INTERNATIONAL
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Use of oilseed rape to clean up fields contaminated with heavy metals

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A thesis submitted for the degree of

Master of Science (MSc) in Sustainable Agriculture and Business

July 2020
Thessaloniki – Greece

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July 2020

Thessaloniki – Greece

Abstract

This dissertation was written as part of the MSc in Sustainable Agriculture and Business at the International Hellenic University.

Food insecurity has become a prominent problem in the modern world, and it has become even more severe due to the anthropogenic pollution of soils with toxic heavy metals. There have been many different strategies proposed to clean up contaminated soils. One of the proposed strategies is the use of hyperaccumulator plants, which are plant that have the ability to absorb and retain large quantities of heavy metals, cleaning up the soil, through a process known as phytoremediation. One of the main hyperaccumulator plants is *Brassica napus*, also known as oilseed rape. The aim of this study is to evaluate the different aspects of oilseed rape as a phytoremediation agent of heavy metals from soil.

For this to happen we conducted a systematic review and analysis of the available literature from the last 10 years (2010-2020), through the search engine Scopus. A total of 74 studies were included. These studied were separated in different groups, based on the subject they were dealing with. This groups included, variation between oilseed rape cultivars, comparisons with other hyperaccumulator plants, effects of heavy metals, tolerance and accumulation mechanisms, effectiveness of different amendments, the role of symbiosis and presence of heavy metals in the final products of oilseed rape. The results of this study indicate a strong interest in the use of oilseed rape in phytoremediation efforts as it is a quite effective way to clean up soils.

On that occasion I should give to thank my supervisor, as well as all the people who contributed to complete this thesis.

Keywords: (hyperaccumulator, heavy metals, oilseed rape, phytoremediation, *Brassica napus*)

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14-Jul-2020

Preface

The present thesis was conducted as part of the MSc in Sustainable Agriculture and Business at the International Hellenic University, Thessaloniki, Greece.

Throughout this thesis, we attempted to sum up the importance of oilseed rape and a phytoremediation agent of heavy metal contaminated soils. It is a growing and quite interesting field of study that, in my personal opinion will thrive in the next years. It is necessary to apprehend the underlying mechanisms behind this phenomenon, as well as the different complex interactions, that take place in nature both between biotic and between biotic and abiotic factors.

At this point I would like to thank my supervisor Prof. Dr. Efimia Papatheodorou for her guidance and the continuous contribution to this dissertation. Additionally, I would like to credit all the teaching staff of the International Hellenic University and, especially, Dr. Dimitrios Schizas with being an absolute helping hand throughout the studies of the MSc Courses. His help was necessary for the completion of our studies.

To conclude , I have to express my gratitude to my family, who supported me all the time with every kind of help, as well as all my friends and and relatives, who empathized with me in cheerful times as also in difficult situations I have faced.

Table of Contents

Introduction

During this section we will primarily analyze the topic of the importance of food shortage in the world these days. In addition, we will discuss the problems deterred by the presence of heavy metals in soils. We will also introduce the main hyperaccumulator plants and discuss the aspects behind phytoremediation.

The issue of food insecurity

Without any doubt, food security has constituted one of the most complex aspects of the world through history. It is admitted that there is the need of sufficient, healthy and clean food in order to be sustained the life of an active and healty individual, regardless of their social, economic or racial status (*Food and Agriculture Association of the United Nations-FAO*). One of the main aspects of food insecurity is the potential limitation of access to a requisite amount of food. This increases the risk of malnutrition for both adults as well as younger children.

Increased exposure to the lack of a sufficient nutrition from an early age can lead to severe consequences that span the individual's life. These include poor health, schooling, and consequent effects on the labor market in later life. Adolescents, who have experienced hunger, are far more likely to develop depressive symptoms and suicidal idealization. At the same time, the potential risk of developing chronic conditions, such as asthma, has substantially increased. In addition, insufficient food intake may lead to several nutrient deficiencies and their subsequent effects. For example, iron deficiency might result in impaired learning and reduced learning productivity in school- aged children, as well as maternal depressive disorder, which includes a variety of conditions that may affect females during pregnancy and up to one year postpartum (Ke and Ford-Jones, 2015). Furthermore, in lower- income countries, food insecurity seems to be one of the main deterring reasons for poverty reduction. This is due to the fact that it keeps the cycle of poverty, poor nutrition, low human capital, and low productivity in motion (Black *et al.*, 2008). In contrast, in higher income countries, food insecurity can lead to the consumption of food with poor nutritional value. This may be accompanied by a substantial rise in the rates of obesity, which may potentially have adverse effects on both the individual as well as the following generations (Perez-escamilla, 2018).

Heavy metals in soil

Heavy metals are a broad and not conclusively defined type of metals, which are primarily characterized by their relatively high densities, atomic weights, and atomic numbers. Some heavy metals, such as iron (Fe), copper (Cu), cobalt (Co) and zinc (Zn) are vital nutrients in human nutrition. Others, such as ruthenium (Ru), silver (Ag) and Indium (In) are harmless in smaller quantities but extremely toxic and dangerous in larger ones. Some others are highly toxic and can be potentially deadly, in case they accidentally come in contact with humans (Khan *et al.*, 2008).

Heavy metals are existing among the contaminants of soils and they have key role it. There are many ways that they can possibly enter and contaminate the soil. These include h emissions from the rapidly expanding industrial areas, usage of high metal wastes, leaded gasoline and paints as fossil fuels, land application of fertilizers, animal manures, sewage sludge, pesticides, wastewater irrigation, coal combustion residues, spillage of petrochemicals, mine tailings and atmospheric deposition. Mainly, the contaminated soils present lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu), mercury (Hg), and nickel (Ni) as contaminated heavy metals(Wuana and Okieimen, 2011).

The heavy metals, which are deposited in soils by the above- mentioned human activities, and are not subjected to microbial oxidization, like organic contaminants. For this reason, they can remain in situ for great time periods . In addition, their presence may restrain the biodegradation of some organic contaminants. Heavy metals pose a considerable danger to the human health. This can be done in a variety of ways, the most important of which are the following:

- Through the direct consumption or getting in touch with contaminated soil
- Through the food chain (soil to plant to human or soil to plant to animal to human interactions)
- Through the drinking of contaminated ground water
- Through the diminution of the quality of food (safety and marketability) via phytotoxicity.
- Through the alleviation in usage of land for agricultural production, which causes food insecurity and land tenure problems.

Their removal from the soil can be achieved through a variety of environmentally friendly techniques. These can include immobilization, soil washing, as well as phytoremediation.

Hyperaccumulator plants

A plant is characterized as a hyperaccumulator provided that it has the ability to grow in soils, which are known as metalliferous soils or in parts of water that present very high concentrations of metals. These kind of plants have the ability to absorb these metals through their roots and converge incredibly high levels of these metals in their tissues. These conditions would, in general, be toxic and, thus, deterring to other species of plants, even to the closely related ones. Hyperaccumulators species have the capability of accumulating metals 100-fold greater than those measured in total in plants characterized as non-accumulator. Thus, a hyperaccumulator plant has the ability to conglomerate more than 10 mg/kg Hg, 100 mg/kg Cd, 1000 mg/kg Co, Cr, Cu, and Pb; 10 000 mg/kg Zn and Ni. In comparison to other non-hyperaccumulator species, hyperaccumulator ones have some distinct adaptations. These are that their roots can extract metals from the soil at a higher rate than other plants, then transfer these metals rapidly to their shoots, and then store large amounts of them in their leaves and roots. The evolutionary reason for these adaptations is that the accumulation of such quantities of metals makes the plants toxic and thus deter herbivores from consuming them (Rascio and Navari-izzo, 2011).

It's been noted that approximately 400 to 450 plants from 45 different families are hyperaccumulators. Some of these families, such as *Brassicaceae*, *Fabaceae*, *Euphorbiaceae*, *Asterraceae*, *Lamiaceae*, and *Scrophulariaceae,* are important crops. Some important hyperaccumulator species are:

- Alpine pennycress (*Thlaspi caerulescens*) for Cd and Zn
- *Ipomea alpine* for Cu
- *Haumaniastrum robertii* for Co
- Cream milkvetch (*Astragalus racemosus*) for Se
- *Astragalus racemosus* for Ni
- Sunflower (*Helianthus annuus)* for Cr, Cu, Mn and Zn
- Rapeseed (*Brassica napus)* for Ag, Cr, Hg, Pb, Se, Zn

The process of regulating the absorption and relocation of heavy metals in the different parts of the plants is regulated by a specific family of genes, known as hyperaccumulation genes (HA). The differentiation of the expression of such genes determines the ability of a plant to accumulate large quantities of heavy metals. Some of the most prevalent genes, that have been well characterized, belong to the ZIP gene family. Zip genes are metal transporter proteins that regulate the transport of cations like zinc, manganese, cadmium and iron. However, there seem to be more gene families involved in the accumulation of heavy metal in plants. Some of these include the HMA, MATE, YSL and MTP families (Verbruggen, Hanikenne and Clemens, 2013).

The exact mode and mechanism that these genes are involved in the facilitation remain currently unknown. However, their expression patterns between different species and different heavy metal concentrations suggest that they play a regulatory role in heavy metal absorption and relocation. Furthermore, the available data suggest that the ability of a species for hyperaccumulation is determined by the exposure of the plant to high concentrations of heavy metals, as well as the expression patterns of HA genes, such as the ZIP genes (Barcelo, Poschenrieder and Tolra, 2006).

Rapeseed plant

Rapeseed (*Brassica napus* sub. *napus*) in a plant belonging to the family of *Brassicaceae* (the mustard or cabbage family). It contains different including winter and spring oilseed, vegetable and fodder rape. It is mainly cultivated for its rich in oil seed, which is the source of canola oil. It is also cultivated as livestock feed and its oil can be used in the production of biodiesel (Rahman, 2013). As seen in figure 1, the general trend of world rapeseed cultivation is increasing, as it went from 39.5 million metric tonnes in 2000 to 75million in 2018, according to the stats of FAO.

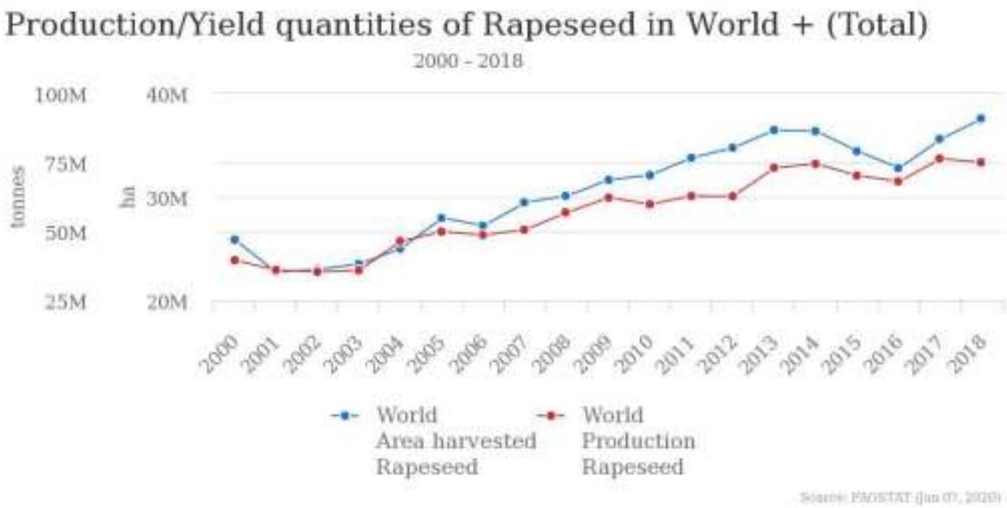

Figure 1: World production and yield quantities of rapeseed between the years 2000 and 2018

Rapeseed is also a hyperaccumulator of heavy metals, such as Ag, Cr, Hg, Pb, Se and Zn, so it has a lot of potential in cleaning contaminated soils, making them safe of crop cultivation (Angelova *et al.*, 2017).

Phytoremediation

The process of phytoremediation, also known as green remediation, botanoremediation, agroremediation, or vegetative remediation is an *in situ* (meaning in place) set technologies that aim at the cleaning of soils, waters and air that are polluted by hazardous contaminants. It can be achieved by using plants and associated with the microbiota. It is a costless and efficient plant-based remediation technique, which takes advantage of the specific aspects of plant metabolism and has different effects on the different pollutant. An organic pollutant can be degraded into less toxic forms, but inorganic pollutants, such as heavy metals cannot, and thus, they have to be accumulated by hyperaccumulator plants (Das, 2018).

It is more or less a new technology it is mainly used in experiments with not many large-scale applications. However, it has shown a lot of promise in the cleaning of polluted soils around metal mines. Phytoremediation has some serious advantages, along with some disadvantages (Wuana and Okieimen, 2011).

The main advantages of phytoremediation are:

- It is a more economic solution than traditional remediation technologies, as it uses the same material and goods as agriculture
- It is less offensive to the environment and there in no need in anticipating the new plant communities to be reinstalled in the site,
- There is no need to develop disposal sites
- It is more likely to be accepted by the community because it is more environmental friendly than traditional methods
- There in no need of excanating and transporting of polluted media, therefore, it reduces the risk of spreading the contamination
- It has the potential to treat sites polluted with more than one type of pollutant

By the way, there are some disadvantages too. These are:

- It heavily depends on the growing conditions required by the plant, such as climate, geology, altitude, and temperature
- Its large scale operations require access to agricultural equipment and knowledge
- Its success depends on how tolerant the plant is to the pollutant

- The contaminants that are collected in senescing tissues could be released back into the environment in autumn
- The contaminants might be collected in woody tissues and subsequently used as fuel
- The estimated time needed in remedation of the soil is bigger than other methods.
- In some cases, the solubility of the contaminant could be increased, thus leading to greater environmental damage and the possibility of leaching
- Different crops and even different varieties and cultivars in the same crop have different phytoremediation potential and ability

The main phytoremediation techniques to clean up contaminated soils of heavy metals include phytoextraction or phytoaccumulation and phytostabilization.

The process of phytoextraction, uses the ability of the plant to collect heavy metals from the soil and then relocate them in specific tissues and organs, especially ones above the ground. Plants that are used in this process need to be tolerant of the heavy metals, have a rapid growing stage with a high biomass yield per hectare, have a high metal-accumulating ability in the foliar parts, have an abundant root system, and a high bioaccumulation factor. There are two different phytoextraction strategies. The first is continuous or natural phytoextraction, which is based on the use of hyperaccumulator plants. The other is known as chemically enhanced or chelate-assisted phytoextraction that uses chelating agents, produced by plants, in order to absorb these heavy metals. Usually, both these techniques need to be done in more than one cultivation periods, in order to achieve a substantial decrease in heavy metal concentrations in the soils. Also, the metals absorbed from the harvested plants can be used in different applications, in a process known as phytomining (Sheoran, Sheoran, and Poonia, 2016).

The process of phytostabilization also referred to as in - place inactivation, involves the reduction of the mobility of soil and its components, for example by reducing leaching. This leads to a reduction in the mobility of the pollutant. Unlike phytoextraction, the plant does not absorb the heavy metal but instead leads to its binding to the soil, near the roots, that is known as the rhizosphere. In this way, the pollutant's availability is reduced. As a technique, phytostabilization has some serious advantages. The main is that there is no need for the disposal of hazardous material/biomass, and it is very effective when rapid immobilization is needed to preserve ground and surface waters (Wuana and Okieimen, 2011).

Aim of the Study

The main goal of the study is to evaluate the effectiveness of rapeseed (*Brassica napus*) as a phytoremediation agent. The main questions that need to be answered are:

- Which is the most efficient way for *Brassica napus* to clean polluted soils; as the plant or as an organic amendment that is incorporated into soil?
- Does the ability of *B. napus* to clean polluted soils vary in relation to different varieties or to different metals?
- Does the mechanism that makes *B. napus* suitable for cleaning polluted soils depend on the type of metal or not?
- What are the effects of toxic metals on oilseed rape?

Methods

Literature search

This is a systematic review and analysis of existing literature regarding the phytoremediation properties and potential of *Brassica napus*. The literature search was performed using the *Scopus* (www.scopus.com) search engine. In order for this to be achieved the keywords, ''*Brassica napus*'', ''rapeseed'', heavy metals'', ''hyperaccumulator'' and ''phytoremediation'' were used as search terms in different combinations.

Study selection criteria

After a preliminary recording of the results of the search that was mentioned before, some additional criteria were applied which concerned the selection and exclusion of the studies. This was done to select the appropriate publications, which will be included in the present study.

In order to select the most recent and modern research available it was decided to include studies that were conducted in the last 10 years (2010 to 2020). Furthermore, as this is a systematic literature review, only research articles were included and no review articles. In addition, only articles that were in English were included and not in other languages. Also, only published studies were included and studied in the pre-print stage were not. Moreover, conference papers or presentations, as well as editorial pieces were also excluded from this study.

After imposing these criteria, the remaining results from the Scopus database were manually searched and studied in order to find the final studies, that would be included in this thesis.

Included data

From each study, specific data were selected and collected, in order to follow with their evaluation and presentation. The main data included the name of the authors, the year of the publication, the type of heavy metal that was studied. The secondary data that were collected, in the case they were mentioned, included if it was compared to the phytoremediating properties of other accumulator plant(s), the type of tissue that was studied, the variety of *Brassica napus*, if rapeseed was used as a whole plant or in other form, the mechanism of action and if there was any alteration to the rapeseed oil composition and quality.

Results and discussion

Selected studies

After the preliminary search, the implementation of the inclusion and exclusion criteria and the manual search the total and final number of studies that will be included in this thesis is 75. These studies are presented in chronological order in table 1. Furthermore, the heavy metal(s) of interest, the measured parameters and the treatments of each study are presented.

Study	Heavy metal	Parameters or measurements	Factors/Treatments
(Cao, Wang, *et al.*, 2020)	Cd and Pb	Accumulation capacity	28 different oilseed rape cultivars
(Wu *et al.*, 2020)	Cd	Antioxidant capacity and cell wall chelation	Effect of boron (B) presence and concentration
(Perotti *et al.*, 2020)	Cr (VI)	Phytoremediation efficiency, mechanisms and post-removal toxicity	Hairy roots
(Zhang, Xiao and Wu, 2020)	Cd	Tolerance mechanism at the initial growth stage	Two different accessions and different Cd concentrations
(Zeng *et al.*, 2020)	Cd	Effect of nitrogen fertilizers	Eight types of fertilizers and three different concentrations of Cd
(Cao, Luo, *et al.*, 2020)	Cd	Soil bacterial community response and Cd phytoextraction	Different cropping systems
(Mera *et al.*, 2019)	Pb	Spatial distribution of Pb in roots and leaves of two different species of plants (*Brassica napus* and *Festuca arundinacea*)	Two different plant species grown in Pb contaminated soil
(Wang, Sun and Li, 2019)	Cd	Physiological, genomic and transcriptomic comparison of two *Brassica napus* cultivars	2 *Brassica napus* cultivars with different Cd tolerance
(Li *et al.*, 2019)	Cr and Cu	Effect of co-contamination of copper (Cu2+) and chromium (Cr6+) on plant growth, photosynthetic parameters, and subcellular distribution in leaves and roots	2 different *Brassica napus* cultivars
(Cao *et al.*, 2019)	Cd and Pb	Concentration and distribution	39 oilseed rape cultivars at 4 locations with different concentrations

(Salam, Shaheen, *et al.*, 2019)	Cu and Pb	Geochemical fractions and Phyto availability in maize	rice straw- and rapeseed residue derived biochars produced under different temperatures, added in soil in different concentrations and under different soil moisture contents
(Salam, Bashir, *et al.*, 2019)	Cu and Pb	Pb and Cu mobility and their accumulation in a high metal accumulating crop such as Chinese cabbage (*Brassica chinensis L.*)	rice straw- and rapeseed residue derived biochars produced under different temperatures, added in soil in different concentrations
(Wang *et al.*, 2019)	Cd	Cadmium accumulation and distribution	32 different oilseed rape varieties
(Alvarez, Paz-ferreiro and M, 2019)	Cu, Pb, Zn and As	(i) The effects of biochar on soil fertility; (ii) Biochar temperature of preparation effect and (iii) Effect of biochar on phytoremediation potential of *Brassica napus*	Soil was treated with rabbit manure biochars prepared at different temperatures, with or without *Brassica napus*
(Li, 2019)	Cd	Characterization of rhizobacteria and their effect on growth and cadmium uptake	13 different novel and uncharacterized Cd-resistant plant-growth-promoting rhizobacteria (PGPR)
(Wu *et al.*, 2019)	Cd	Cadmium phytoremediation and high bioethanol production	2 different rapeseed and 3 different chemical pretreatments for bioethanol production
(Gokul, Cyster and Keyster, 2018)	Vanadium (V)	Effect of Vanadium stress on leaf physiology and biochemistry response	Two different *Brassica napus* cultivars under V stress
(Chen *et al.*, 2018)	Cd	Identification of QTLs related to Cd accumulation	419 *B. napus* accessions and inbred lines were genotyped
(Li *et al.*, 2018)	Cu and Cr	Effects of co contamination of Cu and Cr	Two B. napus cultivars under single and combined Cu and Cr stress

(Ali *et al.*, 2018)	Be	Effects of Be on physiological, ultrastructure, and biochemical attributes of *Brassica napus* seedlings	Two different *B. napus* cultivars and four different Be concentrations
(Mbangi, Muchaonyerwa and Zengeni, 2018)	Zn, Cu, Cr, Ni, Cd and Pb	Phytoremediation potential of different plants	Indian mustard (*Brassica juncea*), lucern (*Medicago sativa*), vetch (*Vicia sativa*), rape (*Brassica napus*) and ryegrass (*Lolium perenne)* on the same soil
(Ferreyroa *et al.*, 2018)	Pb	Pd bioaccumulation at different organs and life stages, as well as the chemical fractionation of Pb in the rhizosphere and bulk soil portions	Four different Pb concentrations, two different life stages
(Lacalle *et al.*, 2018)	Zn, Cu, Cd and diesel	Effectiveness of *Brassica napus L.,* assisted with organic amendment and zero-valent iron nanoparticles (nZVI) in heavy metal accumulation and soil health	Different treatments including: Rapeseed presence, nZVI, organic amendment and contamination
(Ismael *et al.*, 2018)	Cd, Mo, Se	Effect of Mo and Se in Cd toxicity and *Brassica napus* fertility	Single and combined application of Mo and Se
(Nafees *et al.*, 2018)	Cr	Effect of the application of biogas slurry (BGS) and endophytic bacteria on Brassica napus growth and antioxidant activity in Cr-contaminated soil	Inoculation with different bacteria
(Zhang *et al.*, 2018)	Cd	Identification of rapeseed cultivars for potential higher and lower Cd accumulation	64 different *Brassica napus* cultivars
(Franzaringa *et al.*, 2018)	Pb, Zn, As, S, S, W, Mo and Li	Growth responses of five crop species and concentrations of various metals and nutrients in the shoot	Wastes from 2 different mine sites and five different crops
(Lin, 2018)	Cd	Role of xylem proteins in Cd transport	3 different Cd concentrations
(Gill *et al.*, 2017)	Cr	Phenological attributes, cell ultrastructure, protein kinases (PKs) and molecular transporters (MTs) under the combined treatments of Cr stress and reduced glutathione (GSH)	Two different *Brassica napus* cultivars in different treatments with/without Cr and GSH
(Li *et al.*, 2017)	Cd	Comparison of Cd tolerance, growth, and Cd accumulation characteristics of two turnip landraces with three additional commonly known high Cd-accumulating species *(Phytolacca*	Two different turnip landraces and three other species

		americana, Bidens pilosa and *Brassica napus)*	
(Hasanuzzaman *et al.*, 2017)	Cd	Role of externally applied hydrogen peroxide in regulating the antioxidant defense and glyoxalase systems in conferring Cd-induced oxidative stress tolerance in rapeseed	Pretreated rapeseed seedlings grown in two different Cd concentrations
(Yang *et al.*, 2017)	Pb and Cd	Evaluation of oil crop rotation systems for phytoremediation and oil properties	3 plant crop rotation system (rapeseed, sunflower, peanut and sesame)
(Niazi *et al.*, 2017)	As	Effect of phosphate on growth, gas exchange attributes, and photosynthetic pigments of *Brassica napus* and *Brassica juncea* under arsenic stress	3 different phosphate concentrations and 4 different arsenic concentrations
(Dąbrowska *et al.*, 2017)	Cd, Cu, Pb and Zn	Effect of plant growth-promoting rhizobacteria on the phytoextraction of Cd and Zn by Brassica napus	3 different types of bacteria (*Bacteroidetes bacterium, Pseudomonas fluorescens and Variovorax* sp.) alone or in combinations and increasing concentrations of heavy metals
(Mahmud *et al.*, 2017)	Cd	Metal accumulation and tolerance abilities of three Brassica species *(B. napus, B. campestris, and B. juncea*) seedlings	Three different Brassica species and two levels of Cd
(ANGELOVA *et al.*, 2017)	Pb, Zn and Cd	Efficacy of rapeseed (Brassica napus L.) for phytoremediation of contaminated soils in the absence and presence of organic soil amendments (compost and vermicompost)	Absence of organic amendment, compost and vermicompost at two different concentrations
(Shi *et al.*, 2017)	Cd and Pb	Effects of Cd- and Pb-resistant endophytic fungi on growth and phytoextraction of *Brassica napus*	3 different endophytic fungi were isolated and studied
(Shi *et al.*, 2016)	Cd	Cadmium (Cd) accumulation and growth response to Cd stress on 18 plant species	18 different plant species and 3 different Cd concentrations
(Montalbán *et al.*, 2016)	Zn and Cd	Isolating and characterization of a cultivable bacterial community associated with Brassica napus growing on a Zn contaminated site	426 morphologically different bacterial strains were isolated from the soil, the rhizosphere, and

			the roots and stems of B. napus.
(González-valdez *et al.*, 2016)	Pb, As, Hg and Au	Seed germination and dry mass accumulation of five plant species (*Brassica napus L., Brassica rapa L., Celosia cristata L., Tagetes erecta L., and Calendula officinalis L.*) grown in five mine tailings	Seeds from five different species grown in soils from five different mines, contaminated with heavy metals
(Kasiuliene, Paulauskas and Kumpiene, 2016)	Cd and Zn	Influence of nitrogen fertilizer on Cd and Zn accumulation in rapeseed biomass	Cd, Zn contaminated and clean soil, as well as application of liquid and solid nitrogen fertilizer
(Mwamba *et al.*, 2016)	Cd and Cu	Sub- cellular distribution and chemical forms of Cd and Cu in rapeseed	Two different cultivars in different Cd and Cu concentrations
(Gill *et al.*, 2016)	Cr	Transcriptomic analysis of *B. napus* behavior Cr stress and its alleviation through exogenous GSH.	Seedlings of two different cultivars were subjected to different combinations of Cr and GSH
(Feigl *et al.*, 2016)	Zn	Zinc tolerance of Brassica organs and the putative correspondence of it with protein nitration as a relevant marker for nitrosative stress	Two different Brassica species treated with different Zn concentrations
(Feng *et al.*, 2016)	Cd	Profiling of profile long noncoding RNAs in Cd-exposed rapeseed (*Brassica napus*)	Four different Cd concentrations for different periods of time
(Mousa *et al.*, 2016)	Zn	Anatomical and ultrastructural responses of Brassica napus to excess Zn after long-term exposure	Cultivation in the presence and absence of Zn
(Kováčik *et al.*, 2016)	Ni, Pb and Cd	Content of metals and metabolites in different types of honey	Three different types of honey (mixed forest honey, monofloral—black locust and rapeseed honey)
(Al-Dhaibani *et al.*, 2015)	Pb	The capability of Brassica napus to uptake Pb from soil contaminated with Pb-enriched sewage sludge as well as its translocation	Four different concentrations of Pb
(Shaheen and Rinklebe, 2015)	Al, As, Cd, Co, Cr, Cu, Mo, Ni, Pb, Se, V, and Zn	The quantification of the phytoextraction of different heavy metals by Indian mustard, rapeseed, and sunflower from a contaminated riparian soil. The impact of ethylene- diamine-tetra-acetic acid (EDTA), humate (HK),	Three different plant species

		and phosphate potassium (PK) on the mobility and uptake of the elements by rapeseed also was investigated.	
(Mousa, Kouhi and Lahouti, 2015)	Zn	Comparative effects of zinc oxide (ZnO) NPs, ZnO BPs, and zinc ions (Zn^{2+}) on rapeseed after long-term exposure to a wide range of concentrations	Six concentrations of ZnO NPs or ZnO BP as well as a control group
(Mattielli *et al.*, 2015)	Zn	Changes in Zn isotope composition in soil, soil solution, root and shoot on ryegrass *(Lolium multiflorum* L.) and rape *(Brassica napus* L.)	Thee different metal contaminated soils and two plant species
(Mitkova and Markoski, 2015)	Pb, Cd, Zn	Finding of a suitable plant species that could be used for soil remediation in industrial regions	Four plant species (oilseed rape – *Brassica napus* Oleifera D.C., white clover - *Trifolium repens* L., alfalfa - *Medicago sativa* L., and corn – *Zea mays* L.)
(Paduraru *et al.*, 2015)	Zn	Evaluation of the potential of rapeseed waste from biodiesel production as a bio sorbent for Zn ions	Different Zn concentrations
(Contin *et al.*, 2015)	Zn, Ni, Cd and Cu	Effects of addition to soil of sewage sludge (SS) treated and untreated with $FeCl_3$ on the biological availability of Zn, Ni, Cd and Cu to oilseed rape	unamended control soil, sewage sludge amended soils kept bare throughout the experiment and sewage sludge amended soil planted with oilseed rape. Amended soils received either untreated SS or $FeCl_3$ treated SS
(Rinklebe and Shaheen, 2015)	Cu	Suitability and effectiveness of application of different additives as soil amendments together with rapeseed as a possible remediation option for a heavily Cu polluted floodplain soil and potential mobile fraction of Cu	Different soil amendments (activated charcoal, bentonite, biochar, cement kiln dust, chitosan, coal fly ash, limestone, nano-hydroxyapatite, organo-clay, sugar beet factory lime, and zeolite)
(Shaheen, Rinklebe and Selim, 2015)	Ni and Zn	Efficiency of various amendments to immobilize nickel (Ni) and zinc	Different soil amendments (activated carbon,

		(Zn) in soil and reduce their Phyto availability	bentonite, biochar, cement bypass kiln dust, chitosan, coal fly ash, limestone, nano-hydroxyap-atite, organo-clay, sugar beet factory lime, zeolite)
(Wu *et al.*, 2015)	Cd	Physiological and genetic processes involved in Cd uptake and transport of two oilseed rape cultivars	Two different oilseed rape cultivars
(Ullah *et al.*, 2014)	Cu, Pb and Zn	Phytoextraction ability of *Zea mays* and *Brassica napus* plants grown in soils irrigated with municipal wastewater and amended with varying levels of copper, lead and zinc	Two different plant species and four treatments (tap water, municipal wastewater, soil with medium toxicity and soil with high toxicity)
(Kanwal *et al.*, 2014)	Pb	Role of EDTA in the phytoextraction of lead from *Brassica napus* L.	Two different lead levels in the presence or absence of EDTA
(Gill *et al.*, 2014)	Cr	Effect of Cr in different oilseed rape cultivars	4 different cultivars and 4 different Cr concentrations
(Pesko and Kralova, 2014)	Ni	Effect of nickel on two hydroponically cultivated *Brassica napus* L. cultivars	Two different cultivars and six different nickel concentrations
(Kovacik *et al.*, 2014)	Mn	Impact of manganese excess in the shoots of four crops	Four different crops (*Brassica napus, Hordeum vulgare, Zea mays,Triticum aestivum)*
(Ghaderian and Nosouhi, 2014)	Cd, Cu, Pb, and Zn	Capability of stability, growth, and uptake of heavy metals by different plant species	Seven different plant species (*T. caerulescen, Z. mays, H. annuus, E. sativa, B. napus, S. nigrum, M. chenopodiifolia*)
(Shaheen and Rinklebe, 2014)	Cd and Pb	Effects of various amendments on the (im)mobilization of Cd and Pb and their uptake by rapeseed	Various emerging amendments (e.g., nano-hydroxyapatite, biochar, chitosan, and organo-clay) and several of low-cost amendments (e.g., activated carbon, bentonite, cement kiln dust, fly ash, lime stone,

			sugar beet factory lime, and zeolite)
(Ali *et al.*, 2013)	Cd	Ameliorate effects of 5-aminolevulinic acid on the growth of oilseed rape seedlings under Cd stress	4 different 5-aminolevulinic acid concentrations and 3 different Cd concentrations
(Zhang *et al.*, 2013)	Cd	Role of small noncoding RNAs in Cd detoxification	Seven different Cd concentrations
(SUN and GUO, 2013)	Cd	Ethylene levels and root hair initiation under Cd stress	Different treatments with Cd and ACC
(Yunusa *et al.*, 2012)	Zn, Mn, Cu, Mo, Co, Se, B	Evaluate of how acidic and alkaline fly ashes compared with commercial ameliorants two soils and any subsequent influence on yields and trace metal uptake by wheat and oilseed rape, and to tentatively test long-term yield benefit in wheat from addition of coal fly ash	Two different plant species (wheat and oilseed rape) and six different amendment treatments
(Leonardo, Yang and Bitonti, 2012)	Cd	Identification of microRNAs and their targets in Cd stress	Three different Cd concentrations and four different treatment times
(Taylor *et al.*, 2011)	Ni	Effect of putrescine on Ni accumulation by oilseed rape shoots	Four different Ni concentrations and two treatments with putrescine
(Ashraf *et al.*, 2011)	Pb	Investigation of toxic effects of Pb on growth and nutrient uptake in four oilseed rape cultivars	Four different oilseed rape cultivars and four different Pb concentrations
(Nowak and Piotrowski, 2011)	As, Cd, Pb, Zn, Cu, Mn, Ni, Mg, Fe and Cr	Analysis of the content of bio elements and toxic metals in honeys of various botanical origins	Honeys from six different origins from contaminated sites
(Ivanova, Kholodova and Kuznetsov, 2010)	Cu and Zn	Effects of high copper and zinc concentrations and their interaction in oilseed rape plants	Different Zn and Cu concentrations as well as their interactions
(De-chun *et al.*, 2010)	Cd	Effects of oilseed rape phytoremediation on the uptake of Cd from Chinese cabbage	Two different oilseed rape cultivars and presence or absence of phytoremediation

Table 1: Studies included in this thesis

Chronology of the selected studies

As mentioned before, 74 studies very included in this thesis. These publications are the state-of-the-art science of *Brassica napus* phytoremediation that has been conducted from 2010 until today (the last 10 years). This period of time was chosen in order to include the latest available data, but also as, after an initial search, it was found that there is an increase in the interest of using oilseed rape plants as a mean of phytoremediation of heavy metal contaminated soils. More specifically, the studies conducted each year were:

- 2 studies that were published in 2010
- 3 studies that were published in 2011
- 2 studies that were published in 2012
- 3 studies that were published in 2013
- 7 studies that were published in 2014
- 10 studies that were published in 2015
- 10 studies that were published in 2016
- 9 studies that were published in 2017
- 12 studies that were published in 2018
- 10 studies that were published in 2019
- 6 studies that were published in 2020

From these numbers it can easily be understood how much interest the use of oilseed rape in phytoremediation has caused over the last 10 years, with a peak in that interest happening from 2015 until today. In the present year of 2020, there are already 6 studies published, with an unknown number being in line for publication or being conducted at the moment. It is surely quite interesting to monitor if this trend will continue over the next years, as well as the results this will yield.

Heavy metals studied

These 74 publications studied different aspects of a variety of heavy metals both in single treatments and in different combinations. Also, these studies happened in already contaminated soils (for example from mining activities) as well as in soils that were artificially contaminated for the need of the specific experiments. There were 44 studies that examined the effects of only 1 heavy metal, 13 studies that examined the single and combined effects of 2 different heavy metals and 17 studies that examined the effects of 3 or more heavy metals. The heavy metals that were examined in all the studies (both as single and multiple treatments) are as follow:

- 51 studies that examined Cadmium
- 24 studies that examined Lead
- 23 studies that examined Zinc
- 18 studies that examined Copper
- 9 studies that examined Chromium
- 5 studies that examined Arsenic
- 6 studies that examined Nickel
- 4 studies that examined Molybdenum
- 3 studies that examined Selenium
- 2 studies that examined Manganese
- 2 studies that examined Cobaltium
- 2 studies that examined Vanadium
- 1 study that examined Lithium
- 1 study that examined Boron

From these data it can easily be noticed that cadmium is the heavy metal that attracts the majority of the attention of the researchers, as it is involved by far in most of the studies, as 51 of the 74 publications studied cadmium alone or in combination with other heavy metals. The other main heavy metals of interest were lead (24 out of 74 publications), Zinc (23 out of 74 publications) and copper (18 out of 74 publications). There are some reasons why cadmium is the most studied heavy metal, by this far. First of all, it is extremely toxic, carcinogenic, even in low concentrations, and seems to play no role in physiological functions of organisms. Furthermore, there is a variety of ways that soils can be contaminated with cadmium, both naturally and because of human intervention. The main anthropogenic activities that release cadmium in soils are the use of phosphate fertilizers, metallurgical works, wastes from cement industry, sewage sludge, municipal and industrial wastes, , fossil fuel combustion, and mining, smelting and metals ore processing as well. The use of plastic stabilizers, pigments, solar panels, batteries, and steel plating to resist corrosion are daily habits that expand the limits of the problem. (Amjad *et al.*, 2017).

Comparison between oilseed rape varieties

The main objective of 7 out of the 74 publications was to evaluate the variation of different oilseed rape varieties of cultivars regarding the response to different heavy metals. In one of these studies (Cao, Wang, *et al.*, 2020) the researchers studied the accumulation capacity and distribution of cadmium and lead in 28 different oilseed rape cultivars, resulting in the selection of three superior cultivars. In another one of these studies (Cao *et al.*, 2019) the researchers evaluated the contamination and distribution of cadmium and lead in 39 different oilseed rape cultivars at four separately agricultural locations with differences in contamination levels of each, as well as the impact of soil characteristics together with soil total and bioavailable Cd and Pb concentration on metal transfer from soil to oilseed rape. The results of this study showed that Cd and Pb concentration in oilseed rape were correlated with soil organic matter, cation exchange capacity, available phosphorus, available potassium, sand, and total and available Cd and Pb concentration in soil. Also, it showed variation in the absorption between the different genotypes. In another study (Wang *et al.*, 2019) the researchers evaluated 32 different oilseed rape cultivars for growth and accumulation in cadmium contaminated fields. Through these evaluations they were able to identify oilseed rape cultivars that have high tolerance for Cd, high accumulation potential and high yields. This knowledge can be used in direct application to contaminated soils as well as in breeding efforts for more effective Cd accumulating oilseed rape varieties. In another study (Zhang *et al.*, 2018) the researchers evaluated 64 different oilseed rape cultivars for growth and accumulation in cadmium contaminated fields. Again, through these evaluations they were able to identify oilseed rape cultivars that have high tolerance for Cd, high accumulation potential and high yields and can be used either in direct application or in breeding efforts. In another study (Pesko and Kralova, 2014) the researchers compared two different rape cultivars in terms of their response to nickel presence. In another study (Gill *et al.*, 2014) the researchers compared four different rape cultivars in terms of their response to chromium presence. A final study (Ashraf *et al.*, 2011) evaluated the effect of lead on growth and nutrient accumulation in four different oilseed rape cultivars. These studies are extremely useful, as they can lead to the discovery of superior oilseed rape cultivars that can be used directly on contaminated soils or serve as the basis of the creation of new and better cultivars, through breeding efforts.

Comparison between oilseed rape and other species

Out of the included 74 publications 14 were dealing with comparing the effects of oilseed rape and different types of plants in the phytoremediation of different heavy metals. In one study (Mera *et al.*, 2019) the researchers compared the spatial distribution patterns of lead in the species of *Brassica napus* and *Festuca arundinacea.* The results of this study pointed out that the translocation of Pb from the bottom to the upper parts of the plant is more effective in oilseed rape than in *Festuca arundinacea*, that was grown in contaminated soil, where Pb remained at the plant's root system. Furthermore, a co-allocation was observed between Pb and Zn, P, S and Fe in oilseed rape. In another study (Mbangi, Muchaonyerwa and Zengeni, 2018) they compared the phytoremediation properties and potential of Indian mustard (*Brassica juncea*), vetch (*Vicia sativa*), lucern (*Medicago sativa*), oilseed rape (*Brassica napus*) and ryegrass (*Lolium perenne*), using the same heavy metal contaminated soil. Their results showed that, of all the plant species studied Indian mustard and oilseed rape (both belonging to the *Brassica* genus) had the highest biomass and tissue convergence of most of the metals of the present study, indicating their potential as phytoremediation agents. Another study (Franzaringa *et al.*, 2018) inspected the phytoremediation effect of five different plant species (oilseed rape, cucumber, soybean, garden bean and corn) in the treatment of mine waste materials from two different mine sites, one in Italy and one in Germany. Their results showed that, out of all the plant species, oilseed rape had the best phytoremediation properties. Another study (Li *et al.*, 2017) studied the cadmium phytoremediation potential of turnip correlated with three common high Cd accumulating plants (*Phytolacca*

Americana, Bidens pilosa Brassica napus). Their results indicated that although turnip is a potential phytoremediation agent for Cd, its efficiency is still far below than this of the oilseed rape. Another study (Mahmud *et al.*, 2017) evaluated the Cd tolerance of three different *Brassica* species (*B. napus, B. campestris, and B. juncea*). Their results indicated that *B. juncea* had the best response and highest accumulation of all the species. Another study (Shi *et al.*, 2016) investigated the cadmium accumulation and growth response to Cd stress of 18 plant species. Their results indicated that out of all these 18 species Indian mustard and oilseed rape had the highest tolerance and Cd accumulation potential. Another study (González-valdez *et al.*, 2016) assessed the seed germination and dry mass accumulation of five plant species (*Brassica napus, Brassica rapa, Celosia cristata, Tagetes erecta* and *Calendula officinalis*) that were cultivated in five mine tailings from Mexico. The results of the study showed that, in spite of the mining tailings, *B. napus* presented the highest seed germination, tolerance, growth, and total dry mass accumulation of all the different species. In another study (Feigl *et al.*, 2016) the researchers tried to assess the zinc tolerance of *Brassica* organs and the supposed correspondence of it with protein nitration as a relevant marker for nitrosative stress in two different species (*B. napus* and *B. juncea*). Both species proved to be moderate Zn accumulators, however *B. napus* accumulated more Zn in its organs. Also, it showed a smaller amount of damage in its shoots, but more severe damage in its roots. Another study (Shaheen and Rinklebe, 2015) studied the effect of Indian mustard, oilseed rape, and sunflower in the phytoremediation of heavily polluted soil. As their results indicated, Indian mustard presented the highest efficiency for phytoextraction of Al, Cr, Mo, Se, and V; sunflower for Cd, Ni, Pb, and Zn, and rapeseed for Cu. Another study (Mattielli *et al.*, 2015), evaluated the changes in Zn isotope composition in soil, soil solution, root and shoot for ryegrass (*Lolium multiflorum* L.) and oilseed rape (*Brassica napus* L.) grown on three distinct metal-contaminated soils collected near Zn smelters. Their results indicated that the two plant species responded in a similar manner to high Zn concentrations. Another study (Mitkova and Markoski, 2015), evaluated the phytoremediation properties and potential of four different plant species ((oilseed rape, white clover, alfalfa and corn). Their results indicated that for soils with high lead (Pb) correlation, the alfalfa could be useful, for soils with high Cd concentration oilseed rape and white clover are preferred, and for soils with high Zn concentration alfalfa and white clover are preferable. Another study (Ullah *et al.*, 2014), evaluated the phytoextraction capability of *Zea mays* and *Brassica napus* plants sown in soils irrigated with municipal wastewater and amended with varying levels of copper, lead and zinc. Their results indicated that their properties were similar, however, corn gathered two times more heavy metals than oilseed rape, mainly due to its vigorous growth. Another study (Kovacik *et al.*, 2014), evaluated the impact of the excess of manganese in the shoots of four crops (oilseed rape, corn, common wheat and barley). Their results indicated that oilseed rape accumulated the biggest amount of Mn but had the smallest growth. Another study (Ghaderian and Nosouhi, 2014), evaluated the the capability of stability, growth, and uptake of heavy metals by different plant species(*T.caerulescen, Z. mays, H. annuus, E. sativa, B. napus, S. nigrum,* and *M. chenopodiifolia*). Their results indicated that oilseed rape is particularly effective in the phytoremediation of soils contaminated with Cd and Zn.

From all of the mentioned above, we can come to the conclusion that oilseed rape is a very good agent of phytoremediation, compared to different species. However, more research is needed with a view to establish it.

Effect of amendments and additions

14 out of the 74 studies assessed the process of the addition of different amendments or other treatments in the quality of the phytoremediation that was performed by oilseed rape.

Two of these studies evaluated the effects of the use of nitrogen fertilizers. The first study (Zeng *et al.*, 2020), appraised the effect of the application of nitrogen fertilizers in the effectiveness of Cd phytoremediation. The results showed that the physicochemical features of N fertilizers proved to have an impact to the rhizosphere soil pH and promoted Cd phytoextraction and accumulation by oilseed rape. More specifically, the addition of acidic N fertilizer to low Cd contaminated soils provided greater remediation effects, while the physicochemical alkaline N fertilizers were more effective in soils where the Cd concentrations were higher. In another study

(Kasiuliene, Paulauskas and Kumpiene, 2016), they evaluated the influence of both solid and liquid nitrogen fertilizers on Cd and Zn accumulation in oilseed rape biomass. The biomass production was not affected by the high metal concentrations, as this study have shown. However, their accumulation capacity was lowered, meaning they can't be used as a phytoremediation agent but as an energy crop.

Five of these studies included the treatment of the soil with organic materials. In one study (Alvarez, Paz-ferreiro and M, 2019), evaluated how biochar effected the soil fertility, effect of biochar temperature and the effect of biochar on phytoremediation potential, using rabbit manure biochars. Their results showed that the combined effect of biochar and oilseed rape phytoremediation vastly reduced the soil concentration of heavy metals. Furthermore, the biomass production of oilseed rape was increased. In another study (Lacalle *et al.*, 2018), they evaluated the quality of phytoremediation of heavy metals and diesel from oilseed rape in the presence of organic amendment and zero-valent iron nanoparticles (nZVI). Their results showed that, although nZVIs were ineffective, organic matter increased both the accumulation of heavy metals and the biodegradation of diesel, too. In another study (ANGELOVA *et al.*, 2017), they evaluated the efficacy of oilseed rape to phytoremedate the contaminated soils in the lack and adequacy of organic soil amendments (compost and vermicompost). The increasing of compost and vermicompost affected decreasingly to the content of Pb, Zn and Cd, in the roots and stems, whereas the content of Pb and Cd increased in the pods of the rape. The content of the heavy metals of seeds and the oil were reduced by the adding of organic amendments in the soil. In another study (Contin *et al.*, 2015), they evaluated the effect of the treatment of sewage sludge with $FeCl_3$ and its effect on phytoremediation by oilseed rape. Their results showed that the treatment of sewage sludge with $FeCl_3$ and addition to soils reduced the available heavy metal in neutral soils but increased their solubility and bioavailability in acid soils. In a final study (Taylor *et al.*, 2011), they evaluated the usage of exogenous putrescine (Put) in nickel accumulation in oilseed rape shoots. The results of this stydy showed that the application of putrescine increased the absorption of nickel from the oilseed rape plants, thus increasing their phytoremediation potential.

Four of the included studies evaluated the addition of an inorganic material or chemical in the effectiveness of phytoremediation by oilseed rape. The first one (Niazi *et al.*, 2017), described the effect of phosphate on the morphological and physiological response of oilseed rape and Indian mustard. The results of the study showed that phosphate addition gives the ability to improve the efficiency of As phytoextraction, primarly for oilseed rape. This is achieved by decreasing As-induced damage to plant growth, by enhancing the physiological and photosynthetic traits of the plant. Another one of these studies (Kanwal *et al.*, 2014), evaluated the role of EDTA in the phytoextraction of lead from oilseed rape. Their results showed that the supplement of EDTA or mixed with Pb significantly enhanced the plant growth, biomass, chlorophyll content, gas exchange characteristics and antioxidant enzymes activities. In another study (Ali *et al.*, 2013), they evaluated the ameliorate effects of 5-aminolevulinic acid (ALA) on the growth of oilseed rape seedlings in soils with heavy Cd content. It was indicated by the study that in lower loses ALA restored the physiological functions and growth of oilseed rape, however in bigger doses had an adverse effect on growth. A final study (Yunusa *et al.*, 2012), evaluated the application of fly ash in differential growth and yield by oilseed rape and common wheat (*Triticum aestivum* L.). Their results showed no differences in yield (however this could be attributed to the lack of sufficient rainfall during this period). Furthermore, the addiction of fly ash lead to an increase of the accumulation of Mo and Se by oilseed rape, below the phytotoxic threshold.

Three studies compared the effects and potential of different soil amendments. One of these studies (Rinklebe and Shaheen, 2015), evaluated in what rate is suitable and effective the application of activated charcoal, , biochar, bentonite, cement kiln dust, chitosan, coal fly ash, limestone, nano-hydroxyapatite, organo-clay, sugar beet factory lime and zeolite as soil modifiers in combination with rapeseed as bioenergy crop as a possible remediation option for a heavily Cu contaminated floodplain soil. The results of this research proved that both the mobilization and uptake of Cu by oilseed rape were augmented in comparison with the control (except for the organo-clay amendment). In another one of these studies (Shaheen, Rinklebe and Selim, 2015), they assessed the efficiency of various amendments (activated carbon (AC), biochar

(BI), bentonite (BE), cement bypass kiln dust (CBD), chitosan (CH), coal fly ash (FA), limestone (LS), nano-hydroxyapatite (HA), organo-clay (OC), sugar beet factory lime (SBFL), and zeolite (Z)) to incapacitate nickel (Ni) and zinc (Zn) in soil and lessen their phyto availability. This study 's results gave the proof that each amendment has its own benefits and potential for the restraining of Ni and Zn in polluted floodplain soils, with CBD, SBFL, AC, LS, BE and BI having the highest. A final study (Shaheen and Rinklebe, 2014), evaluated the effects of further amendments (nano-hydroxyapatite, chitosan, biochar and organo-clay) and several economic amendments (activated carbon, bentonite, fly ash, lime stone, sugar beet factory lime, cement kiln dust and zeolite) on the immobilization of Cd and Pb and their uptake by oilseed rape. The study 's results showed that the usage of the amendments (except for OC, HA, and FA) augmented the oilseed rape dry biomass yield.

From all of the above, it can be easily understood, how important different kinds of amendments can be in the effectiveness of phytoremediation by oilseed rape. However, it needs further reaserch to be done with the purpose of establishing the best type of amendment for each heavy metal, type of soil and the interaction between different amendments.

Effect of symbiosis

There were five studies that tested the role of symbiosis in the phytoremediation properties of oilseed rape. One of these studies involved fungi and the other four bacteria.

As mentioned, one these studies involved fungi (Shi *et al.*, 2017). More specifically, the main objective of the research was to examine the impact of the Cd and Pb resistant endophytic fungi *(Fusarium, Alternaria* and *Penicillium*) on growth and phytoremediation effectiveness of oilseed rape. The results of the study showed that the colonizing capability of endophytic fungi in roots is crucial to improve phytoremediation effectiveness in oilseed rape.

There were four studies that involved bacteria. In one of these studies (Li, 2019), they characterized Cd-resistant plant-growth-promoting rhizobacteria (PGPR) and evaluated their effects in oilseed rape growth and cd uptake. Out of the 13 different bacteria test three showed promise as they lead to an increase in biomass and better Cd uptake from the oilseed rape plants. In another study (Nafees *et al.*, 2018), evaluated the utilization of biogas slurry (BGS) and endophytic bacteria (*Burkholderia phytofirmans*) as amendments to the improvement of physiology, growth and antioxidant activity of oilseed rape in chromium-contaminated soil. This study's results presented that both the BGS and the bacteria led to the improvement of all these factors. In another case (Dąbrowska *et al.*, 2017), they evaluated the effect of plant growth-promoting rhizobacteria (*Bacteroidetes bacterium* (Ba), *Pseudomonas fluorescens* (Pf) and *Variovorax* sp. (Va)) on the phytoextraction of Cd and Zn from oilseed rape. The results of this study presented that in vitro the combined occurance of the bacteria had a positive effect. However, this was not translated in situ, as they inhibited plant growth. The results were more promising in single inoculations. The final study (Montalbán *et al.*, 2016) intended to isolate the bacterial community affiliated with oilseed rape that grew on a Zn-contaminated soil, with purpose to choose the right cultivable PGPB that may be able to improve the biomass production and metal tolerance, as well as their effects. The study 's results proved that a total of 426 organically different bacterial strains were seperated, some of which showed to potentially have a beneficial role in oilseed rape Zn response.

Over the last years the interactions between host plants and symbiotic bacteria and fungi have gathered a lot of attention, as a potential method of growth regulation and plant defense (Fiorilli *et al.*, 2020).

Different forms of oilseed rape

The vast majority of the studies that are incorporated in this thesis involve the use of oilseed rape in phytoremediation as a whole plant. However, some of them involve the use of it in other forms. These studies are three in total. The first study, (Salam, Bashir, *et al.*, 2019), evaluated the ability of oilseed rape residue (RP), and rice straw (RS)-derived biochars, that were created by unsimilar temperatures on Pb and Cu mobility and their ability to accumulate these heavy metals in Chinese cabbage. The results of this study showed that the combined application of both RP and RS derived biochars yielded the best results, regarding the fractions of Pb and Cu in soil and their availability and solubility, too. The second study (Salam, Shaheen, *et al.*, 2019), assessed the ability of RP and RS derived biochars on the geochemical fractions, Phyto availability, and uptake of Cu and Pb in a polluted mining soil under different moisture contents using maize. This study ‘s results showed that the utilization of both types of biochars led to a reduction on all the parameters. The final study (Paduraru *et al.*, 2015), indicated the capability of oilseed rape waste from biodiesel production as a biosorbent for Zn ions. The results of this study showed that oilseed rape waste has a great potential of absorbing Zn ions from aqueous environments.

All these methods are cost effective alternatives to phytoremediation using living plants and their usage should definitely be considered.

Cropping systems

There were 2 studies that investigated the role of different cropping systems. The first one (Cao, Luo, *et al.*, 2020), evaluated the impact of cropping schemes (monoculture and intercropping) on the rhizosphere bacterial microbiota and their connections with the phytoextraction of cadmium by *Brassica napus, Brassica juncea* and *Sedum alfredii.* The results of the study proved that Cd accumulation in shoots of the *Brassica* species was vastly increased in intercropping schemes. Furthermore, intercropping increased the bacterial variation in soil. The second study (Yang *et al.*, 2017), evaluated the effectiveness of three different plant rotation systems (oilseed rape with either sunflower, peanut or sesame). According to its results, the rotation between oilseed rape and sunflower gave the greatest phytoextraction effectiveness for cadmium.

Effects on plant products

Four different studies investigated the effect of heavy metal contamination in different products of oilseed rape. Two of these involved canola oil and have already been described (ANGELOVA *et al.*, 2017; Yang *et al.*, 2017). In both studies the levels of heavy metals were within the acceptable limits.

One study involved the production of ethanol (Wu *et al.*, 2019) in regards to the Cd accumulation capacity of oilseed rape. It was found that the selection of optimal oilseed rape stalk, along with suitable pretreatments, could lead to a high yield and quality production of ethanol.

Two studies involved the presence of heavy metals in oilseed rape honey (Kováčik *et al.*, 2016: Nowak and Piotrowski, 2011). In both studies they did not find high amounts of heavy metals in oilseed rape honey.

Molecular and biochemical analyses

There were 11 studies that were included in this thesis and involved the unraveling of different molecular (genetic or transcriptomic) or biochemical analyses of different mechanisms. In one study (Wang, Sun and Li, 2019) they compared two oilseed rape cultivars with different Cd tolerance. This lead to the discovery of two different Cd transporter genes associated with enhanced root-to-shoot translocation and accumulation of Cd in the shoot. In another study (Chen *et al.*, 2018), they genotypes 419 oilseed rape accessions and inbred lines and verified different SNP polymorphisms, related to Cd accumulation, as well as some candidate genes. In another study (Lin, 2018) they identified different proteins in the xylem of oilseed rape, that are responsible for Cd transportation. Another study (Gill *et al.*, 2017) showed the alteration in the expression pattern of different proteins. Another study (Hasanuzzaman *et al.*, 2017) they found that, when it is externally applies, hydrogen peroxide (H_2O_2) can regulate the antioxidant defense and glyoxalase systems and confer Cd-induced oxidative stress tolerance in oilseed rape. In another study (Gill *et al.*, 2016), they did a comparative transcriptome profiling of two oilseed rape cultivars under chromium toxicity and also investigated the changes after glutathione treatment. This lead to the unraveling of the expression patterns of several genes and pathways. In another study (Feng *et al.*, 2016), the researchers discovered and characterized a set of long non-coding RNAs that seem to have a crucial role in Cd toxic response in oilseed rape. In another study (Wu *et al.*, 2015), examined the gene expression patterns of Cd transporter proteins in the xylem of different cultivars. In another study (Zhang *et al.*, 2013), the researchers discovered and characterized a micro RNA called miR395 that plays a very important role in the detoxification of Cd in oilseed rape. In another study (SUN and GUO, 2013), it was discovered that the plant hormone ethylene have a significant part in cadmium induced responses and more specifically in root hair development. In the final study (Leonardo, Yang and Bitonti, 2012), the researchers discovered different families of miRNAs that seem to be involved in cadmium responses.

The discovery of all these mechanisms and genes is very important, as it furthers our understanding of this complex phenomenon.

Effects of different heavy metals

Eleven studies were conducted, regarding the single or combined effects of heavy metals on oilseed rape. The first one (Wu *et al.*, 2020), investigates the impact of boron on Cd toxicity and how this is achieved. It was found that boron alleviated Cd toxicity symptoms by heightening the activity of the main antioxidant enzymes and also increased cadmium transportation in the wall of the cell. Another one (Li *et al.*, 2019), investigated the joint effects of Cr and Cu on photosynthetic parameters, subcellular distribution and plant growth. The results showed that together they cause growth defects, along with problems in photosynthesis and other physiological functions. Another study (Gokul, Cyster and Keyster, 2018) indicated the effects caused by Vanadium on leaf physiology and biochemistry response of two rape cultivars. The results showed a differentiation in the symptoms between the two cultivars. Another study (Li *et al.*, 2018), assessed the combined effects of Cu and Cr in two oilseed rape cultivars. According to the results, they cause severe defects in plant growth as they decrease biomass and photosynthetic pigments. Furthermore, their combined effect leads to an increase in the activity of antioxidant enzymes and changes in the structure of the chloroplast. Another study (Ali *et al.*, 2018), checked the toxic effects of Be on ultrastructure, physiological and biochemical attributes in *Brassica napus*. It was observed that high Be content occurred a reduction of the plant growth, chlorophyll contents, biomass production and the total soluble protein contents. These actions led to an increase in antioxidant enzyme activity. Another study (Ismael *et al.*, 2018), examined the role of Selenium and Molybdenum in Cadmium toxicity. It was found that the application of either Se or Mo had protective properties against Cd, while their combination did not. Another study (Mousa *et al.*, 2016), investigated the ultrastructural and anatomical responses of oilseed rape after a long lasting exposure to a plethora of zinc. The results showed that because of the Zn accumulation a number of changes had happened such as decreased number of chloroplasts. Another study (Mousa, Kouhi and Lahouti, 2015) investigated the relative effects of zinc oxide (ZnO) nanoparticles, ZnO bulk particles, and zinc ions on oilseed rape. The results showed changes in growth, as well as other parameter, such as in the antioxidant enzyme activities, soluble proteins, total chlorophyll and soluble sugars of the leaves. The toxicity of the ZnO NPs was lower than those of Zn ions or ZnO BPs. In another study (Al-Dhaibani *et al.*, 2015), they investigated the ability and capability of oilseed rape to uptake Pd from soil that was contaminated with Pd-enriched sewage sludge. All parts of plant growth were affected. Also, Pd content was higher in the lower parts (roots) than the upper parts (shoots) of the plant. Last but not least, oilseed rape demonstrated a very good ability of up taking Pb. In another study (Ivanova, Kholodova and Kuznetsov, 2010), the researchers investigated the biological effects of high Cu and Zn concentrations and their interplay in oilseed rape plants. The results showed that Cu proved to have higher toxic effects than Zn. Furthermore, they discovered many differences including different translocation in the plant. The final study (De-chun *et al.*, 2010) investigates if Chinese cabbages that are grown in soils that were remediated with oilseed rape have lower concentrations of Cd. The results showed no difference.

Other mechanisms

Four studies were conducted, with the subject of unraveling different physiological mechanisms of heavy metal tolerance. The first one (Perotti *et al.*, 2020) involved the investigation of Cr remediation through hairy roots. By the results of this study, it was concluded that hairy roots had the ability to tolerate moderate concentrations, while further concentrations presented toxicity to their development. They also demonstrated a high Cr removal efficiency. Another study (Zhang, Xiao and Wu, 2020), investigated the mechanisms that made tolerant in cadmium oilseed rape plant at the initial growth stage. The results showed that high activity of the different antioxidant enzymes at the early Cd stress stage is the primary detoxification mechanism in Cd tolerant rapeseed. Furthermore, the great Cd transfer factor is the primary mechanism for surviving radicle from the first Cd toxicity in sensitive oilseed rape. Another study (Ferreyroa *et al.*, 2018), investigated the accumulation of Pb in different organs at different ontogenetic stages The outcome was that the higher Pb concentrations were observed in the stems of the plant at physiological maturity. The last study (Mwamba *et al.*, 2016), investigated the difference in subcellular distribution and chemical forms of Cd and Cu, in two different oilseed rape cultivars. The findings proved that Cd was less preserved at cell wall, whereas Cu was found in both the cell walls and the vacuole. Cu accumulated in chloroplasts, while Cd was equally distributed between chloroplasts and mitochondria. Also, they reacted with different ligands inside the cell.

Conclusions

To sum it up, the aspects of phytoremediation using oilseed rapes is an exciting and promising field, with a large number of studies being conducted, especially over the last 10 years.

These studies have unraveled many different physiological, biochemical and molecular mechanisms concerning to the tolerance and accumulation of oilseed rape in different heavy metals.

Furthermore, there has been a large amount of research demonstrating the variation between different oilseed rape genotypes, as well as its superiority as a hyperaccumulator plant, between different species.

However, there is still a lot of work to be done in this field, however the future on this field of study seems quite promising.

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