 (1998).

Koppel AL, Herbert DA Jr, Kuhar TP, Malone S and Arrington M, Efficacy of selected insecticides against eggs of *Euchistus servus* and *Acrosternum hilare* (Hemiptera: Pentatomidae) and the egg parasitoid *Telenomus podisi* (Hymenopera: Scelionidae). J Econ Entomol, **104**: 137-142 (2011).

Korth KL, Doege SJ, Park SH, Goggin FL, Wang S, Gomez SK, Liu G, Jia L and Nakata PA, *Medicago truncatula* mutants demonstrate the role of plant calcium oxalate crystals as an effective defense against chewing insects. Plant Physiol, **141**: 188-195 (2006).

Kortunov P, Vasenkov S, Chmelik C, Kärger J, Ruthven DM and Wloch J, Influence of defects on the external crystal surface on molecular uptake into MFI-type zeolites. Chem Mater, **16**: 3552-3558 (2004).

Kramer LW and Mulla SM, Oviposition attractants and repellents of mosquitoes: oviposition responses of Culex mosquito to organic infusions. Environ Entomol, **8**: 1111-1117 (1979).

Krivoshiev BV, Dardenne F, Blust R, Covaci A and Husson SJ, Elucidating toxicological mechanisms of current flame retardants using a bacterial gene profiling assay. Toxicol In Vitro, **29**: 2124-2132 (2015).

Kühl GH, Catalysis and zeolites: fundamentals and applications. Springer, Heidelberg, Germany, 564 p. (1999).

Kundua S, Pala A and Dikshitb, UV induced degradation of herbicide 2,4-D: kinetics, mechanism and effect of various conditions on the degradation. Sep Purif Technol, **44**: 121-129 (2005).

Kunkel BN and Brooks DM, Cross talk between signaling pathways in pathogen defense. Curr Opin Plant Biol, **5**: 325-331 (2002).

Kvachantiradze M, Tvalchrelidze E, Kotetishvili M and Tsitsishvili T, Application of clinoptilolite as an additive for the photostabilization of the Bacillus thuringiensis formulation. In: Porous materials in environmentally friendly processes, ed. by Kiricsi I, Pal-Borbély G, Nagy JB and Karge HG. Elsevier, Amsterdam, the Netherlands, pp. 731-735 (1999).

Labite H, Butler F and Cummins E, A review and evaluation of plant protection product ranking tools used in agriculture. Hum Ecol Risk Assess, **17**: 300-327 (2011).

Lai T, Cao A, Zucca A and Carucci A, Use of natural zeolites charged with ammonium or carbon dioxide in phytoremediation of lead- and zinc-contaminated soils. J Chem Technol Biotechnol, **87**: 1342-1348 (2012).

Lakso AN, Chapter 2: Apple. In: Handbook of environmental physiology of fruit crops, ed. by Schaffer B and Anderson PC. CRC Press, Boca Raton, Florida, pp. 3-42 (1994).

Lalancette N, Belding RD, Shearer PW, Frecon JL and Tietjen WH, Evaluation of hydrophobic and hydrophilic kaolin particle films for peach crop, arthropod and disease management. Pest Manag Sci, **61**: 25-39 (2005).

Lambers H, Chapin S and Pons TL, Photosynthesis, respiration and long-distance transport. In: Plant physiological ecology, ed. by Lambers H, Chapin S and Pons TL. Springer, Berlin, Germany, pp. 11-162 (2008).

Lanxess, Clean waste water (2009). Available at: http://www.lanxess.com (Accessed: June 2016).

Lanxess, Product information: Lewatit AF 5 (2011a). Available at: http://www.ionexchange.com (Accessed: May 2012).

Lanxess, Product information: Lewatit VP OC 1064 (2011b). Available at: http://www.ionexchange.com (Accessed: May 2012).

Larentzaki E, Shelton AM and Plate J, Effect of kaolin particle film on *Thrips tabaci* (Thysanoptera: Thripidae), oviposition, feeding and development on onions: a lab and field case study. Crop Prot, **27**: 727-734 (2008).

Larsolle A, Wretblad P and Westberg C, A comparison of biological effect and spray liquid distribution and deposition for different spray application techniques in different crops. Department of Agricultural Engineering, Uppsala, Sweden, 32 p. (2002).

Leakey ADB, Bernacchi CJ, Ort DR and Long SP, Longterm growth of soybean at elevated [CO_2] does not cause acclimation of stomatal conductance under fully open-air conditions. Plant Cell Environ, **29**: 1794-1800 (2006).

Lee J, Farha OK, Roberts J, Scheidt KA, Nguyen ST and Hupp JT, Metal-organic framework materials as catalysts. Chem Soc Rev, **38**: 1450-1459 (2009).

Lee JY, Tang CY and Huo F, Fabrication of porous matrix membrane (PMM) using metal-organic framework as green template for water treatment. Sci Rep, **4**: 3740 (2014).

Leggo PJ, Ledésert B and Graham C, The role of clinoptilolite in organo-zeolitic-soil systems used for phytoremediation. Sci Total Environ, **363**: 1-10 (2006).

Lenntech, Regeneration / activated carbon (2016). Available at: www.lenntech.nl (Accessed: February 2016).

Leroux P, Chemical control of Botrytis and its resistance to chemical fungicides. In: Botrytis: biology, pathology and control, ed. by Elad Y, Williamson B, Tudzynski P and Delen N. Kluwer Academic Publishers, Dordrecht, the Netherlands, pp. 195-222 (2007).

Leskey TC, Wright SE, Glenn DM and Puterka GJ, Effect of surround WP on behavior and mortality of apple maggot (Diptera: Tephritidae). J Econ Entomol, **103**: 394-401 (2010).

Leung JW and Webster GRB, Effect of adjuvants on rainfastness and herbicidal activity of glyphosate deposits on trembling aspen foliage. J Environ Sci Health, Part B, **29**: 1169-1201 (1994).

Leus K, Muylaert I, Vandichel M, Marin GB, Waroquier M, Van Speybroeck V and Van der Voort P, The remarkable catalytic activity of the saturated metal organic framework V-MIL-47 in the cyclohexene oxidation. Chem Commun, **46**: 5085-5087 (2010).

Leus K, Bogaerts, T, De Decker J, Depauw H, Hendrickx K, Vrielinck H, Van Speybroeck V and Van Der Voort P, Systematic study of the chemical and hydrothermal stability of selected “stable” Metal Organic Frameworks. Micropor Mesopor Mat, **226**: 110-116 (2016).

Li Z, Use of surfactant-modified zeolite as fertilizer carriers to control nitrate release. Micropor Mesopor Mat, **61**: 181-188 (2003).

Liakopoulos G, Stavrianakou S and Karabourniotis G, Analysis of epicuticular phenolics of Prunus persica and Olea europaea Leaves: evidence for the Chemical Origin of the UV-induced blue fluorescence of stomata. Ann Bot, 87: 641-648 (2001).

Lietti MMM, Botto E and Alzogaray RA, Insecticide resistance in Argentine populations of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Neotrop Entomol, **34**: 113-119 (2005).

Little D, Gouhier-Darimont C, Bruessow F and Reymond P, Oviposition by pierid butterflies triggers defense responses in Arabidopsis. Plant Physiol, **143**: 784-800 (2007).

Liu Z, Yan X, Drikas M, Zhou D, Wang D, Yang M and Qu J, Removal of bentazone from micro-polluted water using MIEX resin: kinetics, equilibrium, and mechanism. J Environ Sci, **23**: 381-387 (2011).

Lloyd J and Farquhar GD, Effects of rising temperatures and [CO_2] on the physiology of tropical forest trees. Philos Trans R Soc Lond B Biol Sci, **363**: 1811-1817 (2008).

Lobo RF, Introduction to the structural chemistry of zeolites. In: Handbook of zeolite science and technology, ed. by Auerback SM, Carrado KA and Dutta PK. Marcell Dekker, New York, New York, pp. 65-90 (2003).

Locke T and Fletcher JT, Incidence of benomyl and iprodione resistance in isolates of *Botrytis cinerea* in tomato crops in England and Wales in 1986. Plant Pathol, **37**: 381-384 (1988).

Logan PA, Casagrande RA, Faubert HH and Drummond FA, Temperature-dependent development and feeding of immature Colorado potato beetles, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) Environ Entomol, **14**: 275-283 (1985).

Loiseau T, Serre C, Huguenard C, Fink G, Taulelle F, Henry M, Bataille T and Férey G, A rationale for the large breathing of the porous aluminum terephthalate (MIL-53) upon hydration. Chem Eur J, **19**: 1373-1382 (2004).

Lombardozzi D, Levis S, Bonan G and Sparks JP, Predicting photosynthesis and transpiration responses to ozone: decoupling modeled photosynthesis and stomatal conductance. Biogeosciences, **9**: 3113-3130 (2012).

Long SP, Ainsworth EA, Rogers A and Ort DR, Rising atmospheric carbon dioxide: plants face the future. Annu Rev Plant Biol, **55**: 591-628 (2004).

Loukehaich R, Elyachioui M, Belhabib N and Douira A, Identifying multiple physiological responses associated with salinity-tolerance for evaluating three tomato cultivars selected from Moroccan territory. J Anim Plant Sci, **10**: 1219-1231 (2011).

Lovatt JC, Nutrient deficiency and correction. In: Citrus production manual, ed. by Ferguson L and Grafton-Cardwell EE. University of California, California, pp. 161-182 (2014).

Lu P, Muller WJ and Chacko EK, Spatial variations in xylem sap flux density in the trunk of orchard-grown, mature mango trees under changing soil water conditions. Tree Physiol, **20**: 683-692 (2000).

Lukens RJ, Chapter 3: fungitoxic barriers. In: Chemistry of fungicidal action, ed. by Lukens RJ. Springer, Berlin, Germany, pp. 13-30 (1971).

Ma LQ, Abney C and Lin WB, Enantioselective catalysis with homochiral metal-organic frameworks. Chem Soc Rev, **38**: 1248-1256 (2009).

Machado EC, Quaggio JA, Lagôa AMMA, Ticelli M and Furlani PR, Trocas gasosas e relações hídricas em laranjeiras com clorose variegada dos citros. Rev Bras Fisiol Veg, **6**: 53-57 (1994).

MacHardy WE, Apple scab: biology, epidemiology and management. The American Phytopathological Society Press, Saint Paul, Minnesota, 546 p. (1996).

MacHardy WE, Gadoury DM and Gessler C, Parasitic and biological fitness of *Venturia inaequalis*: relationship to disease management strategies. Plant Dis, **85**: 1036-1051 (2001).

Maletsika PA and Nanos GD, Leaf and fruit responses to kaolin particle film applied onto mature olive trees. J Biol Agric Healthc, **5**: 17-27 (2015).

Marchi S, Tognetti R, Vaccari FP, Lanini M, Kaligaric M, Miglietta F and Raschi A, Physiological and morphological responses of grassland species to elevated atmospheric CO_2 concentrations in FACE-systems and natural CO_2 springs. Funct Plant Biol, **31**: 181-194 (2004).

Marín-Sánchez JP, Manual de claus per avaluar la severitat de les malalties. Generalitat de Catalunya, Departament d'Agricultura Ramaderia i Pesca, Servei de Protecció dels Vegetals, 40 p. (1990).

Marko V, Blommers LHM, Bogya S and Helsen H, Kaolin particle films suppress many apple pests, disrupt natural enemies and promote woolly apple aphid. J Appl Entomol, **132**: 26-35 (2008).

Marsh H and Reinoso FR, Activated carbon. Elsevier, Amsterdam, the Netherlands, 554 p. (2006).

Martin DE, Lopez JD, Lan Y, Fritz BK, Hoffman WC and Duke SE, Novaluron as an ovicide for bollworm on cotton: deposition and efficacy of field-scale aerial applications. J Cotton Sci, **14**: 99-106 (2010).

Mashaya N, Effect of simulated rain on efficacy of insecticide deposits on tobacco. Crop Prot, **12**: 55-58 (1993).

Masque N, Galia M, Marce RM and Borrul F, New chemically modified polymeric resin for solid-phase extraction of pesticides and phenolic compounds from water. J Chromatogr, **803**: 147-155 (1998a).

Masque N, Marce RM and Borrul F, Comparison of different sorbents for on-line solid-phase extraction of pesticides and phenolic compounds from natural water followed by liquid chromatography. J Chromatogr, **793**: 257-263 (1998b).

Maxwell K and Johnson GN, Chlorophyll fluorescence - a practical guide. J Exp Bot, **51**: 659-668 (2000).

Mazor M and Erez A, Processed kaolin protects fruits from Mediterranean fruit fly infestations. Crop Prot, **23**: 47-51 (2004).

McArthur H and Spalding D, Water. In: Engineering materials science: properties, uses, degradation and remediation, ed. by McArthur H and Spalding D. Horwood Publishing, Chinchester, UK, pp. 129-138 (2004).

McBride J, Whitewashing agriculture. Agric Res, **48**: 14-17 (2000).

McCusker LB and Baerlocher C, Zeolite structures. In: Introduction to zeolite science and practice, ed. by van Bekkum H, Flanigen EM, Jacobs PA and Jansen JC. Elsevier, Amsterdam, the Netherlands, pp. 37-68 (2001).

McCusker LB and Baerlocher C, Zeolite structures. In: Zeolites and ordered mesoporous materials: progress and prospects, ed. by Cejka J and van Bekkum H. Elsevier, Amsterdam, the Netherlands, pp. 41-64 (2005).

McCusker LB and Baerlocher C, Zeolite structures. In: Introduction to zeolite science and practice, ed. by Cejka J, van Bekkum H, Corma A and Schüth F. Elsevier, Amsterdam, the Netherlands, pp. 13-38 (2007).

McPartland JM, Clarke RC and Watson DP, Hemp diseases and pests: management and biological control. Cromwell Press, Trowbridge, UK, 261 p. (2000).

Medlyn BE, Barton CVM, Broadmeadow MSJ, Ceulemans R, De Angelis P, Forstreuter M, Freeman M, Jackson SB, Kellomäki S, Laitat E, Rey A, Roberntz P, Sigurdsson BD, Strassemeyer J, Wang K, Curtis PS and Jarvis PG, Stomatal conductance of forest species after long-term exposure to elevated CO_2 concentration: a synthesis. New Phytol, **149**: 247-264 (2001).

Medrano H, Tomas M, Martorell S, Flexas J, Hernandez E, Rossello J, Pou A, Escalona JM and Bota J, From leaf to whole-plant water use efficiency (WUE) in complex canopies: limitations of leaf WUE as a selection target. Crop J, **3**: 220-228 (2015).

Meek ST, Greathouse JA and Allendorf MD, Metal-organic frameworks: a rapidly growing class of versatile nanoporous materials. Adv Mater, **23**: 249-267 (2011).

Megido RC, Haubruge E and Verheggen FJ, Pheromone-based managent strategies to control the tomato leafminer, *Tuta absoluta* (Lepidoptera: Gelechiidae). A review. Biotechnol Agron Soc Environ, **17**: 475-482 (2013).

Melo CR, Riella HG, Kuhnen NC, Angioletto E, Resmini E, Melo A, Bernardin AM, da Rocha MR and Da Silva L, Synthesis of 4A zeolites from kaolin for obtaining5A zeolites through ionic exchange for adsorption of arsenic. Mater Sci Eng, **177**: 345-349 (2012).

Mengel K, Kirkby EA, Kosegarten H and Appel T, Principles of plant nutrition. Kluwer Academic Publishers, Dordrecht, 849 p. (2001).

Mesnage R, Bernay B and Séralini GE, Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity. Toxicology, **313**: 122-128 (2013).

Microbiotests Inc, Phytotestkit standard operational procedure (2008).

Milenkovski S, Bååth E, Lindgren PE and Berglund O, Toxicity of fungicides to natural bacterial communities in wetland water and sediment measured using leucine incorporation and potential denitrification. Ecotoxicol, 19: 285–294 (2010).

Miller P and Westra P, Herbicide surfactants and adjuvants. Bulletin N_o 0.559. Colorado state university cooperative extension, Fort Collins, Colorado (1996).

Miller M, Lewis G, Gisselquist J, Moscoso J and Patton R, UZM-12 and UZM-12 HS: crystalline aluminosilicate zeolitic compositions and processes for preparing and using the compositions, patent US20060073094 (2004).

Min KS and Suh MP, Silver (I)-polynitrile network solids for anion exchange: anion-induced transformation of supramolecular structure in the crystalline state. J Am Chem Soc, **122**: 6834-6840 (2000).

Ming DW and Boettinger JL, Zeolites in soil environments. In: Natural zeolites: occurrence, properties, applications - reviews in mineralogy and geochemistry, ed. by Bish DL and Ming DW. Mineralogical Society of America and Geochemical Society, Washington DC, USA, pp. 323-345 (2001).

Mintova S and Valtchev V, Synthesis of nanosized FAU-type zeolite. In: Studies in surface science and catalysis: porous materials in environmentally friendly processes - proceedings of the 1st international FEZA conference, ed. by Kiricsi I, Pal-Borbély G, Nagy JB and Karge HG. Elsevier, Amsterdam, the Netherlands, pp. 141-148 (1999).

Mir NA, Wendorf M, Perez R and Beaudry RM, Chlorophyll fluorescence as affected by some superficial defects in stored apples. J Hortic Sci Biotechnol, **73**: 846-850 (1998).

Misra AN, Misra M and Singh R, Chlorophyll fluorescence in plant biology. In: Biophysics, ed. by Misra AN. InTech, Rijeka, Croatia, pp. 171-192 (2012).

Moftah AE and Al-Humaid ARI, Effects of kaolin and Pinolene film-forming polymers on water relations and photosynthetic rate of tuberose (Polianthes tuberosa L.) plants under water deficit conditions. J Appl Hortic, **6**: 16-22 (2004).

Moftah AE and Al-Humaid ARI, Effects of antitranspirants on water relations and photosynthetic rate of cultivated tropical plant (Polianthes tuberosa L.). Pol J Ecol, **53**: 165-175 (2005).

Mogul M, Akin H, Hasirci N, Trantolo D, Gresser J and Wise D, Controlled release of biologically active agents for purposes of agricultural crop management. Resour Conserv Recycl, **16**: 289-320 (1996).

Monkiedje A and Spiteller M, Sorptive behavior of the phenylamide fungicides, mefenoxam and metalaxyl, and their acid metabolite in typical Cameroonian and German soils. Chemosphere, **49**: 659-668 (2002).

Montanari T and Busca G, On the mechanism of adsorption and separation of CO_2 on LTA zeolites: an IR investigation. Vib Spectrosc, **46**: 45-51 (2008).

Moreno SC, Carvalho GA, Picanco MC, Morais EGF and Pereira RM, Bioactivity of compounds from Acmella oleracea against *Tuta* absoluta (Meyrick) (Lepidoptera: Gelechiidae) and selectivity to two non-target species. Pest Manag Sci, **68**: 386-393 (2012).

Morgan JA, Pataki DE, Körner C, Clark H, Del Grosso SJ, Grünzweig JM, Knapp AK, Mosier AR, Newton PCD, Niklaus PA, Nippert JB, Nowak RS, Parton WJ, Polley HW and Shaw MR, Water relations in grassland and desert ecosystems exposed to elevated atmospheric CO_2. Oecol, **140**: 11-25 (2004).

Mota-Sanchez D, Bills SP and Whalon ME, Arthropod resistance to pesticides: status and overview. In: Pesticides in agriculture and the environment, ed. by Wheeler WB. Marcel Decker, Gainesville, Florida, pp. 241-272 (2002).

Mota-Sanchez D, Hollingworth RM, Grafius EJ and Moyer DD, Resistance and cross-resistance to neonicotinoid insecticides and spinosad in the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae). Pest Manag Sci, **62**: 30-37 (2006).

Mravec D, Hudec J and Janotka I, Some possibilities of catalytic and noncatalytic utilization of zeolites. Chem Pap, **59**: 62-69 (2005).

Mumpton FA, La roca magica: uses of natural zeolites in agriculture and industry. Proc Natl Acad Sci USA, **96**: 3463-3470 (1999).

Munoz-Pallares J, Corma A, Primo J, Primo-Yufera E, Zeolites as pheromone dispensers. J Agric Food Chem, **49**: 4801-4807 (2001).

Murray LJ, Dinca M and Long JR, Hydrogen storage in metal-organic frameworks. Chem Soc Rev, **38**: 1294-1314 (2009).

Nation JL, Reproduction. In: Insect physiology and biochemistry, ed. by Nation JL. CRC Press, Boca Raton, Florida, pp. 497-498 (2008).

Naumburg E, Housman DC, Huxman TE, Charlet TN, Loik ME and Smith SD, Photosynthetic responses of Mojave Desert shrubs to free air CO_2 enrichment are greatest during wet years. Global Change Biol, **9**: 276-285 (2003).

Neinhuis C and Barthlott W, Seasonal changes of leaf surface contamination in beech, oak, and ginkgo in relation to leaf micromorphology and wettability. New Phytol, **138**: 91-98 (1998).

Ng EP and Mintova S, Nanoporous materials with enhanced hydrophilicity and high water sorption capacity. Micropor Mesopor Mat, **114**: 1-26 (2008).

Nicot PC and Baille A, Integrated control of *Botrytis cinerea* on greenhouse tomatoes. In: Aerial plant surface microbiology, ed. by Morris CE, Nguyen-The C and Nicot PC. Plenum Press, New York, New York, pp. 169-189 (1996).

Nicot PC, Mermier M, Vaissiere BE and Lagier J, Differential spore production by *Botrytis cinerea* on agar medium and plant tissue under near-ultraviolet lightabsorbing polyethylene film. Plant Dis, **80**: 555-558 (1996).

Nielsen AT, Tolker-Nielsen T, Barken KB and Molin S, Role of commensal relationships on the spatial structure of a surface-attached microbial consortium. Environ Microbiol, **2**: 59-68 (2000).

Nikolov AD, Wasan DT, Chengara A, Koczo K, Policello GA and Kolossvary I, Superspreading driven by Marangoni flow. Adv Colloid Interface Sci, **96**: 325-338 (2002).

Nobels I, Dardenne F, de Coen W and Blust R, Application of a multiple endpoint bacterial reporter assay to evaluate toxicological relevant endpoints of perfluorinated compounds with different functional groups and varying chain length. Toxicol in Vitro, **24**: 1768-1774 (2010).

Nobels I, Spanoghe P, Heasaert G, Robbens J and Blust R, Toxicity ranking and toxic mode of action evaluation of commonly used agricultural adjuvants on the basis of bacterial gene expression profiles. PLoS ONE, **6**: 1-10 (2011).

Norhisham AR, Abood F, Rita M and Hakeem KR, Effect of humidity on egg hatchability and reproductive biology of the bamboo borer (*Dinoderus minutus* Fabricius). Springerplus, **2**: 1-9 (2013).

Norit Americas Inc (Norit), Datasheet: DARCO_ KB-WJ (2009a). Available at: http://www.norit-americas.com (Accessed: May 2012).

Norit Americas Inc (Norit), Datasheet: DARCO_ KB-G (2009b). Available at: http://www.norit-americas.com (Accessed: May 2012).

Norit Americas Inc (Norit), Datasheet: GCN 1240 (2011a). Available at: http://www.noritamericas.com (Accessed: May 2012).

Norit Americas Inc (Norit), Datasheet: GAC 1240 (2011b). Available at: http://www.noritamericas.com (Accessed: May 2012).

Norit Americas Inc (Norit), Datasheet: C GRAN (2011c). Available at: http://www.noritamericas.com (Accessed: May 2012).

Norris LA, Behavior of pesticides in plants. In: Symp on environmental and physiologic chemodynamics, 9 January, Corvallis, Oregon (1969).

Nowak RS, Ellsworth DS and Smith SD, Functional responses of plants to elevated atmospheric CO_2 - do photosynthetic and productivity data from FACE experiments support early predictions? New Phytol, **162**: 253-280 (2004).

O'Keeffe M, Peskov MA, Ramsden SJ and Yaghi OM, The reticular chemistry structure resource (RCSR) database of, and symbols for, crystal nets. Acc Chem Res, **41**: 1782-1789 (2008).

Oleszczuk P, Phytotoxicity of municipal sewage sludge composts related to physico-chemical properties, PAHs and heavy metals. Ecotoxicol Environ Safety, **69**: 496-505 (2008a).

Oleszczuk P, The toxicity of composts from sewage sludges evaluated by the direct contact tests phytotoxkit and ostracodtoxkit. Waste Management, **28**: 1645-1653 (2008b).

Olsen DH, Haag WO and Borghard WS, Use of water as a probe of zeolitic properties: interaction of water with HZSM-5. Micropor Mesopor Mat, **35-36**: 435-446 (2000).

Olvera-Gonzalez E, Alaniz-Lumbrerass D, Ivanov-Tsonchev R, Villa-Hernandez J, de la Rosa-Vargas I, Lopez-Cruz I, Silos-Espino H and Lara-Herrera A, Chlorophyll fluorescence emission of tomato plants as a response to pulsed light based LEDs. Plant Growth Regul, **69**: 117-123 (2013).

Omri, Surround WP crop protectant (2004). Availabe: http://www.omri.org (Accessed: June 2016).

Ooijkaas LP, Tramper J, Buitelaar RM. Biomass estimation of Coniothyrium minitans in solid-state fermentation. Enzyme Microb Technol, **22**: 480-486 (1998).

Ooijkaas LP, Fungal biopesticide production by solid state fermentation, growth and sporulation of Coniothyrium minitans. PhD thesis, Wageningen University, the Netherlands (2000).

Opanasenko M, Dhakshinamoorthy A, Shamzhy M, Nachtigall P, Horacek M, Garciac H and Cejka J, Comparison of the catalytic activity of MOFs and zeolites in Knoevenagel condensation. Catal Sci Technol, **3**: 500-507 (2013).

Orser CS, Capaldi SR, Foong FCF and Farr SB, Use of prokaryotic stress promoters as indicators of the mechanisms of chemical toxicity. In Vitro Toxicol, **8**: 71-85 (1995).

Ozone Biotech, Ozoneem Trishul (2012). Availabe: http://www.ozonebiotech.com (Accessed: June 2016).

PAN, Alphabetic list of all chemicals in the PAN pesticide database (2014). Available at: http://www.pesticideinfo.org (Accessed: December 2015).

Pandey A, Soccol CR and Larroche C, General considerations about solid-state fermentation processes. In: Current developments in solid-state fermentation, ed. by Pandey A, Soccol CR and Larroche C. Springer, New York, New York, pp. 13-25 (2008a).

Pandey A, Soccol CR and Larroche C, Introduction. In: Current developments in solid-state fermentation, ed. by Pandey A, Soccol CR and Larroche C. Springer, New York, New York, pp. 3-12 (2008b).

Pasqualini E, Civolani S and Grappadelli LC, Particle film technology: approach for a biorational control of Cacopsylla pyri (Rhynchota Psyllidae) in Northern Italy. Bull Insectol, **55**: 39-42 (2002).

Paul D, Pandey G, Pandey J and Jain RK, Accessing microbial diversity for bioremediation and environmental restoration. Trends Biotechnol, **23**: 135-142 (2005).

Pavelic KI and Hadzija M, Medical applications of zeolites. In: Handbook of zeolite science and technology, ed. by Auerbach SM, Carrado KA and Dutta PK. Marcel Dekker, New York, New York, pp. 1141-1173 (2003).

Payra P and Dutta PK, Zeolites: a primer. In: Handbook of zeolite science and technology, ed. by Auerbach SM, Carrado KA and Dutta PK. Marcel Dekker, New York, New York, pp. 1-19 (2003).

Penner D, Activator adjuvants. Weed Technol, **14**: 785-791 (2000).

Peralta D, Chaplais G, Simon-Masseron A, Barthelet K, Chizallet C, Quoineaud AA and Pirngruber GD, Comparison of the behavior of metal-organic frameworks and zeolites for hydrocarbon separations. J Am Chem Soc, **134**: 8115-8126 (2012).

Percival GC and Boyle S, Evaluation of film forming polymers to control apple scab (*Venturia inaequalis* (Cooke) G. Wint.) under laboratory and field conditions. Crop Prot, **28**: 30-35 (2009).

Perez-Caballero R, Gil J, Benitez J and Gonzalez L, The effect of adding zeolite to soils in order to improve the N-K nutrition of olive trees. Preliminary results. Am J Agric Biol Sci, **2**: 321-324 (2008).

Phillips C, Keeping forests healthy-good management not good luck. Australian forest growers conference, Mount Gambier, Australia (2010).

Pieterse CMJ, Leon-Reyes A, Van der Ent S and Van Wees SCM, Networking by small-molecule hormones in plant immunity. Nat Chem Biol, **5**: 308-316 (2009).

Plaza G, Nalecz-Jawecki G, Ulfig K and Brigmon R, The application of bioassays as indicators of petroleum-contaminated soil remediation. Chemosphere **59**: 289-296. (2005).

Porcel M, Cotes B and Campos M, Biological and behavioural effects of kaolin particle film on larvae and adults of Chrysoperla carnea (Neuroptera: Chrysopidae). Biol Control, **59**: 98-105 (2011).

Potter KA, Davidowitz G and Woods HA, Insect eggs protected from high temperatures by limited homeothermy of plant leaves. J Exp Biol, **212**: 3448-3454 (2009).

PPDB, Global availability of information on agrochemicals (2014). Available at: http://sitem.herts.ac.uk (Accessed: 1 June 2015).

Puterka GJ, Glenn DMM, Sekutowski DG, Unruh TR and Jones SK, Progress toward liquid formulations of particle films for insect and disease control in pear. Environ Entomol, **29**: 329-339 (2000).

Pyke K, Mesophyll. In: Encyclopedia of life sciences: handbook of plant science, ed. by Roberts K. Wiley and Sons, Chichester, UK, pp. 168-170 (2007).

Qi-Long Z and Qiang X, Metal-organic framework composites. Chem Soc Rev, **43**: 5468-5512 (2014).

Qin QD, Ma J and Liu K, Adsorption of anionic dyes on ammonium-functionalized MCM-41. J Hazard Mater, **162**: 133-139 (2009).

Ramesh K, Biswas AK, Somasundaram J and Rao AS, Nanoporous zeolites in farming: current status and issues ahead. Curr Sci, **99**: 760-764 (2010).

Ramesh K, Reddy DD, Biswas AK and Rao AS, Zeolites and their potential use in agriculture. In: Advances in agronomy, ed. by Sparks DL. Elsevier, San Diego, California, pp. 215-236 (2011).

Raven PH, Evert RF and Eichhorn SE, Biology of plants. W.H. Freeman and Company Publishers, New York, 686 p. (2005).

Ravier I, Haouisee E, Clement M, Seux R and Briand O, Field experiments for the evaluation of pesticide spray-drift on arable crops. Pest Manag Sci, **61**: 728-736 (2005).

Reddy P, Disguising the leaf surface. In: Recent advances in crop protection, ed. by Reddy P. Springer, New York, New York, pp. 91-102 (2012).

Reiley HE and Shry CL, Introductory horticulture. Delmar, New York, New York, 560 p. (2002).

Rennie TJ, Vigneault C, Raghavan GSV and DeEll JR, Effects of pressure reduction rate on vacuum cooled lettuce quality during storage. Can Biosyst Eng, **43**: 3.39-3.43 (2001).

Rezaei F and Webley P, Optimum structured adsorbents for gas separation processes. Chem Eng Sci, **64**: 5182-5191 (2009).

Robbens J, Dardenne F, Devriese L, De Coen W and Blust R, *Escherichia coli* as a bioreporter in ecotoxicology. Appl Microbiol Biotechnol, **88**: 1007-1025 (2010).

Rodgers PB, Potential of biopesticides in agriculture. Pestic Sci, **39**: 117-129 (1993).

Roditakis E, Vasakis E, Grispou M, Stavrakaki M, Nauen R, Gravouil M and Bassi A, First report of *Tuta absoluta* resistance to diamide insecticides. J Pest Sci, **88**: 9-16 (2015).

Rogers A, Ainsworth EA and Kammann C, Perspectives of the future of free air CO_2 enrichment studies. In: Managed ecosystems and CO_2: case studies, processes and perspectives, ed. by Nösberger J, Long SP, Norby RJ, Stitt M, Hendrey GR and Blum H. Springer, Heidelberg, Germany, pp. 431-450 (2006).

Rogiers SY, Whitelaw-Weckert M, Radovanovic-Tesic M, Greer LA, White RG and Steel CC, Effects of spray adjuvants on grape (Vitis vinifera) berry microflora, epicuticular wax and susceptibility to infection by *Botrytis cinerea*. Australas Plant Pathol, **34**: 221-228 (2005).

Rokade YB and Sayyed RZ, Naphthalene derivatives: a new range of antimicrobials with high therapeutic value. Rasayan J Chem, **2**: 972-980 (2009).

Rombouts K, Possibilities of systemic RNAi by feeding experiments in Colorado potato beetle. Master thesis, Ghent University, Belgium (2011).

Romero E, Salido A, Cifuentes C, Fernandez JD and Nogales R, Effect of vermicomposting process on pesticide sorption capability using agro-industrial wastes. Int J Environ Anal Chem, **86**: 289-297 (2006).

Rosati A, Metcalf SG, Buchner RP, Fulton AE and Lampinen BD, Effects of kaolin application on light absorption and distribution, radiation use efficiency and photosynthesis of almond and walnut canopies. Ann Bot, **99**: 255-263 (2007).

Rossi V, Ponti I, Marinelli M, Giosuè S and Bugiani R, Environmental factors influencing the dispersal of *Venturia inaequalis* ascospores in the orchard air. J Phytopath, **149**: 11-19 (2001).

Rosslenbroich HJ and Stuebler D, *Botrytis cinerea* - history of chemical control and novel fungicides for its management. Crop Prot, **19**: 557-561 (2000).

Rouquerol F, Rouquerol J, Sing KSW, Llewellyn P and Maurin G, Adsorption by powder and porous solid. Elsevier, Oxford, UK, 646 p. (2014).

Ryckaert B, Spanoghe P, Haesaert G, Heremans B, Isebaert S and Steurbaut W, Quantitative determination of the influence of adjuvants on foliar fungicide residues. Crop Prot, **26**: 1589-1594 (2007).

Saenger P, Mangrove ecology silviculture and conservation. Kluwer Academic Publishers, Dordrecht, the Netherlands, 363 p. (2002).

Sah A, Precursor film: a key driver to determine wetting behaviour in the vicinity of surface heterogeneity. Soft Matter, **10**: 3890-3896 (2014).

Sakuth M, Meyer J and Gmehling J, Measurement and prediction of binary adsorption equilibria of vapors on dealuminated Y-zeolites (DAY). Chem Eng Process, **37**: 267-277 (1998).

Salvucci ME and Crafts-Brandner SJ, Inhibition of photosynthesis by heat stress: the activation state of Rubisco as a limiting factor in photosynthesis. Physiol Plant, **120**: 179-186 (2004).

Samaras P, Papadimitriou C, Haritou I and Zouboulis A, Investigation of sewage sludge stabilization potential by the addition of fly ash and lime. J Hazard Mater, **154**: 1052-1059 (2008).

Sanchez Y and Lindquist SL, HSP104 required for induced thermotolerance. Science, **248**: 1112-1115 (1990).

Savonnet M, Kockrick E, Camarata A, Bachi DB, Bats N, Lecocq V, Pinel C and Farrusseng D, Combinatorial synthesis of metal-organic frameworks libraries by click-chemistry. N J Chem, **35**: 1892-1897 (2011).

Schrader L, Zhang J and Sun J, Environmental stresses that cause sunburn of apple. Acta Hort, 618, 397-405 (2003).

Schroeder JI, Allen GJ, Hugouvieux V, Kwak JM and Waner D, Guard cell signal transduction. Annu Rev Plant Physiol Plant Mol Biol, **52**: 627-658 (2001).

Schulze ED, Lange OL, Buschbom U, Kappen L and Evenari M, Stomatal response to changes in humidity in plants growing in the desert. Planta, **108**: 259-270 (1972).

Schulze ED, Beck E and Müller HK, Plant Ecology. Springer, Berlin, Germany, 702 p. (2005).

Schüth F, Ordered mesoporous materials - State of the art and prospects. In: Zeolites and mesoporous materials at the dawn of the 21st century, ed. by Galarneau A, Di Renzo F, Fajula F and Vedrine J. Elsevier, Amsterdam, the Netherlands, pp. 1-12 (2001).

Schwabe WFS, Changes in scab susceptibility of apple leaves as influenced by age. Phytophylactica, **11**: 53-56 (1979).

Schwabe WFS, Jones AL and Jonker JP, Changes in the susceptibility of developing apple fruit to *Venturia inaequalis*. Phytopathology, **74**: 118-121 (1984).

Schwarzenbach RP, Gschwend PM and Imboden DM, Environmental organic chemistry. John Wiley and Sons, Edison, New Jersey, 1328 p. (2003).

Scott WJ, Water relations of Staphylococcus aureus at 30°C. Aust J Biol Sci, **6**: 549-564 (1953).

Scott WJ, Water relations of food spoilage microorganisms. Adv Food Res, **7**: 83-127 (1957).

Senkovska I, Hoffmann F, Fröba M, Getzchmann J, Winfried B and Kaskei S, New highly porous aluminium based metal-organic frameworks: Al(OH)**(nds)** (ndc = 2,6-naphthalene dicarboxylate) and Al(OH)(bpdc) (bpdc = 4,40-biphenyl dicarboxylate). Micropor Mesopor Mat, **122**: 93-98 (2009).

Seplyarsky V, Weiss M and Haberman A, *Tuta absoluta* Povolny (Lepidoptera: Gelechiidae), a new invasive species in Israel. Phytoparasitica, **38**: 445-446 (2010).

Shakarova D, Ojuva A, Bergström L and Akhtar F, Methylcellulose-directed synthesis of nanocrystalline zeolite NaA with high CO_2 uptake. Mater, **7**: 5507-5519 (2014).

Shang C and Arshad MA, Sorption of clopyralid, dicamba and MCPA by two soils with conventional and no-till management. Can J Soil Sci*,* **78**: 181-186 (1998).

Shanjiao K, Yanjun G, Tao D, Ying Z and Yanying Z, Preparation and characterization of zeolite beta with low SiO_2/Al_2O_3 ratio. Pet Sci, **4**: 70-78 (2007).

Shapiro AM and De Vay JE, Hypersensitivity reaction of *Brassica nigra* L. (Cruciferae) kills eggs of Pieris butterflies (Lepidoptera: Pieridae). Oecol, **71**: 631-632 (1987).

Sheaffer CC and Moncada MK, Plant disease and insects. In: Introduction to agronomy: food, crops and environment, ed. by Sheaffer CC and Moncada MK. Delmar Cengage Learning, New York, New York, pp. 363-392 (2009).

Shindo S, Takata S, Taguchi H and Yoshimura N, Development of novel carrier using natural zeolite and continuous ethanol fermentation with immobilized Saccharomyces cerevisiae in a bioreactor. Biotechnol Lett, **23**: 2001-2004 (2001).

Sigma Aldrich, Basolite F300 (2012a). Available at: http://www.sigmaaldrich.com (Accessed: May 2012).

Sigma Aldrich, Basolite C300 (2012b). Available at: http://www.sigmaaldrich.com (Accessed: May 2012).

Silva GA, Picanço MC, Bacci L, Crespo ALB, Rosado JF and Guedes RNC, Control failure likelihood and spatial dependence of insecticide resistance in the tomato pinworm, *Tuta absoluta*. Pest Manag Sci, **67**: 913-920 (2011).

Singh BK, Walker A and Wright DJ, Degradation of chlorpyrifos, fenamiphos, and chlorothalonil alone and in combination and their effects on soil microbial activity. Environ Toxicol Chem, 21: 2600-2605 (2002).

Sinha RK, Photosynthesis. In: Modern plant physiology, ed. by Sinha RK. Alpha Science International, Pangbourne, UK, pp. 176-255 (2004).

Siqueira HAA, Guedes RNC and Picanço MC, Cartap resistance and synergism in populations of *Tuta absoluta* (Lep., Gelechiidae). J Appl Entomol, **124**: 233-238 (2000).

Siqueira HAA, Guedes RNC and Picanço MC, Insecticide resistance in populations of *Tuta absoluta* (Lepidoptera: Gelechiidae). Agric For Entomol, **2**: 147-153 (2001a).

Siqueira HAA, Guedes RNC, Fragoso DB and Magalhaes LC, Abamectin resistance and synergism in Brazilian populations of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Int J Pest Manage, **47**: 247-251 (2001b).

Sitea AD, Factors affecting sorption of organic compounds in natural sorbent/water systems and sorption coefficients for selected pollutants: a review. J Phys Chem, **30**: 187-439 (2001).

Skuterud R, Nordby A and Tyldum A, Effects of application methods, spray volumes, pressures and herbicide rates on weed control in spring cereals. Crop Prot, **7**: 303-308 (1988).

Smereka KJ, MacHardy WE and Kausch AP, Cellular differentiation in *Venturia inaequalis* ascospores during germination and penetration of apple leaves. Can J Bot, **65**: 2549-2561 (1987).

Smith EH and Salkeld EH, The use and action of ovicides. Annu Rev Entomol, **11**: 331-368 (1966).

Smith BT and Walker GC, Mutagenesis and more: umuDC and the *Escherichia coli* SOS response. Genetics, **148**: 1599-1610 (1998).

Somervaille A, Betts G, Gordon B, Green V, Burgis M and Henderson R, Adjuvants - Oils, surfactants and other additives for farm chemicals. Grains Research & Development Corporation, Kingston, Australian Capital Territory, 52 p. (2012).

Song W, Justice RE, Jones CA, Grassian VH and Larsen SC, Size-dependent properties of nanocrystalline silicalite synthesized with systematically varied crystal sizes. Langmuir, **20**: 4696-4702 (2004a).

Song W, Justice RE, Jones CA, Grassian VH and Larsen SC, Synthesis, characterization, and adsorption properties of nanocrystalline ZSM-5. Langmuir, **20**: 8301-8306 (2004b).

Sopkova A and Janokova E, An insecticide stabilized by natural zeolite. J Therm Anal Calorim, **53**: 477-485 (1998).

Sotelo-Cuitiva YM, Restrepo-Diaz H, Garcia-Castro A, Ramirez-Godoy A and Florez-Roncancio VJ, Effect of kaolin film particle applications (Surround WP®) and water deficit on physiological characteristics in Rose cut plants (Rose spp L.). Am J Plant Sci, **2**: 354-358 (2011).

Spanoghe P, Effect van additieven en adjuvantia op de efficiëntie van de spuittoepassing van gewasbeschermingsmiddelen. PhD thesis, Ghent University, Belgium (2005).

Spanoghe P, De Schampheleire M, Van der Meeren P and Steurbaut W, Influence of agricultural adjuvants on droplet spectra. Pest Manag Sci, **63**: 4-16 (2007).

Spanoghe P, Dynamica van residu's en pesticiden. Course at Ghent University, Faculty of Bio-science Engineering, Belgium (2014).

Srinivasan R, Hoy MA, Singh R and Rogers ME, Laboratory and field evaluations of Silwet L-77 and Kinetic alone and in combination with imidacloprid and abamectin for the management of the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae). Florida Entomol, **91**: 87-100 (2008).

Srivastava B, Jhelum V, Basu DD and Patanjali PK, Adsorbents for pesticide uptake from contaminated water: a review. J Sci Ind Res India, **68**: 839-850 (2009).

Städler E, Plant chemicals important for egg deposition in herbivorous insects. In: Chemoecology of insect eggs and egg deposition, ed. by Hilker M and Meiners T. Blackwell Publishing Ltd, Berlin, Germany, pp. 171-197 (2002).

Stainier C, Lebeau F, Straet S, Phaleg V, Destain M-F and Schiffers B, Study by image analysis and laser-based droplet size distribution measurement of the influence of eight adjuvants on the spray formation for three agricultural spray nozzles. AFPP, Orléans, France (2004).

Steiman SR and Bittenbender HC, Analysis of kaolin particle film use and its application on coffee. HortScience, **42**: 1605-1608 (2007).

Steppe K, Diurnal dynamics of water flow through trees: design and validation of a mathematical flow and storage model. PhD thesis, Ghent University, Belgium (2004).

Sterk G, Heuts F, Merck N and Bock J, Sensitivity of non-target arthropods and beneficial fungal species to chemical and biological plant protection products: results of laboratory and semi-field trials. In: Proceedings of the 1st International Symposium on biological control of arthropods, ed. by van Driesche RG. Honolulu, Hawaii, pp. 306-313 (2002).

Steurbaut W, Spanoghe P, De Jaeger D and Decadt G, Screening method for the evaluation of adjuvants and additives for fungicides. In: Proceedings of 6th International Symposium on adjuvants for agrochemicals, ed. by de Ruiter H. ISAA, Amsterdam, the Netherlands, pp. 339-348. (2001a).

Steurbaut W, De Smet B and Vercruysse F, Dispersion of plant protection products. In: MIRA-T 2001. Report on the environment and nature in Flanders: scenarios. VMM, Aalst, Belgium (2001b).

Sundaram A, Understanding volatilities of forestry spray mixtures from their viscosities and viscosity-temperature relationships. In: Pesticide formulations and application systems: 5th vol, ASTM special technical publication 915, ed. by Spicer LD and Kaneko TM. American society for testing and materials, West Conshohocken, Pennsylvania, pp. 37-55 (1986).

Surgan M, Condon M and Cox C, Pesticide risk indicators: unidentified inert ingredients compromise their integrity and utility. Environ Manage, **45**: 834-841 (2010).

Taiz L and Zeiger E, Chapter 4: Water balance of plants. In: Plant Physiology (fourth edition). Sinauer Associates, Sunderland, UK, pp. 71-90 (2006).

Taiz L and Zeiger E, Chapter 7: Photosynthesis: the light reactions. In: Plant Physiology (fifth edition). Sinauer Associates, Sunderland, UK, pp. 163-198 (2010).

Tartaglia LA, Storz G and Ames BN, Identification and molecular analysis of oxyR-regulated promoters important for the bacterial adaptation to oxidative stress. J Mol Biol, **210**: 709-719 (1989).

Taylor-Pashow KML, la Rocca J, Xie ZG, Tran S and Lin WB, Post-synthetic modifications of iron-carboxylate nanoscale metal-organic frameworks for imaging and drug delivery. J Am Chem Soc, **131**: 14261-14262 (2009).

Teejet, Catalogus 51 NL - Leiders in precisie, spuit componenten, besturingssysteemtechnologie en beheer van bespuitingsgegevens (2011). Available at: http://www.teejet.com (Accessed: April 2014).

Teejet, Technical information (2014). Available at: http://www.teejet.com (Accessed: April 2014).

Temerak SA, Ovicidal activity of the natural bio-product spinosad through field observation of tagged egg masses of *Spodoptera littoralis* on cotton in five Governorates of Egypt Assiut. J Agric Sci, **36**: 85-95 (2005).

Teng H and Hsieh CT, Influence of surface characteristics on liquid-phase adsorption of phenol by activated carbons prepared from bituminous coal. Ind Eng Chem Res, **37**: 3618-3624 (1998).

Tomé HVV, Cordeiro EMG, Rosado JF and Guedes RNC, Egg exposure to pyripoxyfen in the tomato leaf miner *Tuta absoluta*: ovicidal activity or behavioural-modulated hatching mortality? Ann Appl Biol, **160**: 35-42 (2012).

Tomerlin JR and Jones AL, Development of apple scab on fruity in the orchard and during cold storage. Plant Dis, **67**: 147-150 (1983).

Toole G and Toole S, Part 2: Photosynthesis. In: Essential A2 biology for OCR, ed. by Toole G and Toole S. Nelson Thornes, Cheltenham, UK, pp. 19-31 (2004).

Torrado A and Valiente M, The effect of resin particle size on the rate of ion release: interactions in mixed bed systems. Anal Bioanal Chem, **2378**: 205-213 (2004).

Tosheva L and Valtchev VP, Nanozeolites: synthesis, crystallization mechanism, and applications. Chem Mater, **17**: 2494-2513 (2005).

Trisyono A, Puttler B and Chippendale GM, Effect of the ecdysone agonists, methoxyfenozide and tebufenozide, on the lady beetle, *Coleomegilla maculata*. Entomol Exp Appl, **94**: 103-105 (2000).

Tropea Garzia G, Siscaro G, Biondi A and Zappalà L, *Tuta absoluta*, a South American pest of tomato now in the EPPO region: biology, distribution and damage. EPPO Bull, **42**: 205-210 (2012).

Tu M and Randall JM, Chapter 8: Adjuvants. In: Weed control methods handbook - tools and techniques for use in natural areas, ed. by Tu M, Hurd C and Randall JM. Utah State University, Utah, pp. 172-196 (2001).

Tulod AM, Castillo ASA, Carandang WM and Pampolina NM, Growth performance and phytoremediation potential of Pongamia pinnata (L.) Pierre, Samanea saman (Jacq.) Merr. and Vitex parviflora Juss. in copper-contaminated soil amended with zeolite and VAM. Asia Life Sci, **21**: 499-522 (2012).

Turechek WW, Apple diseases and their management. In: Diseases of fruits and vegetables. Volume I. Diagnosis and management, ed. by Naqvi SAMH. Kluwer Academic Publishers, Dordrecht, the Netherlands, pp. 1-108 (2004).

Tuzet A, Perrier A and Leaning R, A coupled model of stomatal conductance, photosynthesis and transpiration. Plant Cell Environ, **26**: 1097-1116 (2003).

Tzia C and Zorpas A, Zeolites in food processing industries. In: Handbook of natural zeolites, ed. by Inglezakis VJ and Zorpas AA. Bentham Science Publishers, Sharjah, Dubai, pp. 601-651 (2012).

UNFPA, State of World population 2007: unleashing the potential of urban growth. New York, New York, 50 p. (2007).

Uniqema, Creating your advantage in crop protection. Customer seminar at Santa Marta hotel, Barcelona, Spain (2000).

Unruh TR, Knight AL, Upton J, Glenn DM and Puterka GJ, Particle films for suppression of the codling moth (Lepidoptera: Tortricidae) in apple and pear orchards. J Econ Entomol, **93**: 737-743 (2000).

Urbaneja A, González-Cabrera J, Arnó J and Gabarra R, Prospects for the biological control of *Tuta absoluta* in tomatoes of the Mediterranean basin. Pest Manag Sci, **68**: 1215-1222 (2012).

Urbaneja A, Montón H and Mollá O, Suitability of the tomato borer *Tuta absoluta* as prey for *Macrolophus pygmaeus* and *Nesidiocoris tenuis*. J Appl Entomol, **133**: 292-296 (2009).

USDA, New pest response guidelines tomato leafminer (*Tuta absoluta*) (2011). Available at: https://www.aphis.usda.gov (Accessed: September 2015).

US-EPA, PR notice 97-6: Use of the term ''inert'' in the label ingredients statement (1997). Available at: http://www.epa.gov (Accessed: December 2015).

US-EPA, 456/F-99-004: Choosing an adsorption system for VOC: carbon, zeolite, or polymers? (1999). Available at: http://www.epa.gov (Accessed: March 2012).

US-FDA, Database of select committee on GRAS substances (SCOGS) reviews (2006). Available at: http://www.accessdata.fda.gov (Accessed: December 2015).

Valtchev VP, Tosheva L and Bozhilov KN, Synthesis of zeolite nanocrystals at room temperature. Langmuir, **21**: 10724-10729 (2005).

Van Damme V, Moerkens R, Beckmoes E, Wittemans L, De Vis R, Beck B, Nuyttens D, Casteels H, Tirry L and De Clercq P, Control of the invasive leafminer Tuta absoluta by leaf application of entomopathogenic nematodes. IOBC-WPRS Bull, **102**: 219-219 (2014).

van den Bergh J, Nishiyama N and Kapteijn F, Zeolite membranes in catalysis: what is new and how bright is the future? In: Novel concepts in catalysis and chemical reactors: improving the efficiency for the future, ed. by Cybulski A, Moulijn JA and Stankiewicz A. John Wiley and Sons, Weinheim, Germany, pp. 211-238 (2010).

Van Der Walt A, Du Plessis H and Van den Berg J, Using morphological characteristics to distinguish between male and female larvae and pupae of the groundnut leafminer, *Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae). Crop Prot, **25**: 182-184 (2008).

Van Kan JAL, Licensed to kill: the lifestyle of a necrotrophic plant pathogen. Trends Plant Sci, **11**: 247-253 (2006).

Vargas H, Observaciones sobre la biologia enemigos naturales de las polilla del tomate, *Gnorimoschema absoluta* (Meyrick). Idesia, **1**:75-110 (1970).

Verliefde A, Saveyn H and Van der Meeren P, Milieutechnologie: water. Course at Ghent University, Faculty of Bio-science Engineering, Belgium (2011).

Verma LR and Sharma RC, Diseases of horticultural crops: fruits. Indus Publishing Company, New Delhi, India, 718 p. (1999).

Virag D, naar Z and Kiss A, Microbial toxicity of pesticide derivatives produced with UV-photodegradation. Bull Environ Contam Toxicol, **79**: 356-359 (2007).

Virta RL, US Geological survey minerals yearbook: Zeolites (2001). Available at: http://minerals.usgs.gov (Accessed: February 2016).

Volker K, Surround for insect management and protection against heat and light stress (2014). Available at: http://novasource.com (Accessed: June 2016).

Vu JCV, Yelenosky G and Bausher MG, CO_2 exchange rate, stomatal conductance, and transpiration in attached leaves of 'Valencia' orange. HortScience, **21**: 143-144 (1986).

Wagholika SG, Mayadevi S, Mirajkar SP and Sivasanker S, Rearrangement of allyl aryl ethers over zeolites. In: Studies in surface and catalysis: recent advances in the science and technology of zeolites and related materials, ed. by van Steen E, Claeys M and Callanan LH. Elsevier, Cape Town, South Africa, pp. 2731-2738 (2004).

Walke CJ, Life in the phyllosphere. Maine organic farmers and gardeners association (2015). Available at: http://www.mogfa.org (Accessed: December 2015).

Walker DA and Herold A, Can the chloroplast support photosynthesis unaided? Plant Cell Physiol, **51**: 295-310 (1977).

Walsh BD, The new potato bug, and its natural history. Practical Entomologist, **1**: 1-4 (1865).

Walters DR, Disguising the leaf surface: the use of leaf coatings for plant disease control. Eur J Plant Pathol, **114**: 255-260 (2006).

Walters DR, Control of plant disease by disguising the leaf surface. In: Disease control in crops: biological and environmentally friendly approaches, ed. by Walters D. Wiley-Blackwell, Oxford, UK, pp. 237-245 (2009).

Walters DR, Growth, development and yield of infected and infested plants and crops. In: Physiological responses of plants to attack, ed. by Walters DR. John Wiley and Sons, Chichester, UK, pp. 24-40 (2015).

Wang A, Korotev RL, Jolliff BL, Haskin LA, Crumpler L, Farrand WH, Herkenhoff KE, de Souza Jr P, Kusack AG, Hurowitz JA and Tosca NJ, Evidence of phyllosilicates in Wooly Patch, an altered rock encountered at West Spur, Columbia Hills, by the Spirit rover in Gusev crater, Mars. J Geophys Res: Planets, **111**: 1-22 (2006).

Wang CJ and Liu ZQ, Foliar uptake of pesticides - present status and future challenge. Pestic Biochem Physiol, **87**: 1-8 (2007).

Wang CF, Li HS, Sun X, Wang LJ and Sun XY, Evaluation of zeolites synthesized from fly ash as potential adsorbents for wastewater containing heavy metals. J Environ Sci, **21**: 127-136 (2009).

Wang SB and Peng YL, Natural zeolites as effective adsorbents in water and wastewater treatment. Chem Eng J, **156**: 11-24 (2010).

Weber DC and Ferro DN, Colorado potato beetle: diverse life history poses challenge to management. In: Advances in potato pest biology and management, ed. by Zehnder GW, Powelson ML, Jansson RK and Raman KV. APS Press, St. Paul, Minnesota, pp. 54-70 (1994).

Weber DC, Colorado beetle: pest on the move. Pestic Outlook, **14**: 256-259 (2003).

Weibezahn J, Tessarz P, Schlieker C, Zahn R, Maglica Z, Lee S, Zentgraf H, Weber-Ban EU, Dougan DA, Tsai FTF, Mogk A and Bukau B, Thermotolerance requires refolding of aggregated proteins by substrate translocation through the central pore of ClpB. Cell, **119**: 653-665 (2004).

Weiss S, Lebuhn M, Andrade D, Zankel A, Cardinale M, Birner-Gruenberger R, Somitsch W, Ueberbacher BJ and Guebitz GM, Activated zeolite-suitable carriers for microorganisms in anaerobic digestion processes? Appl Microbiol Biotechnol, **97**: 3225-3238 (2013).

Wells B and Fishel FM, Rainfastness of pesticides. UF/IFAS EDIS Publication PI-238 (2014). Available at: http://edis.ifas.ufl.edu (Accessed: January 2015).

Whalon ME, Hollingworth RM and Mota-Sanchez D, The MSU database of pesticide resistance (2005). Available at: http://www.pesticideresistance.org (Accessed: January 2015).

Whiting D, Insect anatomy and growth. CMG Garden notes 312, Colorado state university extension, 9 p. (2014).

Williamson B, Tudzynski B, Tudzynski P and Van Kan JAL, *Botrytis cinerea*: the cause of grey mould disease. Mol Plant Pathol, **8**: 1464-6722 (2007).

Wimpenny J, Manz W and Szewzyk U, Heterogeneity in biofilms. FEMS Microbiol Rev, **24**: 661-671 (2000).

Wolf RE, Prather DM and Shea FM, Growth-rate-dependent alteration of 6-phosphogluconate dehydrogenase and glucose 6-phosphate dehydrogenase levels in *Escherichia coli* K-12. J Bacteriol, **139**: 1093-1096 (1979).

Wolowicz A and Hubicki Z, Carbon-based adsorber resin Lewatit AF 5 applicability in metal ion recovery. Micropor Mesopor Mat, 224: 400-414 (2016).

Woods HA and Singer MS, Contrasting responses to desiccation and starvation by eggs and neonates of two lepidoptera. Physiol Biochem Zool, **74**: 594-606 (2001).

Woods HA, Water loss and gas exchange by eggs of *Manduca sexta*: trading off costs and benefits. J Insect Physiol, **56**: 480-487 (2010).

Wullschleger SD, Tschaplinski TJ and Norby RJ, Plant water relations at elevated CO_2 - implications for water-limited environments. Plant, Cell Environ, **25**: 319-331 (2002).

Wünsche JN, Lakso AN, Robinson T, Lenz F and Denning SS, The bases of productivity in apple production systems: the role of light interception by different shoot types. J Amer Soc Hort Sci, **121**: 886-893 (1996).

Wünsche JN, Lombardini L and Greer DH, Surround particle film applications - effects on whole canopy physiology of apple. Acta Hortic, **636**: 565-571 (2004).

Xu XM and Robinson J, Modelling the effects of wetness duration and fruit maturity on infection of apple fruits of Cox's Orange Pippin and two clones of Gala by *Venturia inaequalis*. Plant Pathol, **54**: 347-356 (2005).

Xu R, Pang W, Yu J, Huo O and Chen J, Chemistry of zeolites and related porous materials: synthesis and structure. In: Structural chemistry of microporous materials. John Wiley and Sons, Singapore, Malaysia, 616 p. (2007).

Yaghi OM and Li H, Hydrothermal synthesis of a metal-organic framework containing large rectangular channels. J Am Chem Soc, **117**: 10401-10402 (1995).

Yang C, Hamel C, Vujanovic V and Gan Y, Fungicide: modes of action and possible impact on nontarget microorganisms. ISRN Ecology, 2011: 1-8 (2011).

Yourman LF and Jeffers SN, Resistance to benzimidazole and dicarboximide fungicides in greenhouse isolates of *Botrytis cinerea*. Plant Dis, **83**: 569-575 (1999).

Yousef RI, El-Eswed B and Al-Muhtaseb AH, Adsorption characteristics of natural zeolites as solid adsorbents for phenol removal from aqueous solutions: kinetics, mechanism, and thermodynamics studies. Chem Eng J, **171**: 1143-1149 (2011).

Yoshizawa K and Lienhard C, Bridging the gap between chewing and sucking in the hemipteroid insects: new insights from Cretaceous amber. Zootaxa, 4079: 229–245 (2016).

Yu Q, Zhang Y, Liu Y and Shi P, Simulation of the stomatal conductance of winter wheat in response to light, temperature and CO_2 changes. Ann Bot, **93**: 435-441 (2004).

Yu Y, Zhu H, Frantz JM, Reding ME, Chan KC and Ozkan HE, Evaporation and coverage area of pesticide droplets on hairy and waxy leaves. Biosyst Eng, **104**: 324-334 (2009).

Zabkiewicz JA, Gaskin RE and Balneaves JM, Effect of additives on foliar wetting and uptake of glyphosate into gorse (Ulex europaeus). In: Symposium on application and biology - Monogram 28, ed. by Southcombe ESE. British crop production council, London, UK, pp. 127-134 (1985).

Zekaria-Oren J and Eyal Z, Effect of film-forming compounds on the development of leaf rust on wheat seedlings. Plant Dis, **75**: 231-234 (1991).

Zeolyst, Datasheet: zeolite Y (2011). Available at: http://www.zeolyst.com (Accessed: February 2014).

Zhang R and Somasundaran P, Advances in adsorption of surfactants and their mixtures at solid/solution interfaces. Adv Colloid Interface Sci, **123**: 213-229 (2006).

Zhang HY, Kim Y and Dutta PK, Controlled release of paraquat from surface-modified zeolite Y. Micropor Mesopor Mat, **88**: 312-318 (2006).

Zhang J, Yan W, Ding H, Liu Y, Tang K, Yu J and Xu R, Microemulsion-directed synthesis of zeolite A nano-crystals. In: Studies in surface science and catalysis: from zeolites to porous MOF materials - the 40th anniversary of international zeolite conference, ed. by Xu R, Gao Z, Chen J and Yan W. Elsevier, Amsterdam, the Netherlands, pp. 475-479 (2007a).

Zhang CQ, Yan SK, Sun HY, Qi ZQ, Zhou MG and Zhua GN, Sensitivity of *Botrytis cinerea* from vegetable greenhouses to boscalid. Plant Pathol, **56**: 646-653 (2007b).

Zhao D, Wan Y and Zhou W, Ordered mesoporous materials. Wiley VCH, Weinheim, Germany, 522p. (2013).

Zhu X, Li B, Yang J, Li Y, Zhao W, Shi J and Gu J, Effective adsorption and enhanced removal of organophosphorus pesticides from aqueous solution by Zr-based MOFs of UiO-67. ACS Appl Mater Interfaces, **7**: 223-231 (2015).

Zion RC, Zeolite (natural and synthetic) market for catalysts, adsorbents, detergent builders and other applications - Global industry perspective, comprehensive analysis, size, share, growth, segment, trends and forecast, 2014-2020 (2015). Available at: http://www.marketresearchstore.com (Accessed: March 2016).

Zolkiewski M, ClpB cooperates with DnaK, DnaJ, and GrpE in suppressing protein aggregation. A novel multi-chaperone system from *Escherichia coli*. J Biol Chem, **274**: 28083-28086 (1999).

Zschintzsch J, O'Brien RD and Smith EH, The relation between uptake and toxicity of organophosphates for eggs of the large milkweed bug. J Econ Entomol, **58**: 614-621 (1965).