UNIVERSITY OF HELSINKI
DEPARTMENT OF AGRICULTURAL SCIENCES
Plant Production Sciences
PUBLICATION no 22

TOWARDS SUSTAINABLE INTENSIFICATION OF FEEDSTOCK PRODUCTION WITH NUTRIENT CYCLING

DOCTORAL THESIS

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ACADEMIC DISSERTATION

To be presented, with the permission of the Faculty of Agriculture and Forestry of the University of Helsinki, for public criticism in Viikki, Auditorium B3, on April 4th, 2014, at 12 o'clock noon.

HELSINKI, FINLAND

2014

Seleiman, M.F. Towards sustainable intensification of feedstock production with nutrient cycling
Keywords: bioenergy crops, feedstock quality, growth, mycorrhizal fungi, sludge

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Cover photograph	Mahmoud Fathy Seleiman
	“Maize, hemp and oilseed rape fertilized with sludge”

ISBN 978-952-10-8879-7 (PDF)
ISBN 978-952-10-8878-0 (paperback)
ISSN 1798-744X (PDF)
ISSN 1798-7407 (paperback)
ISSN-L 1798-7407

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ABSTRACT

Sludge contains valuable nutrient sources such as N (3.1%), P (2.6%) and micronutrients as well as organic matter. Nevertheless, depending on the feedstock materials, sludge contains heavy metals and metalloids that can be partly taken up by plants. The continuing need for disposal of sludge is a challenge due to the increasing world population.

Experiments were conducted at glasshouse and field at Viikki Experimental Farm, University of Helsinki, Finland, during 2008-2012, in order to study the suitability of sewage sludge and digested sludge as nutrient sources for bioenergy crops. Leaf N content, leaf area index, leaf area formation, net photosynthesis, water relations and biomass accumulation were determined. In addition, the effects of sludge on feedstock quality in terms of macronutrient and trace element content in crop biomass, C:N mass ratio and ash content as well as higher heating value and gross energy yield were studied. Further attention was paid to the role of mycorrhizal colonization in the improvement of N and P availability to the host plants.

In the first glasshouse experiment, sewage sludge was applied in a high dose (1 kg sludge per 5 kg soil), a low dose (50% of high), or a low dose premixed with an equal mass of peat. In the subsequent glasshouse and field experiments, treatments for each crop species were standardized on the basis of total N, where 100% represented 120 kg ha^{-1} of N for maize, 90 kg ha^{-1} for oilseed rape, and 60 kg ha^{-1} for fibre hemp and ryegrass. The second glasshouse experiment comprised five treatments (soil + synthetic fertilizer, soil + sewage sludge, soil + digested sludge, sand + synthetic fertilizer, sand + sewage sludge), while the field experiments comprised six treatments (100% synthetic fertilizer, 50% + 50% synthetic fertilizer in a split application, 50% synthetic fertilizer + 50% sewage sludge, 100% sewage sludge, 150% sewage sludge and 100% digested sludge). Each experiment was arranged in a randomized complete block design with 3-6 replicates.

Sewage sludge-peat mixtures significantly increased leaf area, and improved net photosynthesis of maize and hemp. Sewage sludge resulted in higher biomass accumulation in maize and hemp at 90 and 150 DAS than in the other treatments in the field study, while biomass accumulation of oilseed rape was equally high following applications of both sewage sludge and synthetic fertilizer throughout most of sampling

dates. Sewage sludge application resulted in more numerous fungal spores in soil and increased root colonization in comparison to synthetic fertilizer. The highest root colonization rate was in maize, followed by hemp. Sewage sludge and synthetic fertilizer applications resulted in higher N uptake in maize, hemp and oilseed rape than in the other treatments. However, sewage sludge resulted in the highest P uptake in maize and hemp, while high sewage sludge and digested sludge resulted in the highest P uptake in oilseed rape. Also, sewage sludge resulted in the highest accumulation of many heavy metals and metalloids in plant biomass. Sewage sludge application provided the optimum feedstock quality in maize, hemp and oilseed rape, in terms of reducing the content of alkali metals, Cl and ash, while sewage sludge and synthetic fertilizer applications gave the optimum C:N ratio for methane production in maize and hemp.

Thus, recycling nutrients from sludge provides an opportunity to minimize the use of synthetic fertilizer with its high consumption of fossil fuel, and improves sustainability in agriculture. Sewage sludge improved plant growth, mycorrhizal colonization of roots and plant uptake of N and P, and feedstock quality. However, the potential losses of P can be a serious environmental issue in long-term sludge use, and the high content of Cu in sludge makes it the heavy metal of greatest concern.

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following journal articles, which are referred to by the Roman numerals in the text **I- III.** The papers are reprinted with the permission of the publishers.

I **Seleiman, M.F., Santanen, A., Stoddard, F.I. and Mäkelä, P.** 2012. Feedstock quality and growth of bioenergy crops fertilized with sewage sludge. Chemosphere 89: 1211-1217.

II **Seleiman, M.F., Santanen, A., Kleemola, J., Stoddard, F.I. and Mäkelä, P.S.A.** 2013. Improved sustainability of feedstock production with sludge and interacting mycorrhiza. Chemosphere 91: 1236-1242.

III **Seleiman, M.F., Santanen, A., Jaakkola, S., Ekholm, P., Hartikainen, H., Stoddard, F.L. and Mäkelä, P.S.A.** 2013. Biomass yield and its quality of bioenergy crops grown with synthetic and organic fertilizers. Biomass and Bioenergy 59: 477-485.

CONTRIBUTIONS

The contributions of authors to the original publications of this thesis:

Publication I

All authors contributed to the research plan for this investigation. The experimental work, measurements and laboratory analyses were carried out by Mahmoud Seleiman with laboratory guidance from Arja Santanen. Statistical analysis of obtained data was done by Mahmoud Seleiman with guidance from Pirjo Mäkelä and Fred Stoddard. Mahmoud Seleiman was responsible for the writing of the manuscript and incorporating the input of the other authors.

Publication II

All authors contributed to the plan of this study. The experimental work and all measurements were done by Mahmoud Seleiman. Mycorrhiza analyses were done by Mahmoud Seleiman with guidance from Arja Santanen. Statistical analysis of data was done by Mahmoud Seleiman under guidance from Pirjo Mäkelä and Fred Stoddard. Mahmoud Seleiman was responsible for the writing of the manuscript and incorporating the input of the other authors.

Publication III

All authors except Seija Jaakkola contributed to the research plan of this study. The field experimental work, measurements, laboratory analyses and statistical analyses were done by Mahmoud Seleiman, with suggestions from the other authors. Seija Jaakkola gave her assistance for energy analysis. Mahmoud Seleiman was responsible for the writing of the manuscript and incorporating the input of the other authors.

1. INTRODUCTION

1.1. Bioenergy

In the 21st century, the world faces problems as a result of increasing energy usage and decreasing supplies of fossil fuels, which are non-renewable or finite sources (Hein 2005). The consumption of global primary energy, including fossil fuel, nuclear and renewable energy, was 280 EJ in 2000, and it is expected to be 470 EJ in 2030 (Bakkes et al. 2008). Around 80% of global primary energy is from fossil fuels, namely petroleum, gas and coal (El Bassam 2010, REN21 2011, BP 2012). Petroleum contributes the largest part of fossil fuels, followed by coal and natural gas (BP 2012). Renewable energy, including biomass, hydropower, geothermal, wind and solar energy, contributes around 18% of the worldwide primary energy consumption (El Bassam 2010), while nuclear energy provides around 2.8% (El Bassam 2010, REN21 2011).

1.1.1 Biomass

Biomass refers to any biological material of organisms living or recently alive, and includes plant materials (i.e. trees, grasses, and crops), animal manure, municipal biosolids (sludge) and microorganisms such as algae (Montross and Crofcheck 2010). Biomass contributes 13-15% of the global primary energy demand (AEBIOM 2010, El Bassam 2010, BP 2012, REN21 2012) and more than 60% of total renewable energy (AEBIOM 2010, REN21 2011). The contribution of biomass to the energy supply has increased from 53 Tg in 2000 to 105 Tg in 2008 (EUROSTAT 2010). This was due to the replacement of fossil fuel by biofuel. Biofuel is obtained by converting biomass into solid, gas or liquid fuel through thermal, chemical or biochemical means (Figure 1). The energy derived from biomass in the European Union in 2008 was 13% liquid biofuel, 11% electricity and 76% heat (AEBIOM 2010). Biodiesel and bioethanol are usually used for transport, where the biofuel is either mixed with traditional transport fuels (e.g. biodiesel with petroleum diesel or bioethanol with gasoline) for standard engines or used on its own in vehicles with specialized engines (Demirbas 2009, AEBIOM 2010, El Bassam 2010).

Biofuel is classified as "first" or "second" generation based on production technology and feedstock. First-generation biofuels (i.e. bioethanol 85% and biodiesel 15% of current global production) are usually made from the edible parts of starch, sugar and oil crops by simple means such as fermentation of sugars and starch to ethanol and transesterification of vegetable oil to fatty acid methyl esters (Larson 2008, El Bassam 2010, Pedroli et al. 2012). Second-generation biofuels are commonly made from non-edible biomass by more advanced technologies such as conversion of cellulose to ethanol (Larson 2008, El Bassam 2010), using crops that produce plentiful biomass with high land use efficiency and relatively little competition with food crops (Larson 2008). Worldwide, total biofuel production increased from 32 billion litres in 2004 (bioethanol 30 billion litres and biodiesel 2 billion litres) to 100 billion litres in 2010 (bioethanol 80 billion litres and biodiesel 20 billion litres) (REN21 2010). The European Union is third in biofuel production globally after Brazil and the United States. In 2012, the European Union consumed about 6 billion litres of bioethanol and about 14 billion litres of biodiesel, and 16% of the bioethanol and 22% of the biodiesel were imported, mainly from Brazil and the United States (Flach et al. 2012).

1.1.2 Biomass conversions into biofuels

Thermo-chemical conversions use heat, often in combination with varied catalysts, to break biomass down into smaller basic units, which are further processed to produce fuel (El Bassam 2010, Pedroli et al. 2012). The combustion of biomass pellets or bales is a thermo-chemical process that produces hot gases which can be used directly to dry other products (Figure 1). These hot gases can also be converted in a heat exchanger to hot water, air or steam, which can be used for district heating or in steam turbines for power generation (El Bassam 2010). Gasification (Figure 1) is also a thermo-chemical process in which biomass is partially oxidized by heating at 1200°C to produce fuel gas (Dumbleton 1997). The fuel gas, which can be used in electricity, is mostly methane, carbon monoxide and hydrogen, with low amounts of ethane and ethylene (El Bassam 2010). Pyrolysis is also a thermo-chemical degradation of biomass in the absence of oxygen at a wide range of temperatures (350-800°C), to produce gaseous (syngas), liquid (pyrolysis oils) and solid (biochar) fuels (Figure 1) (El Bassam 2010). Syngas (i.e. CO, CO_2, H_2 and H_2O) can be converted into ethanol, methanol and butanol through the Fischer-Tropsch process (El Bassam 2010).

Fermentation is a biochemical process in which sugars are fermented in the presence of yeast and enzymes and converted directly to bioethanol (Figure 1). Starch has to be broken down by acids or hydrolytic enzymes into simple sugars before fermentation (El Bassam 2010). Even lignocellulosic biomass can be converted into bioethanol after thermo-chemical, chemical and biological pretreatments (Sun and Cheng 2002, Sipos et al. 2010) that facilitate the enzymatic accessibility of cellulose and remove hemicellulose and lignin (Sun and Cheng 2002). The cellulose is hydrolysed in the presence of enzymes, saccharified into simple sugars, and fermented to bioethanol (Olofsson et al. 2008). Residues from the fermentation process can be used as feedstock for anaerobic biogas production (Barta et al. 2010, Kreuger et al. 2011) or combusted for heat and power production (Sassner et al. 2008).

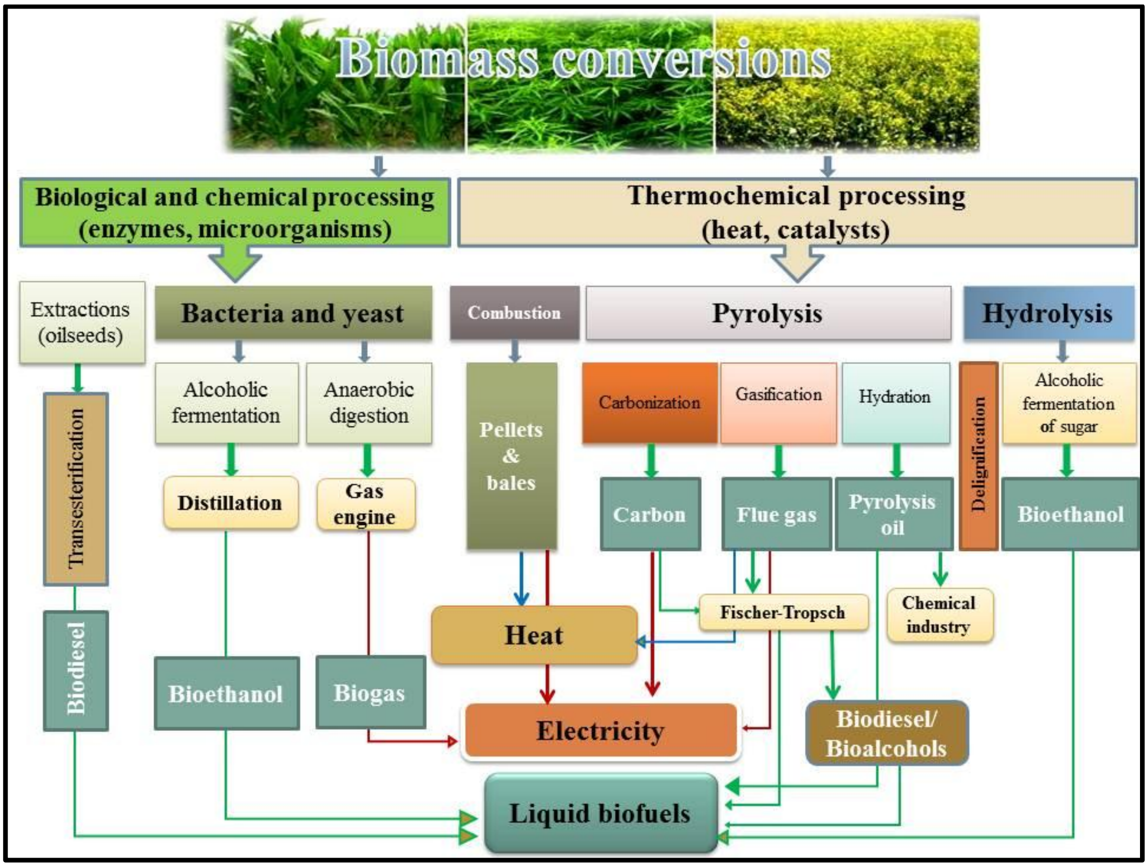

Figure 1. Conversion of biomass into heat, electricity and liquid biofuels (Modified from Bassam 2010, Mäkelä and Santanen 2012).

Transesterification (Figure 1) is a conversion process of triglyceride (i.e. vegetable oil or animal fat) with an alcohol in the presence of a catalyst such as NaOH or KOH into biodiesel and glycerol (Stephenson et al. 2008). Biogas consists of about 60% methane and 40% carbon dioxide, and is produced by biological conversion of biomass into methane through anaerobic digestion (Chynoweth et al. 2001, El Bassam 2010). It can be used as a source of power and heat production, as well as can be purified to natural gas (El Bassam 2010). Most of the nitrogen and minerals, along with the lignin, remain in the digestate that can be used as a fertilizer.

1.1.3 Bioenergy crops

Bioenergy crops can be defined as those that are planted and harvested in order to produce biomass that can be converted into solid, liquid or gaseous fuels (Tuck et al. 2006, Montross and Crofcheck 2010). Bioenergy crops are composed of protein, carbohydrates, oils, lipids, and fibres depending on the plant species and environmental conditions. Some 450 000 plant species have been identified globally as potential bioenergy crops, and 3000 of those are currently used as food, feed, fibre and fuel (El Bassam 2010). Most of the cultivated bioenergy crops are more suitable for utilization as solid fuel than those cultivated for bioethanol and biodiesel fuels (El Bassam 2010). Annual bioenergy crops can be fitted into a crop rotation with food and feed species, which is also an essential feature for sustainability in bioenergy production (El Bassam 2010, Zegada-Lizarazu and Monti 2011). In this case, alternative genotypes that require lower energy inputs than food genotypes are highly recommended to get high gross energy yield at the end (Venturi and Venturi 2003, El Bassam 2010, Zegada-Lizarazu and Monti 2011). Perennial bioenergy crops are favored for biofuel production, since they require infrequent planting, less weed control, decrease soil erosion, and are more drought resistant than annual crops (Montross and Crofcheck 2010).

Generally, bioenergy crops have numerous benefits in comparison to other renewable energy sources (i.e. wind and photovoltaic) and fossil fuel, since their cultivation can decrease the dependence on short-term weather variations, support the local economy and create new jobs in rural areas (European Commission 2005, Ruane et al. 2010). Bioenergy crops are considered CO_2 neutral, since C released during the combustion process has been absorbed by the plants from the atmosphere during their growing period (El Bassam 2010). Bioenergy crops have some beneficial effects on the environment, such as reducing GHG emissions and air pollution, and improving land use efficiency and water use (Miguez et al 2006, Demirbas and Urkmez 2006, Balat 2008, Demirbas 2009, El Bassam 2010). The cultivated area of bioenergy crops has increased 10-fold over the last 10 years (Zegada-Lizarazu and Monti 2011), and was about 5.5 M ha in 2008 in the

European Union (EUROSTAT 2009). The area has been estimated to increase to approximately 17.5 M ha by 2020 in order to supply the energy sector with 10% of its needs as biofuel (European Commission 2007, AEBIOM 2010). Changing the use of agricultural lands from food cropping to dedicated bioenergy cropping is difficult, because of the increasing global population, demand for animal protein and economics. In contrast, converting forest and pastureland, or land otherwise unsuitable for food use to dedicated bioenergy crops is considered an important impact for the sustainability, heating energy value, and renewable energy sources (Montross and Crofcheck 2010). Using underutilized agricultural lands (385 to 472 M ha worldwide) is an important option to increase biomass production that could be 4.3 t ha^{-1} per year and could account for 10% of the primary energy supply in regions such as North America, Europe and Asia (Campbell et al. 2008).

Maize (*Zea mays* L.) is one of the most important and widely cultivated bioenergy crops for grain bioethanol, cellulosic bioethanol, and whole-crop biomethane (Ericsson and Nilsson 2006). It has been used as source for food, feed and bioenergy through the last decade (Yuan et al. 2008). New varieties of maize can produce over 30 t ha^{-1} biomass for biogas production (Kreps 2008). Hemp (*Cannabis sativa* L.) is an annual plant, grown mainly for fibre production (Pahkala et al. 2008). Seeds of hemp are considered an important food oil source due to their composition of unsaturated fatty acids (Patel et al. 1994). Fibre hemp has high land-use efficiency because of its high biomass production, and it requires relatively little input of agrochemicals (Montford and Small 1999). Hemp can be grown for bioenergy purposes (i.e. solid biofuel, biogas and bioethanol), although knowledge of its potential biomass and energy productivity is still limited (Castleman 2006, Prade et al. 2011). The stem of fibre hemp contains approximately 44% cellulose, making it an appreciated crop for bioethanol production (Sipos et al. 2010). Rapeseed produces the highest yield among different oil crops in European Union, since it can grow better in cool and temperate conditions than other oil crops (Stephenson et al. 2008). The bioethanol yield obtained from straw of oilseed rape [*Brassica napus* L. ssp. *oleifera* (Moench.) Metzg.] was higher than obtained from straw of winter rye (*Secale cereale* L) or faba bean (*Vicia faba* L.), while biogas yield was almost equivalent (Petersson et al. 2007).

1.1.4 Biomass quality

The heating value of biomass is an important factor, when plant species are selected for bioenergy purposes. The heating value is the amount of heat released from biomass during combustion (Montross and Crofcheck 2010), and it is associated with the presence of cellulose, hemicellulose and lignin. Hemicellulose has the lowest heating value, while lignin provides the highest heating value due to its high degree of oxidation (Jenkins et al. 2011). The heating value is associated negatively with ash content, and positively with C content in biomass. Each 1% increase in ash content leads to a decrease of 0.2 MJ kg^{-1} in heating values of biomass (Jenkins 1989), since the ash does not provide substantially to the total heat released during combustion process. Each 1% increase in C content leads to an increase of 0.4 MJ kg^{-1} in heating values of biomass (Jenkins 1989) due to the association between the heating value and the amount of O_2 needed to complete combustion (Shafizadeh 1981). The quality of biomass ash (i.e. Si and alkali metals including Na, Ca, and K) is also more important than its quantity (Grammelis et al. 2011), and lower content of ash means high quality of biomass. Cl is vaporized during biomass combustion, forming alkali chlorides, HCl and Cl_2, that cause corrosion in combustion tubes and facilitate the transport of volatile heavy metals from the fuel ash to aerosol particles (Grammelis et al. 2011). During combustion, Cl increases the vaporization of the alkali metals more than the alkali metal itself. The rest of alkali metals in fuel ash act with Si and S to form silicates and sulphates (Grammelis et al. 2011).

1.1.5 Recycling biomass ashes

After thermal combustion has converted plant biomass into heat or electricity, the ash contains most of macronutrients (i.e. Ca, K, Mg, P, S) and trace elements (i.e. Cu, Cr, Ni, Mo, Zn), with the composition depending on plant species, plant part used for combustion, and growing environment (Demeyer et al. 2001, Knapp and Insam 2011, Pels and Sarabèr 2011). These nutrients can be recycled either alone or in combination with synthetic fertilizers, but this depends on the composition of the ash (Pels and Sarabèr 2011). Using the ash in forestry is considered the most suitable route, because the P is in weakly soluble form and needs a long time to become available to plants, particularly in acidic soil (Knapp and Insam 2011, Pels and Sarabèr 2011). Biomass ashes generally contain little or no N or C, because their oxides are gaseous (Steenari et al. 1999). Biomass ash should not be applied to crop or forest land if its content of plant nutrients is outside established limits (Table 1). Low content of macronutrients generally indicates incomplete combustion, so the ash can be used again for fuel production using gasification (Pels and Sarabèr 2011). Other uses for ash include as a raw material for synthetic fertilizer production, or as filler in cement and concrete for building (Pels and Sarabèr 2011).

Table 1. Recommended maximum contents of trace elements and minimum contents of macronutrients when ash is to be used as a forest fertilizer (data from Samuelsson 2002)

Trace elements		Macronutrients	
Element	Maximum content (mg kg^{-1} ash)	Element	Minimum content (g kg^{-1} ash)
As	30	Ca	125
Cd	30	K	30
Ni	70	Mg	20
Cr	100	P	10
Pb	300		
Cu	400		
B	500		
Zn	7000		

1.2. Plant nutrition

1.2.1 Macronutrients and micronutrients

There are 17 essential elements for plant growth and reproduction, which are divided into macro- and micro-nutrients based on the quantity needed by the plant. Macronutrients are required in the range of 1000 mg kg^{-1} DM or more, while micronutrients are required in the range of 100 mg kg^{-1} DM or less (Oertli 1979; Table 2). Carbon (C), hydrogen (H), oxygen (O), nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S) are the macronutrients, while boron (B), chlorine (Cl), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni) and zinc (Zn) are the micronutrients (Fageria et al. 2011). Roughly 95% of the plant biomass is C, H, and O, with the remaining 5% being the other elements (Fageria and Moreira 2011). Plants obtain C, H and O from atmospheric CO_2 and from soil water, while the other elements are absorbed from the soil solution (Fageria and Moreira 2011). Nutrients are needed for metabolism and structure of plants, and their deficiency or excess decreases and sometimes inhibits plant growth (Fageria 2009, Fageria et al. 2011, DalCorso 2012), so when the supply of essential plant nutrients is inadequate, it should be supplemented. Sometimes, the differences between the content of macronutrients (i.e. S or Mg) and micronutrients (i.e. Fe or Mn) are smaller than those shown in Table 2 (Mengel and Kirkby 2001).

Table 2. The adequate tissue content of macro- and micronutrients required by higher plants (Source: Epstein 1972, 1999)

Macronutrients (g kg^{-1} DM)		Micronutrients (mg kg^{-1} DM)	
H	60	Cl	100
C	450	Fe	100
O	450	B	20
N	15	Mn	50
P	2	Na	10
K	10	Zn	20
Ca	5	Cu	6
Mg	2	Ni	0.1
S	1	Mo	0.1
Si	1		

N is considered the most important plant nutrient (Havlin et al. 2005, Fageria 2009, Fageria and Moreira 2011). It is an important component of proteins, nucleic acids, secondary compounds, chlorophyll and cell walls (Mengel and Kirkby 2001, Fageria 2009). Usually, proteins contain about 85% of the total N in plants, with most of the remaining 15% being in nucleic acids (Li et al. 2013). Roots absorb most of a plant's N from the soil, and leaves and stems can take up small quantities of reactive N from the atmosphere (Fageria and Moreira 2011, Li et al. 2013). N content in plants varies from 1 to 6%. Generally, roots absorb more NO_3^- than NH_4^+, due to its high availability in soil solution and low cellular toxicity (Mengel and Kirkby 2001, Havlin et al. 2005). The plant metabolizes NO_3^- to NH_4^+ and then to amino acids, and finally into proteins and nucleic acids (Mengel and Kirkby 2001, Havlin et al. 2005). The reduction of nitrate requires reducing power from NADPH (in leaves containing chloroplasts) or NADH (in roots), so it represents an energy cost to the plant (Mengel and Kirkby 2001). The absorption of NH_4^+ increases the absorption of anions (i.e. $H_2PO_4^-$, SO_4^{-2} and Cl^-) and decreases the absorption of cations (i.e. Mg^{+2}, Ca^{+2}, and K^+) from the soil solution, while absorption of NO_3^- has the opposite effect and increases rhizosphere pH (Havlin et al. 2005). Plants can take up very small quantities of organic N in the form of amino acids that pass through the cell wall to the plasma membrane via the apoplast and cytoplast systems (Li et al. 2013). Also, organic N can be absorbed through the plasma membrane in an active (sugar/proton cotransport) or even passive process (Li et al. 2013). Within the plant, amino acids can be quickly assimilated and transformed into other amino acids by transamination (Li et al. 2013). Usually, the period of vegetative growth is reduced and plants mature earlier when suffering from N deficiency (Mengel and Kirkby 2001).

P is an essential macronutrient present in molecules such as phospholipids, sugar phosphates and nucleic acids (Starnes et al. 2008). The P content in plants varies from 0.1 to 0.5%. P plays an important role in the activity of enzymes involved in plant growth, reduces respiration, preventing energy losses, increases translocation of sugars and starch to storage organs, increases protein content in plants, maintains turgor and reduces water loss (Fageria and Gheyi 1999, Havlin et al. 2005). Plants absorb inorganic P from soil mainly in the forms of dihydrogen phosphate ($H_2PO_4^-$) or monohydrogen phosphate (HPO_4^{-2}) (Marschner 1995, Havlin et al. 2005), taking up more $H_2PO_4^-$ than HPO_4^{-2} in acidic soil and vice versa in alkaline soil (Marschner 1995, Havlin et al. 2005, Smith and Read 2008). Plants can also absorb some organic P compounds such as small nucleic acid fragments and phytin that are produced during decomposition of organic matter (Havlin et al. 2005). Around 50-80% of the total P in soil is in organic forms (Turner et al. 2002) that are usually unavailable to plants until mineralized. Therefore, P deficiency often limits plant growth in natural ecosystems (Starnes et al. 2008). This could be attributed to the rapid immobilization of P in organic or insoluble forms, even when it has been added to the soil in an inorganic form (Wetterauer and Killon 1996). Soil pH is the primary factor affecting the availability of P, and high concentrations of soluble Fe, Al and Ca in the soil solution cause the precipitation of P, making it unavailable to plants. In soil with pH lower than 5, inorganic P precipitates as Fe/Al-P that can be adsorbed onto clay minerals and Fe/Al oxide (Havlin et al. 2005). However, inorganic P precipitates as Ca-P and Mg-P or can be adsorbed to surfaces of clay minerals and $CaCO_3$ when soil pH is greater than or equal to 7.8 (Havlin et al. 2005).

K is, after N, the second largest absorbed nutrient by plants. The total content of K in soils ranges from 5 to 25 g kg^{-1} DM, and mineral K accounts for 90 to 98% of total K (Havlin et al. 2005). However, the readily available part (i.e. exchangeable and solution) of K in soil ranges from 0.1 to 2% of the total K due to the adsorption of K^+ by 2:1 layer silicate minerals (Mengel and Kirkby 2001, Havlin et al. 2005, Fageria 2008). K is absorbed by plant roots in the form of K^+, and its content in plant biomass ranges from 5 to 60 g kg^{-1} DM (Havlin et al. 2005). K is involved in water relations and osmotic pressure of plant cells due to its high mobility in the plant. Furthermore, it is important to photosynthesis because it is involved in the production and activity of RuBP carboxylase enzyme, ATP synthesis and CO_2 absorption via leaf stomata (Havlin et al. 2005). CO_2 is assimilated within leaves into sugars during photosynthesis, then sugars are transported to seeds, roots or tubers using ATP energy which requires K in its synthesis (Havlin et al. 2005). Excess supply of K can affect the crop yield and its quality by reducing the uptake of other nutrients such as Mg^{2+}, Ca^{2+} and Na^{+}, which is known as ion antagonism (Barker and Pilbeam 2006).

The term "heavy metal" refers to metallic elements with an atomic number of 24 or higher. Metalloids are elements with transitional properties between metals and non-metals on the diagonal of the periodic table. The essential heavy metals and metalloids are the micronutrients Ni, Cu, Mn, Zn and Se that participate in redox reactions, electron transfer and play an integral part of several enzymes (Gerendas et al. 1999, Taiz and Zeiger 2006, Nagajyoti et al. 2010). The non-essential or toxic heavy metals and metalloids are arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), mercury (Hg) and silver (Ag) (DalCorso 2012). The plant has no specific transporters for these elements (Manara 2012), but they may be co-transported across the plasma membrane in the roots. The accumulation of heavy metals depends not only on their availability in the soil solution, but also on the absorption efficiency of the root system (Liu et al. 2000), which is affected by the presence of mycorrhizal fungi that can affect the availability of sparingly soluble ions with narrow diffusion zones (Lambert et al. 1979, Liu et al. 2000). Mycorrhizal fungi can solubilize different minerals, including heavy metal-containing rock phosphates, by producing organic acids and releasing protons (Leyval et al. 1997). This leads to a potential increase in the availability of heavy metals and metalloids in the rhizosphere. Once these ions have entered the hyphae, they can be sequestered there or transferred to the crop roots and then transported to the shoots (Leyval et al. 1997). All micronutrient heavy metals can be toxic to the plant when present at too high a concentration.

1.2.2 Plant nutrient sources

1.2.2.1 Synthetic fertilizers and fossil energy

Synthetic fertilizers contain the most important nutrients, particularly N, P, and K that are needed in high amounts and rapidly absorbed by plants (Mengel and Kirkby 2001). The global production of synthetic fertilizers has steadily increased from 33 Tg in 1961 to 180 Tg in 2007 (IFA 2010, FAOSTAT 2012). Between 2002 and 2009, N accounted for 58%, P 24% and K 18% of the total production of fertilizers (Figure 2) (FAOSTAT 2012). China is the largest producer of synthetic fertilizers with 33% of the global production, followed by USA (10%), India (9%) and Russia (9%) (FAOSTAT 2012). About 30 Tg of fertilizers are transported yearly across the globe (IFA 2010). Fertilization of bioenergy crops will account for 1 to 8% of global synthetic fertilizer use in 2015, and this consumption is expected to double by 2030 (Smeets and Faaji 2005).

Higher plants cannot directly uptake and metabolize atmospheric N_2, and it has to be converted into available forms through symbiotic or nonsymbiotic soil microorganisms or manufacture of synthetic N fertilizers (Havlin et al. 2005). N fertilizers are mostly manufactured in three forms, namely nitrate, urea and ammonium, with some other sources such as isobutylidene urea and urea formaldehyde which are slow-release N sources (Mengel and Kirkby 2001). World production of synthetic N fertilizers increased from 11.6 Tg in 1961 to 106 Tg N in 2009 (IFA 2010, FAOSTAT 2012), mostly due to the need to increase crop yields (Smil 2011). The amount of synthetic N fertilizers applied to agricultural crops has increased over 7 fold, while the crop yield generally has increased only by 2.4 times (Tilman et al. 2002).

P fertilizers are mainly made in the form of orthophosphate, but there are some products in which P is present as polyphosphates (Mengel and Kirkby 2001). The total production of P fertilizer increased from 10 Tg in 1961 to 38 Tg in 2009 (Figure 2) (IFA 2010). Asia was the largest producer of P fertilizer (21 Tg) during 2009, followed by Northern America (9 Tg) and Europe (5 Tg) (FAOSTAT 2012). Around 82% of total P_2O_5 produced is used as fertilizer and 18% for industrial purposes (Heffer and Prud’homme 2010). The production of K fertilizers has been relatively stable since 1977 (Figure 2). K fertilizers are mainly presented in the form of chloride, sulphate or magnesium sulphate (Mengel and Kirkby 2001, Havlin et al. 2005).

About 5% of the total commercial energy in the world is used in agriculture, and about 40% of that is used to produce nitrogen fertilizers (Isherwood 2000). Production of synthetic N fertilizer consumes higher energy than synthetic P and K fertilizers (Table 3) due to the large input required to reduce N_2 to ammonia in the Haber-Bosch process (Mudahar and Hignett 1985, Helsel 1992). Natural gas accounts for 80% of the fuel used in the ammonia production (Mudahar and Hignett 1985), and 1.6 kg of CO_2 are released during the production of each kg of synthetic N fertilizer (Nemecek and Kägi 2007). Therefore, energy input is a main indicator of the environmental impact, not only for the production of conventional crops but also for bioenergy crops (Lewandowski and Schmidt 2006). The energy needed for single superphosphate production is 16 MJ kg^{-1} P_2O_5 and triple superphosphate 14 MJ kg^{-1} P_2O_5, with CO_2 being released at 1 kg kg^{-1} of P_2O_5 (Elsayed and Mortimer 2001). Producing K fertilizer requires the lowest energy input of the three main fertilizer components (Table 3).

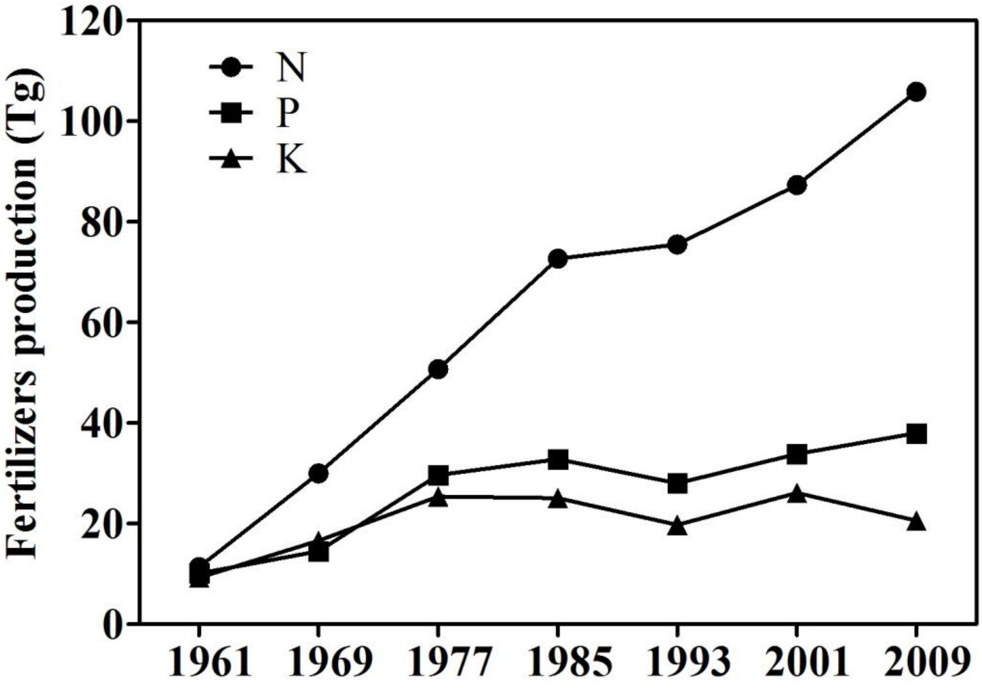

Figure 2. World production of N, P and K fertilizers during 1961-2009 (IFA 2010, FAOSTAT 2012).

Table 3. Energy inputs (MJ kg^{-1}) in producing synthetic fertilizers: N, P_2O_5 and K_2O (Source: Helsel 1992[a], Mikkola and Ahokas 2009[b])

Nutrient	Production	Packaging	Transportation	Application	Total
N	49.2[b]	2.6[a]	4.5[a]	1.6[a]	78.2[a]
P_2O_5	7.7[a]	2.6[a]	5.7[a]	1.5[a]	17.5[a]
K_2O	6.4[a]	1.8[a]	4.6[a]	1.0[a]	13.8[a]

1.2.2.2 Organic fertilizers

There are many other sources for plant nutrients besides synthetic fertilizers, including sludge (Epstein 2003, Bozkurt et al. 2006, Boeira and Maximiliano 2009), manure

(Hooda et al. 2000, Epstein 2003) and meat and bone meal (Jeng et al. 2006). Animal manure, as used in fertilization, includes decomposed straw as well as faeces and urine (Mengel and Kirkby 2001). It contains 1-6% N, 0.2-2.9% P and 0.3-2.0% K, along with other nutrients. About 25 to 50% of the total N in manure is NH_4^+, and the remainder is in organic forms (Havlin et al. 2005). Meat and bone meal contain also valuable nutrients such as N (~8%), P (~5%), Ca (~10%) and K (~0.4%) (Jeng et al. 2006).

Sewage sludge is a solid or semi-solid product that originates from domestic or industrial wastewater treatment plants through aerobic or anaerobic digestion process (Gardiner et al. 1995, Epstein 2003). The disposal of sewage sludge in a safe way is considered a major environmental concern all over the world. The alternatives for the disposal of sludge are land application, incineration and landfill (Epstein 2003). Land application is usually considered as the most economical and sustainable option to cope with sewage sludge, as it recycles the nutrients (Shammas and Wang 2007). Anaerobic digestion of wastewater sludge results in the production of methane, a valuable biofuel (Berktay and Nas 2008). Digested sludge can be used as nutrient source on cropland if its composition is appropriate. Thus, the utilization of sewage sludge recycles not only nutrients but also provides some energy (Gilbert et al. 2011). About 12 Tg (DM) of sludge is produced in the European Union annually, of which about 45% is applied to cropland (EUROSTAT 2010). Half or more of the sludge produced in Cyprus, the Czech Republic, Denmark, France, Germany, Ireland, Luxembourg, Spain and the UK is applied to cropland, while less than 5% of the sludge produced in Finland and Romania is used in this way, and none at all in Greece and Malta (Table 4). The remaining part of sewage sludge is used in landfill (18%), combustion (23%), composting (7%) and other uses (7%) (Alabaster and LeBlanc 2008).

Sludge contains macronutrients such as N, P, K, Ca and Mg, and micronutrients such as B, Cu, Fe, Ni and Zn (Epstein 2003, Bozkurt et al. 2006, Kidd et al. 2007, Yan et al. 2009, Du et al. 2012, Gu et al. 2013). Sewage sludge contains up to 40 g kg^{-1} DM of inorganic and organic N, and up to 28 g kg^{-1} DM of inorganic and organic P depending on the product quality (U.S. EPA 1994, Yan et al. 2009). The content of K in sewage sludge is much lower (less than 5 g kg^{-1} DM) than N and P due to the solubility of K compounds in the wastewater that does not settle in sludge (Epstein 2003, Yan et al. 2009).

Table 4. Annual sewage sludge production in European Union (EUROSTAT 2010)

Member state	Total amount (t DM per year)	Agriculture and compost use (%)
Austria	255 000	44.3
Belgium	112 000	18.7
Bulgaria	40 000	15.0
Cyprus	9 000	55.5
Czech Republic	172 000	78.5
Denmark	200 000	50.0
Estonia	31 000	16.1
Finland	160 000	2.5
France	1060 000	59.6
Germany	2049 000	52.6
Greece	126 000	0.0
Hungary	286 000	45.8
Ireland	88 000	71.6
Italy	1056 000	44.1
Latvia	23 000	43.5
Lithuania	76 000	42.1
Luxembourg	12 000	66.6
Malta	400 000	0.0
Netherlands	541 000	15.3
Poland	1088 000	15.0
Portugal	237 000	50.0
Romania	758 000	2.6
Slovakia	332 000	11.7
Slovenia	21 000	19.0
Spain	1065 000	64.5
Sweden	210 000	49.5
UK	1771 000	69.7

Supplemental synthetic fertilizer can be added with sewage sludge on cropland to balance the nutrition, or more sewage sludge can be added for further nutrients as long as the heavy metal content does not exceeded the limit values (Yan et al. 2009). It is generally recommended that sewage sludge should be applied based on crop N requirements in order to avoid ammonia volatilization and denitrification, excessive soil acidification resulting from nitrification of ammonia, inhibition of microbial activity, and increased nitrate leaching from soils (U.S. EPA 1995, Smith and Doran 1996, Wong et al. 1998, Henry et al. 1999, Yan et al. 2009). Sewage sludge has a high organic matter content which serves as a soil conditioner, improves soil physical conditions by increasing waterholding capacity and water infiltration, and stimulates soil microbial activity (Jarausch-Wehrheim et al. 1999, Epstein 2003, Samaras et al. 2008, Yan et al. 2009).

Mineralization of organic N sources is considered the most important process that can influence the supply of mineral-N for plant uptake and leaching (Havlin et al. 2005, Tian et al. 2008, Boeira and Maximiliano 2009). It is the combination of ammonification and nitrification processes, whereby the organic-N is converted to NH_4^+ and then to NO_3^-. Mineralization is the process by which soil organic N is converted to inorganic N (i.e. NH_4^+) through aminization and ammonification (Figure 3) with the help of heterotrophic bacteria (Havlin et al. 2005, Fageria 2009). The NH_4^+ form is available to plants, and is generally not leachable, since the positively charged NH_4^+ cation is held on the surface of negatively charged soil particles (Epstein 2003, Havlin et al. 2005). Nevertheless, NH_4^+ can be leached in sandy soil where the cation exchange capacity is low, so it can cause contamination of surface water by runoff (Epstein 2003). NH_4^+ can be converted into NO_3^- through nitrification, in two steps (Figure 3). It is oxidized to NO_2 in the presence of *Nitrosomonas* spp. bacteria, and then NO_2 is oxidized in presence of *Nitrobacter* spp. bacteria to NO_3 (Epstein 2003, Havlin et al. 2005). NO_3^- is easily taken up by plants, but it can be converted by denitrifying bacteria into N_2, N_2O or NO that escape to the atmosphere, particularly in anaerobic conditions (Fageria 2009). These processes recycle substantial quantities of organic-N back to the soil and thus are considered as important in the N cycle (Figure 3). Worldwide, almost 40% of N_2O emissions result from agriculture, transportation and industry activities (USEPA 2010). Usually, N_2O is emitted when N fertilizers are applied into soil (USEPA 2010, Linquist et al. 2012), and it can also be emitted through the breakdown of nitrogenous compounds in livestock manure and other organic materials (USEPA 2010). NO_3^- can leach and percolate into groundwater, and hence can act as a pollutant, because it is negatively charged and not adsorbed onto soil particles (Mengel and Kirkby 2001, Epstein 2003). Organic N and NO_3^- can be immobilized and used by soil organisms that decompose the organic matter when biosolids are incorporated into the soil (Figure 3) (Fageria 2009, Yan et al. 2009). This can decrease leaching of NO_3^- during those times in the growing season when plant uptake of N is low (Epstein 2003, Fageria 2009). Volatilization of NH_3 can occur during the first few days after sludge is applied to the surface of cropland (Epstein 2003, Yan et al. 2009). Thus, sludge should be incorporated into soil promptly after it is applied (Yan et al. 2009).

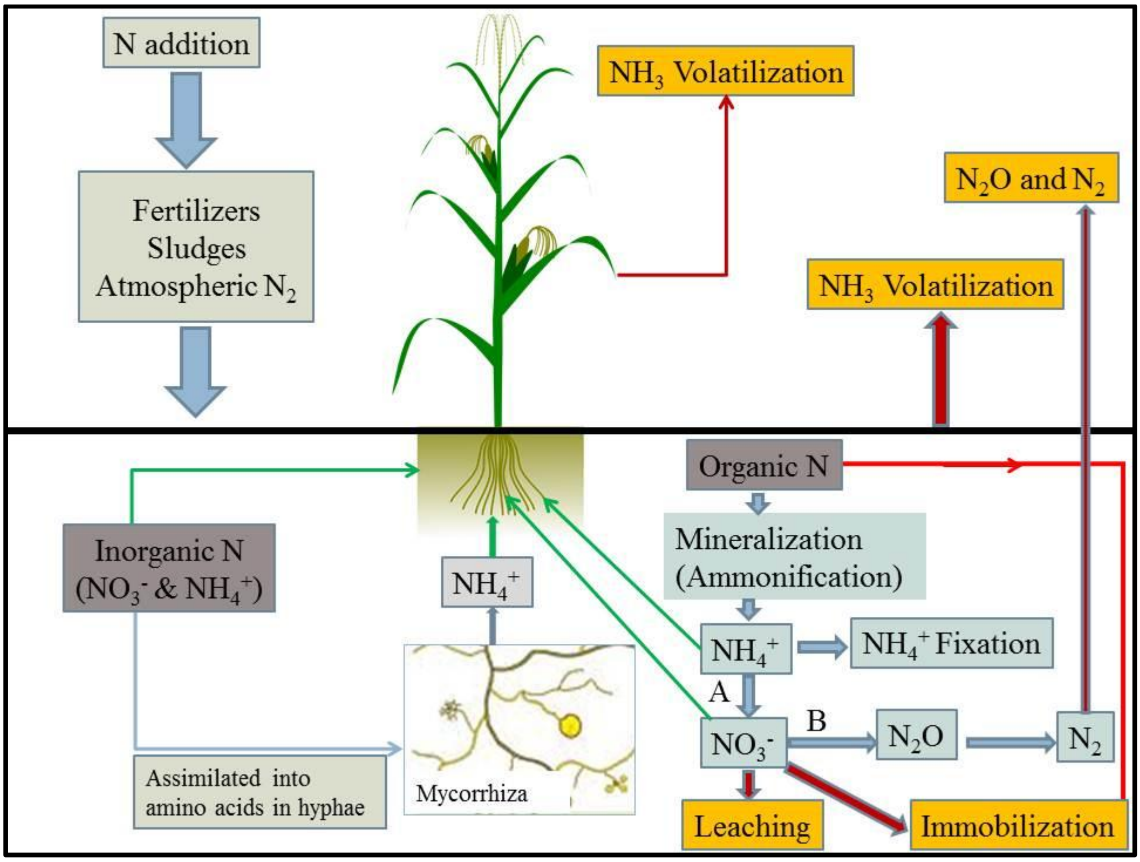

Figure 3. A simplified version of direct and indirect pathways of N uptake by plants and the nitrogen cycle in soil – plant system. Modified from Fageria (2009). Plants can directly uptake organic N in the form of NO_3^- and NH_4^+, or indirectly via extraradical hyphae of AMF which take up and assimilate NH_4^+, NO_3^- and amino acids. The amino acids are converted into NH_4^+which is then translocated to the host plants. The third pathway is organic N which is converted into NH_4^+-N via ammonification and then NH_4^+-N is converted into NO_3^- via nitrification.

A = Nitrification; B = Denitrification

Sludge generally contains heavy metals and metalloids (Pepper et al. 2006, Singh and Agrawal 2008, Yan et al. 2009), some of which, such as Cu, Mo, Ni and Zn, are micronutrients, but others, such as As, Cd, Cr and Pb, are toxic (Epstein 2003). The potential risk of those elements for plants, animals and human is low due to their low content and bioavailability in sewage sludge (Epstein 2003). The limit values of heavy metals and metalloids in soil to which sludge is applied and limit values for heavy metals in sludge are presented in Table 5. Heavy metals are considered non-biodegradable, but can be taken up by plants and stored in their different tissues, which can then contaminate the food chain (Wagner 1993, McLaughlin et al. 1999). Soil factors that affect the uptake of trace element include pH, organic matter content, cation exchange capacity, elemental interactions, water content, temperature and aeration (Epstein 2003). Firstly, soil pH is considered the most important factor affecting the solubility of trace elements, as the solubility of all essential trace elements except Mo and Se increases at low pH (Epstein 2003), so their potential uptake by plants increases. Secondly, organic matter can control the availability of some trace elements, such as Cd, Cu, Ni and Zn, because it has higher cation exchange capacity than the mineral fraction of soils. Furthermore, organic compounds in soil can chelate trace elements, reducing their availability (Epstein 2003). Thirdly, soil cation exchange capacity can bind all the trace elements except those that occur as anions in the soil solution. The binding of trace elements in clay soil is higher than that in sandy soils due to the high cation exchange capacity of clay soil (Epstein 2003). Fourthly, the interactions between certain elements and trace elements can reduce their availability in soil. For instance, phosphate interacts with trace elements to form soluble or insoluble compounds depending on soil pH (Epstein and Chaney 1978). Finally, soil water can affect the availability of trace elements in soil solution, as in dry soil they precipitate or adsorb onto soil colloids (Epstein 2003).

Table 5. The European limits for heavy metals in sludge and in soil to which sludge is applied (European Commission 2001, 2002, Alabaster and LeBlanc 2008)

Source material	Cd	Ni	Cu	Cr	Pb	Zn
Soil treated with sludge (mg kg^{-1} DM)	3	75	140	150	300	300
Current rules						
Heavy metals in sludge (mg kg^{-1} DM)	40	400	1750	1000	1200	4000
Maximum load of heavy metals to agricultural soil (g ha^{-1} year^{-1})	150	3000	12000	3000	1500	30000
Proposed 2015						
Heavy metals in sludge (mg kg^{-1} DM)	5	200	800	800	1500	2000
Maximum load of heavy metals to agricultural soil (g ha^{-1} year^{-1})	15	600	2400	2400	1500	6000
Proposed 2025						
Heavy metals in sludge (mg kg^{-1} DM)	2	100	600	600	200	1500
Maximum load of heavy metals to agricultural soil (g ha^{-1} year^{-1})	6	300	1800	1800	600	4500

Sewage sludge often also contains pathogens (i.e. bacteria, viruses, protozoa, and eggs of parasitic worms) (Epstein 2003, Yan et al. 2009) that pose a risk for human health if they are transferred to food crops grown on soil treated with sewage sludge (Yan et al. 2009).

The typical values of different pathogens in sewage sludge are shown in Table 6. Furthermore, sludge can contain a range of toxic organic compounds, including chlorinated compounds, alkylphenol ethoxylates, volatile compounds, dioxins, phthalates, and polycyclic aromatic hydrocarbons (Epstein 2003, Yan et al. 2009). The potential effect of toxic organic compounds on the environment, humans and animals occurs through the food chain when sewage sludge is applied on cropland (Epstein 2003). Soluble organic chemicals can be taken up from soil through roots and translocated to leaves via xylem, and other organic compounds can be taken up by leaves from the atmosphere and be translocated via the phloem to the other plant parts. Both of these pathways depend on factors such as lipophilicity and water solubility, ambient temperature, content of the organic compound in the soil, and plant species (Simonich and Hites 1995).

Most products of sewage sludge contain low levels of organic compounds which do not cause a significant risk for humans or the environment, depending on the efficiency of the treatment used in the wastewater plant (Yan et al. 2009). Further treatments of the sewage sludge can significantly decrease the content of undesirable organic compounds. For example, chlorinated compounds can be biodegraded in anaerobic digestion (Ballapragada et al. 1998). Other organic compounds can be biodegraded during composting, and can be volatilized during heating and drying (Epstein 2003, Yan et al. 2009). Nevertheless, these additional processes and treatments reduce the availability of macronutrients, in particular N, by NH_3 volatilization (Cogger et al. 1999, Richards et al. 2000, Yan et al. 2009). Specific requirements for organic compounds in sewage sludge are not included in Directive 86/278/EEC, because their concentration in the sludge is very low, but several national regulations include limitations concerning organic compounds for sludge applied on cropland in order to reduce potential health risks (Table 7).

Table 6. Maximum values of different pathogens in sewage sludge (European Commission 2001, 2002, Alabaster and LeBlanc 2008)

	Salmonella	Other pathogens
Finland	Was not detected in 25 g	*Escherichia coli*: <1000 cfu
Poland	Sludge cannot be applied on cropland if it contains *salmonella*	-
Italy	1000 MPN per g DM	-
France	8 MPN per 10 g DM	*Enterovirus*: 3 MPCN per 10 g DM Helminths eggs: 3 per 10 g DM
Luxembourg	-	*Enterobacteria*: 100 per g, and no eggs of worm expected to be contagious
Denmark	-	*Faecal streptococci*:< 100 per g

MPN = most probable number

MPCN = most probable cytophatic number

Table 7. Maximum values of different organic compounds in sewage sludge (European Commission 2002, Langenkamp et al. 2001, Alabaster and LeBlanc 2008)

	LAS	AOX	DEHP	NP/NPE	PAH	PCB	PCDD/F
	mg kg^{-1} DM						ng Teq kg^{-1} DM
EC 2000	2600	500	100	50	6^a	0.8^b	100
Finland	-	-	23-270	-	0.01-13	<0.2	<0.02
Sweden	-	-	-	50	3^a	0.4^b	-
Denmark	1300	-	50	10	-	3^b	-
France	-	-	-	-	-	0.8^b	-

LAS = linear alkylbenzene sulphonates

AOX = adsorbable organohalogen compounds

DEHP = di(2-ethylhexyl)phthalate

NP/NPE = nonylphenole and nonylphenole ethoxylates with 1 or 2 ethoxy groups

PAH = polynuclear aromatic hydrocarbons

PCB = polychlorinated biphenyls

PCDD/F = polychlorinated dibenzo-p-dioxins and -furans

a = sum of 9 congeners: acenapthene, fluorene, phenanthrene, fluoranthene, pyrene, benzo(b+j+k)fluoranthene, benzo(a)pyrene, benzo(ghi)perylene, indeno(1,2,3-c,d)pyrene

b = sum of 7 congeners: PCB 28, 52, 101, 118, 138, 153, 180

EC = European Commission

Ng = nanogram

Teq = toxicity equivalent

1.3. Mycorrhizal fungi and their role in plant nutrition

There are six types of mycorrhiza, namely arbuscular, arbutoid, ecto-, orchid, ericoid and monoptropoid, which are classified by their morphological properties (Garg et al. 2006, Wang and Qiu 2006). Worldwide, the majority of terrestrial plant species (250 000) have symbiosis with mycorrhizal fungi (Harley and Harley 1987, Peterson et al. 2004, Wang and Qiu 2006, Smith and Read 2008, Helgason and Fitter 2009). Normally, species in the Brassicaceae and Chenopodiaceae families do not have a symbiotic relationship with mycorrhizal fungi (Newman and Reddell 1987, Peterson et al. 2004). The arbuscular mycorrhizal fungus (AMF) is considered the most important type among the microbial populations that can influence plant growth and soil fertility (Johansson et al. 2004, Gosling et al. 2006, Smith et al. 2011). Furthermore, AMF can increase the host plant resistance to drought or to pathogens (Smith and Read 2008).

Root mycorrhizal colonization has several advantages, including plant uptake of the most important macronutrients such as N, P, K and Mg (Hodge et al. 2001, Leake et al. 2004, Smith and Read 2008, Smith et al. 2011), and of some micronutrients such as Cu and Zn (Gildon and Tinker 1983, Faber et al. 1990, Kothari et al. 1991, Li et al. 1991, Azaizeh et al. 1995, Taiz and Zeiger 2006). In return, the plant provides the required organic carbon (from 4 to 20%) for the formation, maintenance and function of the AMF (Graham 2000, Smith and Read 2008, Smith and Smith 2011). The symbiosis process between the AMF and its host starts with the colonization of roots by the hyphae and asexual spores (Requena et al. 1996, Smith et al. 2011). The hyphae start to penetrate the root cortical cell walls and form morphologically different structures, for instance branched structures called arbuscules and oval structures called vesicles that interface with their host cytoplasm (Figure 4) (Lambert et al. 1979, Smith and Read 2008 Smith et al. 2010, Smith and Smith 2011). Spores of AMF grow in the soil and germinate spontaneously and freely of plant-derived signals. Following root colonization, the mycelium grows out of the root exploring the soil, accumulating nutrients and water, and translocating them to the roots, and it can colonize other susceptible roots (Peterson et al. 2004, Smith and Read 2008, Smith et al. 2010, Smith and Smith 2011). The arbuscules are considered the site of nutrient transfer between the mycorrhizal fungus and its host plant (Taiz and Zeiger 2006). Vesicles work as storage organs for lipids, which indicates that they can act as propagules for AMF (Peterson et al. 2004, Smith and Read 2008).

Organic sources of nutrients and slow-release mineral fertilizers can stimulate AMF (Harinikumar and Bagyaraj 1989, Baby and Manibhushanrao 1996, Dann et al. 1996, Kabir et al. 1998, Joner 2000, Alloush and Clark, 2001, Smith et al. 2011), whereas most synthetic fertilizers are known to suppress mycorrhizal colonization (Kothari et al. 1991, Liu et al. 2000). AMF are particularly important in plant P nutrition, increasing total plant uptake of this element, and sometimes also affect P use efficiency (Koide et al. 2000), often resulting in increased growth and crop yield (Osonubi et al. 1991, Vosatka 1995, Ibibijen et al. 1996, Koide et al. 2000, Smith et al. 2011). AMF grows extensive below-ground extraradical hyphae fundamental for the uptake of inorganic phosphate, and other immobile nutrients from the soil and transporting them to the host plant (Giovannetti et al. 2006, Smith and Read 2008). Thus, the main function of AMF is to supply colonized plant roots with P (Bucher 2007). The P depletion zone is closely linked to root hair length in non-mycorrhizal plant (Marschner and Dell 1994), but it exceeds the root hair zone in mycorrhizal plants, which shows that unavailable P to the plant is associated with the fungal hyphae (Garg and Chandel 2010). The metabolic routes of symbiotic P acquisition begin with the absorption of inorganic P via fungal high-affinity transporters (Harrison and van Buuren 1995, Maldonado-Mendoza et al. 2001). Inside the AMF, inorganic phosphate is condensed in polyphosphate (Solaiman et al. 1999, Ohtomo and Saito 2005). The polyphosphate becomes depolymerized into inorganic P before it is released into the periarbuscular interface (Solaiman et al. 1999, Ohtomo and Saito 2005). Then, P is transferred from the interface by phosphate transporters to the cells of plant roots (Solaiman et al. 1999).

As shown in Figure 3, AMF can take up the N from organic sources, convert it into inorganic N, and transfer it to the host plant (Hodge et al. 2001). The hyphae of AMF are also able to take up NH_4^+ and NO_3^- (Johansen et al. 1996, Smith and Read 2008) and amino acids (Hawkins et al. 2000, Hodge et al. 2001) from their surroundings and translocate N in inorganic form to their host plants (Hawkins et al. 2000, Azcón et al. 2001, Vazquez et al. 2001). Assimilation of NH_4^+ is a key aspect of N absorption in AMF (Hawkins et al. 2000, Toussaint et al. 2004). NH_4^+ is taken up by the extraradical mycelium, assimilated into amino acids, translocated from extra- to intraradical fungal structures as arginine and then transported as NH_4^+ to the host plant (Figure 3) (Govindarajulu et al. 2005, Jin et al. 2005, Chalot et al. 2006).

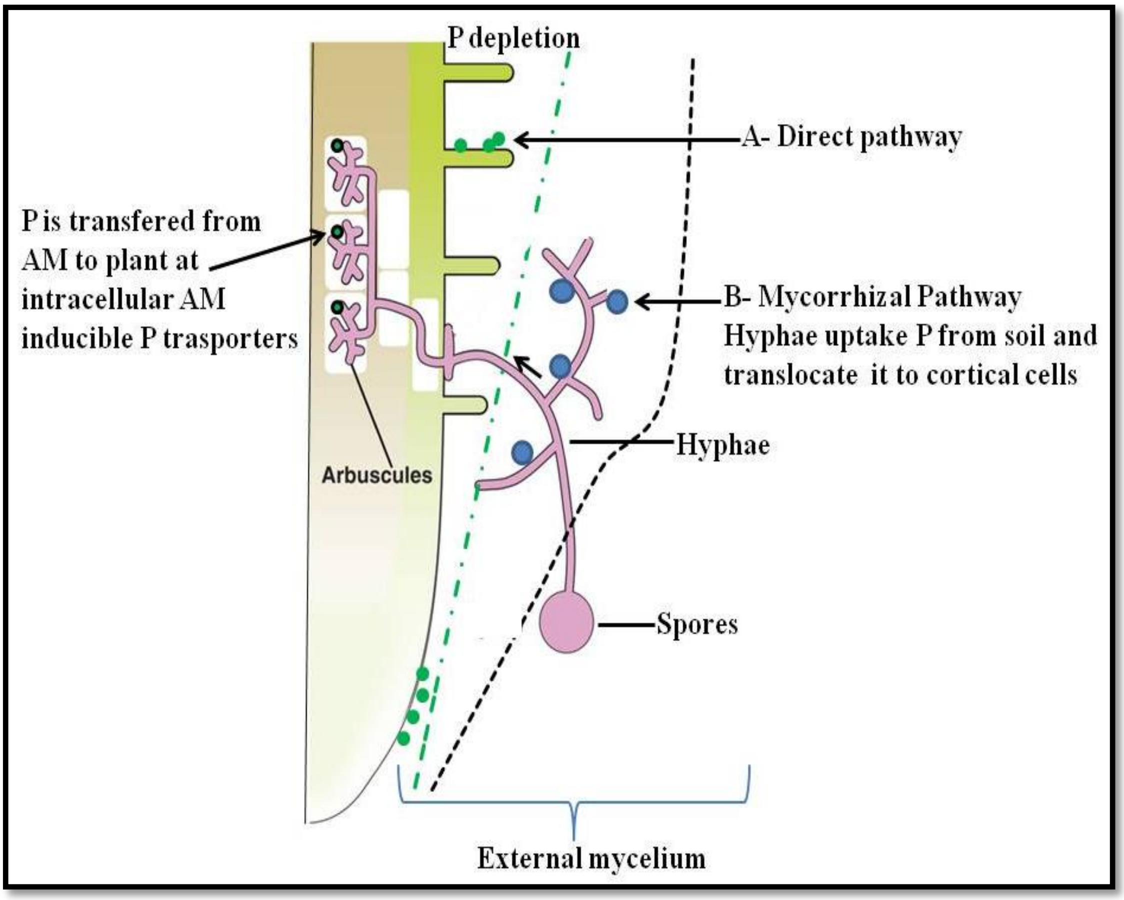

Figure 4. Direct and mycorrhizal pathways of plant P uptake (Modified from Smith et al. 2011). In the direct pathway, P is taken up from the rhizosphere by plant P transporters in the epidermis and root hairs (green circles) from the root zone. In the mycorrhizal pathway, P is taken up by fungal P transporters (blue circles, behind the root apex) into hyphae and translocated to arbuscules and hyphal coils in root cortical cells. Plant P transporters (black circle) translocate P from the interfacial apoplast to plant cortical cells.

1.4.Objectives of this study

The purpose of this study was to investigate the suitability of sewage sludge and digested sludge in comparison to synthetic fertilizer on growth and feedstock quality of maize, hemp, oilseed rape and Italian ryegrass. Further attention was paid to the role of mycorrhizae in the improvement of N and P uptake, and to the potential for uptake of heavy metals and metalloids in the feedstock as either undesirable contaminants or as evidence of phytoremediation of the soil.

The research questions were:

1. Can sewage sludge or digested sludge be used as a compatible fertilizer for bioenergy crops without synthetic fertilization?
2. Would the bioenergy crops contain heavy metals and metalloids resulting from sewage sludge or digested sludge application? Do these elements impede the growth, and which of them are translocated to the above-ground parts of plants?
3. Does the use of sewage sludge and digested sludge have significant effects on feedstock quality?

The main working hypotheses tested were:

1. Sludge application does not have adverse effect on physiological and growth parameters, and increases accumulation of biomass, heavy metals and metalloids in plant biomass.
2. Sludge improves growth and increases N and P plant uptake through N-mineralization over time and with mycorrhizal colonization. Also, it increases the density of fungal spores in sand and soil.
3. Sludge increases leaf N content, biomass accumulation and gross energy yield as well as improves feedstock quality. In addition, it saves energy used in synthetic fertilizer manufacturing.

2. MATERIALS AND METHODS

The experimental part of the work is described here as a general outline. It is presented in more details in the original publications (I-III).

2.1 Plant material and experimental design (I-III)

Experiments were conducted at glasshouse (I-II) and field (III) at Viikki Experimental Farm (60° 13' 38" N, 25° 10' 00" E, 3 m amsl), University of Helsinki, Finland, during 2008-2012. Three plant species were used in all experiments (I-III): maize (cv. Ronaldino), fibre hemp (cv. Uso 31) and oilseed rape (cv. Wildcat). In addition, Italian ryegrass (*Lolium multiflorum* L. ssp. *italicum*, cv. Barmultra) was used in publication II. All treatments for each crop species were standardized on the basis of total N as follows: maize was fertilized with 120 kg ha^{-1} of N, oilseed rape with 90 kg ha^{-1} of N, and fibre hemp and ryegrass with 60 kg ha^{-1} of N (II - III). The experiments were arranged in randomized complete block designs with three to six replicates (I-III).

Table 8. Amounts of N, P and K (kg ha^{-1}) added to soil by application of synthetic fertilizer, sewage sludge or digested sludge, on the basis of total N for each species

	Synthetic fertilizer	Sewage sludge	Digested sludge
Maize			
N	120	120	120
P	13	100	162
K	21	8	18
Hemp			
N	60	60	60
P	6	50	81
K	11	4	9
Oilseed rape			
N	90	90	90
P	10	75	121
K	16	6	12

Sewage sludge was pretreated with $FeSO_4$, while the digested sewage sludge received biological pretreatment alone.

Table 9. Plant species, treatments, analyses and measurements presented in the original publications I-III.

Paper no.	Exp no.	Treatments	Species	Measurements
I	1	Sewage sludge Sewage sludge mixed with peat	Maize Oilseed rape	Water relations Photosynthesis Whole plant leaf area Plant dry weight Ash content
	2	High sewage sludge Low sewage sludge (50% of high SS) Sewage sludge mixed with peat	Maize Hemp Oilseed rape	Water relations Photosynthesis Whole plant leaf area Whole plant biomass Elemental analysis Ash content
II	1, 2	Synthetic fertilizer, single application Synthetic fertilizer, multiple application[a] Sewage sludge Digested sludge	Ryegrass	Biomass Relative growth rate
	3, 4	Soil + synthetic fertilizer Soil + sewage sludge Soil + digested sludge Sand + synthetic fertilizer Sand + sewage sludge	Maize Hemp Oilseed rape	Germination % Shoot dry weight Root dry weight Shoot height Root length Elemental analysis N uptake P uptake Mycorrhizal analysis
III	1-3	100% synthetic fertilizer Split synthetic fertilizer (50 + 50%) [b] Split nutrient sources (50% N+ 50% SS) 100% sewage sludge High sewage sludge (150% SS) 100% digested sludge	Maize Hemp Oilseed rape	Leaf N content Leaf area index Biomass Elemental analysis N uptake P uptake Ash content C:N ratio Energy analysis

a = after each harvest, a further 60 kg N ha^{-1} was applied

b = all treatments in III were applied prior to sowing except 50% + 50% synthetic fertilizer which was applied 50% prior to sowing and 50%.at mid-season

2.2 Methodology

2.2.1 Soil analysis (I-III)

Soil samples were taken before the establishment of experiments and stored in a freezer at -20°C until chemical analysis. Samples were analysed at Viljavuuspalvelu Oy, Mikkeli (I-III) and Suomen Ympäristöpalvelu Oy, Oulu (III) (Table 10). Except for N, elements in soil in experiments during 2009 and 2010 (I, II and III) were extracted for the analysis using the ISO/IEC 17025 method, while those elements in soil in experiments during 2011 (III) were extracted for analysis using the US EPA 3051a method. The measurements were done using ICP-OES. N was analyzed by the Kjeldahl method (Bremner 1960).

Table 10. Chemical properties of soil, and sewage and digested sludge used in experiments during 2009-2011

	Soil properties				Sludge properties (I, II, III)	
	I & II	III				
		2009	2010	2011	Sewage	Digested
DM %	86.0	90.0	93.0	94.0	30.0	30.5
pH (1:5)	6.4	6.4	6.3	6.2	7.2	6.9
N	<10.0[b]	20.4[b]	14.1[b]	14.6[b]	31.0[c]	7.4[c]
P	20.0[b]	17.0[b]	19.0[b]	1650.0[a]	26.0[c]	9.9[c]
K	100.0[b]	300.0[b]	300.0[b]	8050.0[a]	2.1[c]	1.1[c]
Ca	1100.0[b]	2000.0[b]	2400.0[b]	7430.0[a]	38.0[c]	ND
Mg	110.0[b]	230.0[b]	230.0[b]	7540.0[a]	3.3[c]	ND
Na	<20.0[b]	20.0[b]	ND	550.0[a]	ND	ND
S	7.7[b]	9.0[b]	13.6[b]	1220.0[a]	ND	ND
Mn	7.1[b]	9.3[b]	4.0[b]	270.0[a]	220.0[a]	56.9[a]
Cd	0.1[a]	0.3[a]	0.6[a]	0.5[a]	0.4[a]	<0.5[a]
Cr	7.5[a]	35.0[a]	48.0[a]	72.0[a]	30.0[a]	11.0[a]
Cu	7.9[a]	53.0[a]	77.0[a]	91.0[a]	270.0[a]	88.0[a]
Pb	7.9[a]	30.0[a]	74.0[a]	97.0[a]	20.0[a]	4.9[a]
Ni	3.2[a]	14.0[a]	16.0[a]	30.0[a]	20.0[a]	5.5[a]
Zn	32.0[a]	96.0[a]	180.0[a]	90.0[a]	470.0[a]	130.0[a]
As	<5.0[a]	7.1[a]	7.8[a]	9.6[a]	5.0[a]	1.3[a]

a = mg kg^{-1} DM
b = mg L^{-1} DM
c = g kg^{-1} DM
ND = Not Detected
One L DM soil = 1.29 Kg DM soil
N was expressed as a mineral form in soil, but it was expressed as a total in sludges
Data show concentrations of soluble form of elements from N to Mn in soil 2009 and 2010 and soil used in I and II. Otherwise, the data show the total concentrations.

2.2.2 Growth and physiological measurements

2.2.2.1 Germination (II)

Germination of maize, hemp and oilseed rape was recorded at 7 and 14 d after sowing (DAS).

2.2.2.2 Water relations (I)

Leaf water potential (Ψ_w) was measured at 29, 44 and 59 DAS from the uppermost fully expanded leaf as described by Mäkelä et al. (1998) using a pressure chamber (Soilmoisture Equipment Crop., Santa Barbara, CA, USA). Osmotic potential (Ψ_s) was analysed with a freezing point depression osmometer (Micro-Osmometer 3300 M, Advanced Instruments, Norwood, MA, USA). Turgor (Ψ_p) was calculated as follows:

$$\Psi_p = \Psi_w - \Psi_s \qquad (1)$$

2.2.2.3 Photosynthesis (I)

Photosynthesis was measured at 30, 45, 60 and 75 DAS with a portable photosynthesis meter (LI-6400, LI-COR, Lincoln, NE, USA). A 2×3 cm leaf chamber with a LED light source (6400-02B, 90% red and 10% blue) was attached with the photosynthesis meter. Photosynthesis photon flux density was 1000 µmol m^{-2} s^{-1}. A CO_2-injecting cartridge was attached to the system to control reference CO_2 concentration at 400 µmol mol^{-1}. The flow rate was 400 µmol s^{-1}. The measurement was conducted between 9 and 11 am using the youngest fully expanded leaf.

2.2.2.4 Whole plant leaf area (I)

Whole plant leaf area excluding dead leaves was determined at 30, 45, 60 and 80 DAS with a portable leaf area meter (LI-3000, LI-COR, Lincoln, NE, USA).

2.2.2.5 Leaf area index (III)

Leaf area index (LAI) was measured with a SunScan portable canopy analysis system equipped with a BF3 sunshine sensor (SS1-UM-2.0, Delta-T Devices Ltd., Cambridge, UK) at 29, 44, 59, and 74 DAS.

2.2.2.6 Biomass (I- III)

Biomass samples were collected at 30, 45, 60, 80 DAS and at maturity (BBCH stage 97; Meier, 2001), 100 DAS (I). Shoots and roots of randomly selected plants (maize, hemp, and oilseed rape) were separated to measure root and shoot length, and for determination of biomass accumulation (II). Biomass accumulation of ryegrass was analysed every 20 days from sowing until senescence (at 20, 40, 60, 80, 100, 120, 140 DAS) (II). In field experiments, plants were collected from 1.0 m^2 of the plot for biomass analysis at 30, 60, 90, 120, and 150 DAS during growing seasons 2009 - 2011 (III). Samples were dried at 65 – 70°C for 2 3 days and weighed (I-III). Dried samples were ground (Retsch ZM 200, Retsch GmbH, Haan, Germany) into fine powder (0.5 mm mesh size) and stored at room temperature for elemental and energy analyses.

2.2.2.7 Relative growth rate (II)

Relative growth rate was calculated from ryegrass as follows:

$$\mathrm{RGR} = \frac{(\ln \mathrm{W}_2 - \ln \mathrm{W}_1)}{(\mathrm{t}_2 - \mathrm{t}_1)} \qquad (2)$$

Where W_1 is dry weight at time t_1, and W_2 is dry weight at time t_2.

2.3 Feedstock quality (I- III)

2.3.1 Elemental analyses

Concentrations of macro- and micro-elements (P, Ca, K, S, Si, Mg, Na, As, Cd, Cr, Cu, Mn, Ni, Pb and Zn) were determined in ground plant samples of maize, hemp and oilseed rape. Elements were analyzed at 60 DAS and maturity (I), at 14 DAS (II), and at maturity (III). Ground plant samples (300 mg) were weighed into PTFE Teflon tubes (CEM, Matthews, North Carolina, USA). On each sample, 6 mL of nitric acid (67-69%, VWR International BVBA, Geldenaaksebaan, Leuven, Belgium) and 1 mL of hydrogen peroxide (30%, Merck KGaA, Darmstadt, Germany) were added for microwave digestion (MARSXpress, MARS 240/50, CEM, Matthews, NC, USA). After digestion, samples were filtered through Whatman paper (Grade No. 42, pore size 2.5 μm, GE Healthcare Companies, UK) and diluted in distilled water up to 50 mL, and then stored at -20°C overnight. Elemental analysis was run with Inductively Coupled Plasma-Optical Emission Spectrometry (iCAP 6200, Thermo Fisher Scientific, Cambridge, UK). Cl (I and III) was analysed from ground plant samples (0.5 g) according to Mäkelä et al. (2003) using a Corning M926 chloride analyser (Corning Ltd., Halstead, Essex, UK). The total N and C contents (I-III) were analysed from ground plant samples (200 mg) by the Dumas combustion method using a Vario MAX CN (Elementar Analysensysteme GmbH, Hanau, Germany).

2.3.2 N and P uptake

N and P uptake (II-III) by maize, hemp and oilseed rape was calculated as follows:

$$\text{N or P uptake}\left(\frac{\text{kg}}{\text{ha}}\right)=\frac{\text{Biomass yield}\left(\frac{\text{kg}}{\text{ha}}\right)\times\text{N or P content in plant biomass}\left(\frac{\text{g}}{\text{kg}}\right)}{1000}\qquad(3)$$

2.3.3 Ash content

Ash content (I and III) was determined from the ground plant samples. Samples (1.0 g) were dried in an oven at 105 °C overnight. The dry weight was determined (W_1). Samples were placed in a muffle furnace (LV 15/11/P320, Nabertherm GmbH, Bremen, Germany) for 18 h at 600 °C, cooled in a desiccator, and weighed again (W_2). The ash content was calculated as follows:

$$\text{Ash (\%)}=\frac{W_2}{W_1}\times 100\qquad(4)$$

2.3.4 Energy analysis (III)

The higher heating value of crop biomass was analysed from 0.5 g subsamples of the ground biomass using an adiabatic bomb calorimeter (Parr 1241EA, Parr Instrument Co., Moline, IL, USA). Benzoic acid pellets (1.0 g, Parr Instrument Co., Moline, IL, USA) were used as standards. Samples were compressed into pellets using a Pellet Press (Parr Instrument Co., Moline, IL, USA) prior to weighing and analysis. The higher heating value (MJ kg^{-1}) was determined by complete combustion with excess O_2 at 3.04 MPa in a sealed steel bomb.

Gross energy yield was calculated as follows:

$$\text{Gross energy yield}\left(\frac{\text{GJ}}{\text{ha}}\right)=\frac{\text{Energy content}\left(\frac{\text{MJ}}{\text{kg}}\right)\times\text{Biomass yield}\left(\frac{\text{kg}}{\text{ha}}\right)}{1000}\qquad(5)$$

2.3.5 Calculations of the limiting elements for long-term sludge application

Since sludge contains non-nutrient minerals, it was necessary to estimate how long it could be used on cropland before the heavy metal accumulation would reach the limits of European regulations (Table 5; European Commission 2001, 2002). The annual load of the heavy metal to agricultural land through sludge application was calculated from the content of each element in the sludge (mg kg^{-1}, see Table 10) multiplied by the annual sludge application for each plant species (kg ha^{-1} of DM, III). The heavy metal accumulation in the whole biomass above ground at maturity was obtained from the content of each element in plant biomass (mg kg^{-1}) multiplied by the biomass production (kg ha^{-1}) (III). The soil weight (kg ha^{-1}), which depends on the bulk density and the depth of harrowing, was used in the calculation to get the remaining annual content of each element in the soil (mg kg^{-1}) after sludge application. From these details, we calculated the number of years in which sludge can be applied for the long term, as follows:

$$\mathrm{X} = \mathrm{Max}_{\mathrm{V}} - \mathrm{E}_{\mathrm{Soil}} \qquad (6)$$

$$\mathrm{Net}_{\mathrm{E}} = \frac{\mathrm{E}_{\mathrm{Input}} - \mathrm{E}_{\mathrm{output}}}{\mathrm{W}_{\mathrm{Soil}}} \qquad (7)$$

$$\mathrm{Y} = \frac{\mathrm{X}}{\mathrm{Net}_{\mathrm{E}}} \qquad (8)$$

Where: X = limiting concentration of any given element; $\mathrm{Max}_{\mathrm{v}}$ = European limits for that element in soil (mg kg^{-1} DM); $\mathrm{E}_{\mathrm{Soil}}$ = concentration of the element in the soil at sowing (mg kg^{-1} DM); $\mathrm{Net}_{\mathrm{E}}$ = the different between input and output of the element (mg kg^{-1} DM); $\mathrm{E}_{\mathrm{input}}$ = amount of element added to the soil through sludge application (mg ha^{-1}); $\mathrm{E}_{\mathrm{output}}$ = element content in above-ground biomass at maturity (mg ha^{-1}); $\mathrm{W}_{\mathrm{Soil}}$ = soil weight (kg ha^{-1} DM); Y = number of years.

2.4 Mycorrhiza analyses (II)

2.4.1 Number of fungal spores

Number of fungal spores was counted according to the modified method of Allen et al. (1979). Soil samples were taken at 15 DAS from pots using a cork borer (∅ 5 cm). Samples were mixed and sieved (1 and 63 µm). A subsample of 10 g was placed in a centrifuge tube containing 15 mL of distilled water. Soil samples were hydrated for 15 min, and centrifuged for 10 min at 2000 rpm at 10°C to remove organic matter. Samples were resuspended in 20 mL of 2 M sucrose solution and centrifuged. The liquid, containing the spores, was slowly dripped into a separatory funnel for 10 min, after which the liquid was then slowly drained (10 mL min^{-1}). Spores were carefully washed from the funnel walls with 2 mL of purified water into a Petri dish (Tissue Culture Plate 6-Well Flat Bottom, Sarstedt, USA). The number of spores was determined immediately with a stereo microscope (Leica MZ FL III, Fluorescent Stereo Microscope, Heerbrugg, Germany).

2.4.2 Mycorrhizal root colonization

Lateral and small roots were randomly sampled at 15 DAS and washed under tap water. A 1-g sample of fresh root was cut into 2 cm pieces, placed in a bottle containing 40 mL of 10% KOH (Merck KGaA, Darmstadt, Germany), and placed in a water bath at 60–90 ºC for 2–4 h (Phillips and Hayman, 1970). The samples were rinsed with 10 mL of 10% HCl (ACS reagent, Sigma-Aldrich, Germany), followed by distilled water, and stained with cotton blue (Riedel-de Haën AG, Seelze, Germany) according to Grace and Stribley (1991). Roots were viewed with light microscope (Leitz, Wild Leitz GmbH, Wetzlar, Germany) with an attached CCD camera for AM mycelium and vesicles.

2.5 Statistical analyses

Data of different traits measured were subjected to ANOVA using R program (version 2.11.1) in I, and using PASW statistics 20.0 (IBM, Chicago, IL, USA) in II-III to compare the effects of sludge and synthetic fertilizer treatments on growth and feedstock quality parameters of crops as well as mycorrhizal analysis. Significant differences between means of treatments were compared by Tukey’s test (I-III). Simple correlation coefficient was calculated in order to study the relationship between the number of fungal spores in soil and the content of P or N in the plant biomass (II). Growth of the ryegrass fertilized with sewage and digested sludge and synthetic fertilizer (II) was fitted to Gompertz curves using PASW.

$\mathrm{Biomass} = \mathrm{A} + \mathrm{C} \times \mathrm{Exp}(-\mathrm{Exp}\left(-\mathrm{B} \times \left((\mathrm{DAS} \div 20) - \mathrm{M}\right)\right)$, where A = the lower asymptote; C = the upper asymptote; B = the rate of increase; DAS = the days after sowing; M = the point of inflection.

3. RESULTS AND DISCUSSION

3.1. Growth of bioenergy crops fertilized with sludge (I-III)

In the current study, the germination percent at 7 DAS, and root and shoot dry mass at 14 DAS of maize, oilseed rape and hemp were a little higher in the sludge treatments than in those given synthetic fertilizer (II). This shows that there were no adverse effects due to the heavy metals when sludge was applied. Similarly, Qasim et al. (2001) reported that there was no significant difference between the effects of sewage sludge and inorganic fertilizers on seed germination of maize. On the other hand, seed germination percent of faba bean (*Vicia faba* L.) fertilized with untreated sludge was lower than in those fertilized with treated sludge, due to the excesses of various cations and anions (Singh et al. 2002). Also, lower seed germination of wheat (*Triticum aestivum* L.) and maize was obtained when sewage sludge was applied than in lime-stabilized and composted sludge and control treatments (Du et al. 2012). However, increasing the application of sewage, lime-stabilized and composted sludge from 3 to 6 g kg^{-1} soil, increased the germination rate of wheat and maize (Du et al. 2012). This suggests that the adverse effect of heavy metals or organic compounds on seed germination is reduced when sludge is applied according to land use standards.

Root and shoot masses of hemp and oilseed rape were improved when sewage sludge was mixed with sand in comparison to results from synthetic fertilizer (II). The increase in shoot mass may be mainly attributable to improved root growth and consequent greater nutrient transport to the above-ground parts of the plant (Zhang and Barber 1993, Durieux et al. 1994). The results of root and shoot growth (II) agree with the conclusion of Qasim et al. (2001) who reported that sludge application resulted in an increase in root and shoot growth of maize in comparison to those fertilized with synthetic fertilizer. On the other hand, root growth of plants grown with sludge extracts in another experiment was retarded, not due to the heavy metals but due to the effects of other substances, since electrical conductivity in heavy metal mixtures was lower than sludge extracts (Wong et al. 1981). Shoot mass of all plant species fertilized with either sewage sludge or synthetic fertilizer and grown in sand was lower than those grown in soil, although the N content of those species was higher when grown in sand than in those grown in soil (II). In very early stages of vegetative growth for plants such as maize, the relationship between biomass production and N concentration is different (Herrmann and Taube 2004). The higher N content could be a consequence of lower yield.

The sewage sludge-peat mixture improved maize and hemp growth in terms of net photosynthesis at 60 DAS, and in leaf area formation and biomass accumulation at most sampling dates, in comparison to plain sludge applications (I). This could be attributed to the uptake of easily absorbed plant nutrients, N and P, from soil by roots and translocation to shoots and leaves, since the increase of nutrient in leaves can increase photosynthesis (Fageria and Moreira 2011). It could also be attributed to lower heavy metal accumulation in plants fertilized with the sludge-peat mixture (I), since the peat in (I) was an adjuvant to reduce the stickiness, density and nutrient richness of the sludge. Usually, photosynthetic capacity of leaves is associated with the leaf N content, since proteins of the Calvin cycle and thylakoids represent the majority of leaf N in the plant (Evans 1989). Thus, improved photosynthesis and more efficient development of LAI for increased light interception will cause an increase in biomass accumulation in terms of converting solar radiation into stored chemical energy, i.e., biomass (Beadle and Long 1985).

The sludge added in the high sewage sludge treatment in the first glasshouse experiment was equivalent to 1200 kg ha^{-1} N, meaning 10 times normal the fertilization rate for maize, 15 times that for oilseed rape and 20 times that for hemp. The amounts of all macronutrients and trace elements were increased correspondingly. In maize and hemp, plain sludge applications resulted in a reduction in net photosynthesis at 60 DAS, and in leaf area formation and biomass accumulation at most sampling dates (I). This might be attributed to the increased Cr and Ni content in those crops over those fertilized with the sewage sludge-peat mixture (I). The disorganization of the chloroplast ultrastructure and inhibition of electron transport processes are linked to Cr uptake, and a change of electrons from the electron-donating side of photosystem-I can reduce the photosynthetic rate (Shanker et al. 2005).

Sewage sludge and synthetic fertilizer applications resulted in significant increases in leaf N content of maize and hemp, particularly at 60 DAS, in comparison to other treatments (III). Biomass accumulation of maize and hemp fertilized with sewage sludge was significantly increased at 90 and 150 DAS (III). At 120 DAS, there was no significant difference between biomass accumulation in maize whether it was fertilized with sewage sludge or synthetic fertilizer, whereas sewage sludge significantly increased biomass accumulation of hemp in comparison to all other treatments except split synthetic fertilizer (III). However, leaf N content, LAI and biomass accumulation of oilseed rape were equally high following applications of both sewage sludge and synthetic fertilizer throughout most of sampling dates (III). This could be due to the increase in mineralization of organic N during growing season into NH_4^+ which is absorbed by roots or converted into NO_3^- which is rapidly taken up by roots (Wong et al. 1998, Hernández et al. 2002, Epstein 2003, Sing and Agrawal 2008, Mbakwe et al. 2013). Leaf N content started to decline after 60 DAS in maize and hemp, and after 45 DAS in oilseed rape (III). This may have been due to the translocation of N and amino acids to the storage parts, since photosynthetic proteins and chlorophylls are degraded (Novoa and Loomis 1981, Fageria et al. 2011, Yang et al. 2012). In addition, it can partly be due to the reduction in the N-mineralization rate as the organic N content in the soil decreased over time (Wong et al. 1998). Sewage sludge improved growth of ryegrass in terms of increasing biomass accumulation and consequently increasing relative growth rate (II). Relative growth rate was highest (0.0025 g g^{-1} d^{-1}) at 100 DAS following sewage sludge application in comparison to singly applied synthetic fertilizer (0.0016 g g^{-1} d^{-1}) or digested sludge (0.0014 g g^{-1} d^{-1}) (II). It remained the highest in ryegrass fertilized with sewage sludge until the last harvest. This implies that N was mineralized from the sludge at a comparable rate to the available N from other nutrient sources.

Table 11. Biomass (Mg ha^{-1} DM) of maize, hemp and oilseed rape at maturity following different fertilizer treatments. Data show means over three years (2009-2011). S.E.M.[a] = Standard error of means for 100% SF and 100% SS (n = 12); S.E.M.[b] = Standard error of means for all treatments except 100% SF and 100% SS (n = 8).

Treatment	Biomass production at maturity (Mg ha^{-1} DM)		
	Maize	Hemp	Oilseed rape
100% SF[a]	27.4	12.6	10.2
50% SF+50% SF[b]	24.5	14.0	8.2
50% SF+50% SS[b]	28.9	13.2	8.4
100% SS[a]	29.6	15.0	9.9
150% SS[b]	28.1	13.5	8.5
100% DS[b]	27.7	12.4	7.7
S.E.M.[a]	0.9	0.42	0.14
S.E.M.[b]	1.1	0.52	0.18

100% SF = synthetic fertilizer; 50% SF + 50% SF = split synthetic fertilizer; 50% SF + 50% SS = split nutrient sources; 100% SS = sewage sludge; 150% SS = high sewage sludge; 100% DS = digested sludge.

Bleken et al. (2009) reported that N influences the plant growth and biomass accumulation through LAI by increasing radiation interception. On the other hand, N deficiency is known to decrease LAI, radiation use efficiency and photosynthesis activity in plants (Muchow 1988, Sinclair and Horie 1989, Fageria and Baligar 2005). Sludge application resulted in an increase in the biomass yield of different crops including maize and barley (Hernández et al. 1991), sunflower (*Helianthus annuus* L.) (Morera et al. 2002), rice (*Oryza sativa* L.) (Singh and Agrawal 2010), lettuce (*Lactuca sativa* L.) (Zhao et al. 2012), and ryegrass (Gu et al. 2013). Increases in biomass in the sludge treatment were usually attributed to the improvement in the soil conditions, in addition to the supply of mineralized N and other nutrients from the sludge (Christie et al. 2001, Tanu et al. 2004, Bozkurt et al. 2006). Furthermore, the increased crop biomass may have been partly as a result of increased mycorrhizal root colonization (II), which could have contributed to improved biomass through the increased N and P uptake (III).

3.2. Root mycorrhizal colonization of bioenergy crops fertilized with sludge (II).

The roots of maize and hemp fertilized with sewage and digested sludge were colonized with arbuscular mycorrhiza (II), more so in maize than in hemp. This interspecific difference may have been attributable to the morphological differences in root systems, along with qualitative and quantitative differences in root rhizodeposition attracting and promoting the growth of beneficial soil microorganisms. Hyphae and arbuscules were detectable in maize roots, and vesicles and hyphae were detectable in hemp roots when sewage sludge was applied (II). In oilseed rape roots, only vesicles were observed following sewage sludge application (II). The inhibition of root mycorrhizal colonization and the reduction of spore numbers in soil have been associated with N and P fertilizer application (Johnson 1993, Miller and Jackson 1998, Liu et al. 2000, Treseder and Allen 2002, Lin et al. 2012, Ortas 2012). The number of fungal spores in soil and sand treated with sewage sludge was 30-40% higher than in those treated with synthetic fertilizer (II). Application of P fertilizer (45 kg P ha^{-1} per year) reduced spore density in the soil by 50% over five years (Martensson and Carlgren 1994). Sewage sludge used in the current study did not contain spores. Thus, it enhanced fungal spore counts in both soil and sand due to the low amounts of inorganic N and P in sludge. Colonization was not detectable in the roots of any crop fertilized with synthetic fertilizer in sand or soil (II).

3.3. Sludge application increased gross energy yield (III)

Low biomass quality can drastically decrease the net energy output by limiting the effectiveness of conversion plants (Jenkins et al. 1998). In addition, it reduces the heating value by 0.2 MJ kg^{-1} for each 1 % increase in the ash content (Cassida et al. 2005). In the current study, feedstock of hemp fertilized with sewage sludge or synthetic fertilizer contained the highest heating value (18.1 and 17.2 MJ kg^{-1} DM, respectively) and lowest ash mass fraction (59.3 and 60.1 g kg^{-1} DM, respectively) in comparison to split synthetic fertilizer, split nutrient sources or digested sludge treatments (III). Nevertheless, the higher heating values and ash content varied little between different fertilizer treatments in maize (higher heating values 3%, ash 8%) and oilseed rape (higher heating values 10%, ash 14%) without significant differences (III). Biomass yield is considered the major factor that determines the gross energy yield (McKendry 2002). In the current study, the highest gross energy yield was obtained in maize (438 GJ ha^{-1}) and hemp (274 GJ ha^{-1}), when sewage sludge was applied. However, oilseed rape fertilized with sewage sludge and synthetic fertilizer produced equivalent highest gross energy yield (172 GJ ha^{-1}). The order of gross energy yield followed the biomass yields, maize > hemp > oilseed rape, because the feedstock of those crops had comparable higher heating value. This was due to the variation among biomass accumulation of different plant species (III), which indicates the importance of plant species biomass when it is selected for energy purposes.

3.4. Biomass quality of bioenergy crops fertilized with sludge

Bioenergy crops seem to be a good choice to be grown with sewage sludge, as the contamination of the food chain with heavy metals is avoided. Also, all mineral nutrients except N in crop feedstock can be recovered in the form of ash after thermo-chemical conversion (El Bassam 2010). This form of ash may be recyclable as fertilizers to cropland (Pels and Sarabèr 2011) if its composition is appropriate.

Sewage sludge application resulted in higher N content in biomass of maize and hemp grown in soil than synthetic fertilizer, while synthetic fertilizer resulted in higher N content in all plant species grown in sand than in those fertilized with sewage sludge (II). Generally, the N content of all plant species fertilized with sewage sludge or synthetic fertilizer and grown in sand was higher than that in those grown in soil (II). This might be due to the increase of nitrification level through oxidation of NH_4^+ to NO_3^- in well aerated soils (Chesworth 2008). NO_3^- is not adsorbed on soil particles, which means that NO_3^- in rooting zone is available for plant uptake (Chesworth 2008). Furthermore, N in sandy soils is more rapidly mineralized than in loamy and clay soils (Mengel 1996), because proteins and enzymes that are involved in the mineralization process can be adsorbed to clay minerals (Loll and Bollag 1983). The highest sewage sludge application (150%) did not result in a further an increase in N content or yield of plant biomass over that achieved at the normal application rate (III). This may be attributed to an adverse effect on nitrification accompanied by the decrease of N mineralization following the high dose (Wong et al. 1998). Sewage sludge and synthetic fertilizer applications resulted in the highest N uptake in maize (320 kg ha^{-1}), hemp (175 kg ha^{-1}) and oilseed rape (135 kg ha^{-1}) compared to other treatments (III). This implies that N was exhausted from synthetic fertilizer and sewage sludge at similar times. The highest crop N uptake with sewage sludge or synthetic fertilizer treatments was accompanied by an increase in both biomass yield and its N content. The N uptake by different crops was higher than the total N introduced with different treatments (III), showing that additional synthetic fertilizer can be added on the long-term of sludge application. The high N uptake in different plant species might was attributed to the uptake of inorganic N from the soil, mineralized N from sludge, inorganic N introduced with different treatments and N released from the microbial biomass. Also, it can partly be due to the acquisition of N by external hyphae of AMF (Figure 3). Thus, depletion of soil N by hyphae can prevent or reduce the leaching of NO_3^- and denitrification (Frey and Schuepp 1993, Smith and Read 2008).

P is an important nutrient for plant growth and metabolism and accounts 0.2% of DM in plants. However, it is one of the most difficult nutrients for plants to acquire, since it is poorly available in soil due to its low solubility (Schachtman et al. 1998). Sewage sludge resulted in the highest content of P in maize, hemp and oilseed rape at 14 DAS and at maturity (II, III). The correlation of the abundance of fungal spores in the rhizosphere with the P content of the studied plant species may show that sewage sludge addition influences soil microorganisms and their contribution to P availability by plants (II). AMF increases the plant uptake of P from soil (Figure 4). Nevertheless, the differences in P content among plant species could not be attributed to the root colonization with mycorrhizal fungi, but mainly were due to the plant species-specific demand for P. Sewage sludge application resulted in the highest P uptake in maize (57.5 kg ha^{-1}) and hemp (34.5 kg ha^{-1}), while high sewage sludge and digested sludge applications resulted in the equal highest P uptake (about 25 kg ha^{-1}) in oilseed rape (III). The high amount of P that was added through sludge application could be a primary reason, besides AMF, for the high P content and uptake in the present study. The total P applied through 100% sewage sludge application was 100 kg ha^{-1} for maize, 50 kg ha^{-1} for hemp and 75 kg ha^{-1} for oilseed rape, while the total P applied into soil through digested sludge was 162 kg ha^{-1} for maize, 81 kg ha^{-1} for hemp, and 121 kg ha^{-1} for oilseed rape. This implies that an excess of P was introduced with sewage and, especially, digested sludge (Table 8), which can increase the potential risk of P losses in runoff and leaching (Penn and Sims 2002, Kidd et al. 2007).

N and P are considered the main causes of concern regarding sludge application on cropland, due to their potential for leaching and pollution for groundwater (Chaney 1990, Korboulewsky et al. 2002). Some studies have emphasized the potential risk of excess P and consequently the leaching or runoff of P after sewage sludge application (Korboulewsky et al. 2002, Kidd et al. 2007). Usually, the amount of sewage sludge applied is based either on the required N for the plant species, as in the current study, or on total heavy metal content. The P content (26 g kg^{-1} DM) in the sewage sludge was close to the N content (31 g kg^{-1} DM), so the N:P ratio was low, and application based on the N requirements could provide excessive P (Korboulewsky et al. 2002). Adequate tissue content of N and P in higher plants is about 15 and 2 g kg^{-1} DM, respectively (Epstein 1972 and 1999). In the current study, it seems that either there was no potential risk of N leaching or N leaching was very low, since the N accumulated in biomass of maize and hemp (III) was three times more than that applied through sewage sludge or synthetic fertilizer applications (Table 8). On the other hand, based on P applied through sewage and digested sludge applications (Table 8) and on the results of P uptake (III), it is clear that more P was added than was absorbed by the crops (III). Consequently, P can accumulate in agricultural soils up to a level that can pose a potential risk to surface and ground water during long-term sludge application.

K is the second highest element absorbed by plant roots after N (Havlin et al. 2005). In the current study, split application of nutrient sources (50% N + 50% sewage sludge) resulted in the highest K uptake in maize (430 kg ha^{-1}) and hemp (171 kg ha^{-1}), while high sewage sludge application resulted in the highest K uptake in oilseed rape (141 kg ha^{-1}). These results indicate that the plants will take up more K than their needs when the K is available. This phenomenon is known as luxury uptake, and in forage grasses, high content of K in herbage might contribute to metabolic disorders in animals (Kayser and Isselstein, 2005). The phenomenon of luxury K uptake has been demonstrated in maize (Setiyono et al. 2010) and in hemp (Finnan and Burke 2013). The variation of K uptake among the different plant species could be attributed to the differences in their root structure (i.e., root density and its depth, and root hair length) (Zörb et al. 2013). The correlations between K uptake and root characteristics (i.e. hair length and density) in K-depleted soils were found to be positive in maize and oilseed rape (Jungk 2001). In the present study, the results of K uptake show that additional synthetic K fertilizer should be added to avoid the effect of K deficiency on plant growth in long-term sludge application. Alternatively, additional sewage sludge can be added to supply further nutrients, as long as the heavy metal content does not exceed the European limits (See Table 5).

Sewage sludge application improved feedstock quality in terms of relatively low contents of Cl in all plant species and alkali metals such as K and Ca in maize and hemp in comparison to synthetic fertilizer application (III). In the current study, feedstock of different crops had higher K content than other alkali metals. This was consistent with the conclusion of Baxter et al. (1998) who reported that K is considered the major alkali metal in most feedstocks. Sewage sludge resulted in lower content of Cl in feedstock of maize (58%), hemp (25%) and oilseed rape (40%) fertilized with sewage sludge than in those fertilized with synthetic fertilizer (III). Cl can act as a catalyst in association with K and Na to facilitate the transport of alkali metals from the fuel to combustor surface, where the alkali metals can react with Si and S to form sulfates or silicates, and cause corrosion and slagging of the combustor (Baxter et al. 1998, Jenkins et al. 1998, McKendry 2002). Slagging is linked to the low melting point of deposits, which results in the formation of a glassy layer on the heat transfer surfaces that has to be removed, causing extra expenses in maintenance (Reumerman and van den Berg 2002). The optimum mass ratio of C:N for anaerobic digestion in terms of methane production ranges from 10 to 30 (Schattauer and Weiland 2004). Synthetic fertilizer and sewage sludge applications resulted in the lowest and most related C:N mass ratio of maize and hemp for methane production (III). This could be explained by higher N content in maize and hemp fertilized with synthetic fertilizer than those fertilized with other treatments.

In this study, S content was 4 times higher in oilseed rape than in maize and hemp (III). This may have been due to the high demand of S by oilseed rape (3.5 g kg^{-1} in comparison with 1.2 in maize), which is demonstrated by its sensitivity to S deficiency at relatively high tissue S content (Barker and Pilbeam 2006), probably because of cysteine-rich antifungal and anti-microbial proteins and glucosinolates, synthesised in oilseed rape (Dubuis et al 2005). Na content was also higher in oilseed rape than in maize and hemp (II-III). This could be linked to mycorrhizal colonization of maize and hemp roots in the current study, since AMF can provide a protection against excessive uptake of this highly soluble cation (Muhsin and Zwiazek 2002).

Sewage sludge application increased content of heavy metals and metalloids in all three species (I-III). At 14 DAS, maize fertilized with sewage sludge had the highest Cr (4.3 mg kg^{-1}), Cu (4.6 mg kg^{-1}), Ni (2.1 mg kg^{-1}) and Pb (0.9 mg kg^{-1}) (II). Also, oilseed rape fertilized with sewage sludge had the highest Cr (3.3 mg kg^{-1}), Cu (2.9 mg kg^{-1}), Ni (2.3 mg kg^{-1}). This was similar to earlier observations, in which the contents of particularly Zn, Cu, Cr and Cd increased in plant biomass with increasing sludge application (Hernández et al. 1991, Bozkurt et al. 2006, Singh and Agrawal 2007). This might be due to the interactions of N with micronutrients. The uptake of NH_4^+ by plants decreases soil pH, which increases the uptake of most micronutrients (Fageria 2009). In the current study, crops grown on sand and fertilized with either sewage sludge or synthetic fertilizer had the highest Mn content in comparison to those grown on soil and fertilized with the same treatments. This could be attributed to increased nitrification in sand-peat mixtures, which decreased the soil pH, which in turn would have enhanced the availability of Mn to be taken up by the roots (Mukhopadhyay and Sharma 1991).

The concentration of all heavy metals in maize and hemp were higher at maturity than at 60 DAS, while heavy metal content in oilseed rape was higher at 60 DAS than at maturity (I). This could be associated partly with the shedding of leaves by oilseed rape toward maturity, and partly to the longer growing season of maize and hemp, which allowed them to accumulate more heavy metals. In the field experiments, all crops accumulated the most Cr and As when sewage sludge was applied, while maize and hemp accumulated the highest Mn when synthetic fertilizer was applied (III). Increased Mn in plants fertilized with synthetic fertilizer could partly be attributed to antagonism between Mn and Cd (Singh and Agrawal 2007), since crops fertilized with sludge in the current study contained higher Cd than those fertilized with synthetic fertilizer (III). It can also be due to the high P content in the soil treated with sludge, because plant nutrient sources that contain high P are known to aggravate the reduction of Mn content in plants such as cereal species (Kabata-Pendias 2011).

Zinc was the most heavily accumulated trace element, followed by Mn, Cu, Ni or Cr, As, Pb and finally Cd (I-III). This was attributed to their relative contents in sewage sludge, digested sludge and soil, in particular Zn and Cd, which were present in the highest and lowest concentrations, respectively in the sludge and soil. Furthermore, Zn mobility and bioavailability increased as a result of soil pH reduction, such as happens when sludge is applied into soil (Korboulewsky et al. 2002, Bozkurt et al. 2006). Moreover, Zn has lower affinity for organic matter, which makes it more available than other trace elements (Planquart et al. 1999). The content of Zn has been shown to increase in line with the amount of sludge applied to soil (Reed et al. 1991, Bozkurt et al. 2006, Bose and Bhattacharyya 2008), which indicates a reduction of the potential risk of Zn losses in runoff and leaching, when sludge is applied on cropland.

Table 12. Number of years for which sludge can be applied into soil as fertilizer for bioenergy crops without exceeding the limit values of heavy metals and metalloids in soil according to European Union regulations.

Treatment	50% N+ 50% SS	100% SS	150% SS	100% DS
Maize	650	350	150	160
Hemp	1700	680	360	400
Oilseed rape	700	360	220	240

N = synthetic fertilizer
SS = sewage sludge
DS = digested sludge

Heavy metal accumulation differed among the plant species (III). Hemp accumulated more Pb, Ni, Mn and Cu than maize and oilseed rape, whereas maize accumulated higher Cr than hemp and oilseed rape (III). This indicates that hemp can be fertilized with sewage sludge for a longer period than maize and oilseed rape without exceeding the limit values of heavy metal and metalloids in the soil set by the European Commission (2001, 2002). Given the inputs from sludge and the uptake by crops, sewage sludge can be used as fertilizer on the same soil for many centuries (Table 12), particularly for hemp. Copper was the first limiting element in these calculations, owing to its high content in sewage sludge (270 mg kg^{-1}) in comparison to the content of Cr (30 mg kg^{-1}) or Ni (20 mg kg^{-1}).

This shows that there is a risk of Cu leaching and losses into ground water from the soil treated with sludge. However, P accumulation in soil can limit sludge application sooner than heavy metal accumulation in soil.

4. CONCLUDING REMARKS

The growth of maize and hemp was improved when sludge was applied, possibly due to mycorrhizal colonization of crop roots linked to sludge application and partially to the slow release of the different nutrients, in particular N and P, from their organically bound forms over time. The occurrence of fungal spores in soil across the different treatments correlated well with plant P content, which indicates a beneficial effect on P plant uptake.

The results demonstrated that there was no difference of N availability in soil treated with sludge or synthetic fertilizer, so mineralization of organic N originating from sludge was sufficient for growth. This was indicated by the high leaf N content of maize and hemp fertilized with sewage sludge or synthetic fertilizer in comparison to other treatments particularly at 60 DAS. Sewage sludge improved crop growth in terms of increased biomass accumulation in maize and hemp. Sewage sludge significantly increased gross energy yield of hemp in comparison to other treatments, while sewage sludge and synthetic fertilizer resulted in nearly equivalent gross energy yield in maize and oilseed rape whereas other treatments yielded less.

Sewage sludge application improved biomass quality in terms of reducing the content of alkali metals, Cl, Si, S and ash. In addition, sewage sludge and synthetic fertilizer applications resulted in the optimal C:N ratio of plant biomass for methane production in comparison to other treatments. Crops fertilized with sewage sludge accumulated higher contents of heavy metals and metalloids in their biomass than those fertilized with other treatments. According to the current study, Cu is the first heavy metal that can limit sludge application on the long-term due to its high content in sludge and soil.

Using sludge as fertilizer for bioenergy crops (Figure 5) could be a more suitable option than using it for food crops, since heavy metals can be transferred to the food chain, which would pose a serious risk to human health. Using sludge as fertilizer for bioenergy crops would provide an opportunity to reduce the use of industrially synthetic fertilizer, which consumes large amounts of fossil fuel, and improve the sustainability in agriculture. However, sludge application to cropland can be associated with some potential problems such as ground and surface water contamination, and introduction of pathogens, antibiotics and organic chemicals which are difficult to remove. According to the current study, the most limiting heavy metal that in the long-term use of sludge was

Cu, due to its high content in sludge and soil. There was a potential risk for P loss that could be a serious environmental problem in long-term sludge applications. However, there was no indication for a potential risk of N leaching, since sludge was added on the basis of required N for each species.

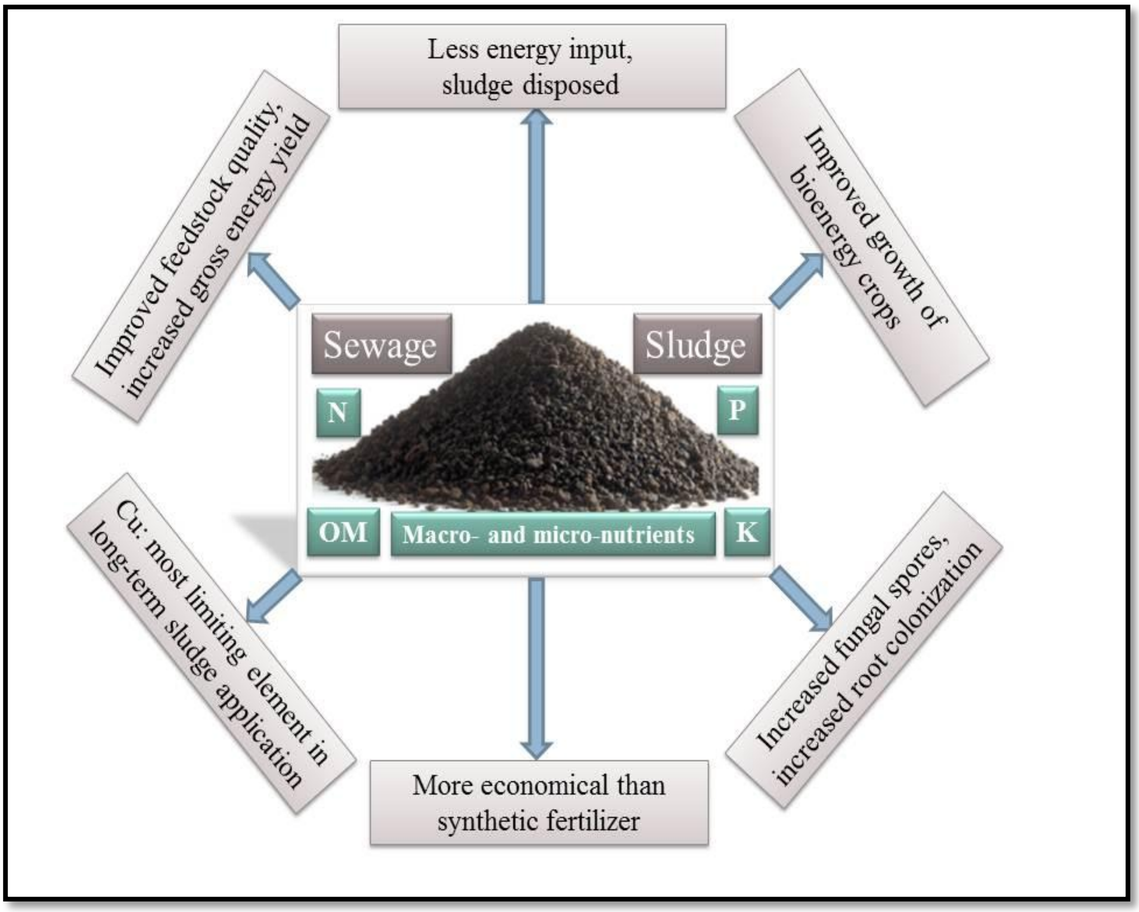

Figure 5. The beneficial effect of using sludge as fertilizer for bioenergy crops

5. ACKNOWLEDGEMENTS

With finishing this dissertation, firstly my thanks go to my supervisor Prof. Pirjo Mäkelä for her help, encouragement, and friendship. Indeed, I could not finish this work without her encouragement. There are not enough words to express how much she has done for me by always encouraging forward and sharing her endless ideas. I am deeply grateful to my other supervisors, Dr. Frederick Stoddard, Dr. Arja Santanen for their calm and always supportive attitude towards my work. I am very grateful to all of you for sharing your vast experience with me and spending pleasant moments discussing numerous issues. I wish to thank Prof. Markku Yli-halla, Prof. Juha Helenius, Dr. Jouko Kleemola, Dr. Seija Jaakkola, Dr Päivi Ekholm, Prof. Helinä Hartikainen and Dr Kari Jokinen for sharing their expertise of preservation and giving valuable opinions during the work.

My warm thanks also go Petra Egilmez for always being there when I needed help in my studies when I came to Finland. I owe endless thanks to our group of crop sciences for living this experience with me. Also, thanks to all short-term visitors, as well as all my colleagues in the C-building. They made it worth it to come to the office with the nice atmosphere. I would also like to thank the staff and colleagues at the Department of Agricultural Sciences for supportive attitude towards my work and for many pleasant and memorable moments.

I want to thank all of my present and former colleagues in Viikki. I have been fortunate to get to know so many great people during these years, and it has been a pleasure working with you. I am grateful to the office neighbours. Thanks for all other staffs for their hidden work that made my work much easier. I wish to express my gratitude to Marjo Kilpinen and Ritva Löfman for help in the laboratory. My special thanks to Markku Tykkyläinen, Jouko Närhi, Miika Kahelin and Sini Lindström for their help in the field and glasshouse.

My warmest thanks go to Prof. Marc Janssens, Prof. El Shawadfy Mousa, Prof. Shaaban El-Shamarka, Dr. Juan Carlos Torrico, Dr. Osama Ali, Dr. Khaled Abass, Dr. Nagy Elkalashy, and Dr. Muzamir Esa. I thank my Professors and colleagues in the Faculty of Agriculture, Minufiya University, Egypt. Also, the Sector of Cultural Affairs and Missions in Egypt is acknowledged. Special thanks go to my supervisors on my M.Sc. thesis, Prof. Sayed Abd El-aal and Prof. Mahmoud Ibrahim for supporting me and helping

me from the first step. I give special thanks to the current president (Prof.Sobhy Ghoniem) and former presidents (Prof. Abbas Elhefnawy, Prof. Mohamed Abd El Maksoud, Prof. Ahmed Zaghlol) of Minufiya University, and many thanks to the Dean of Faculty of Agriculture (Prof. El-Said Khalifa) and the Head of Crop Sciences Department (Prof. Abu Zaid Khalil), Minufiya University.

Finally, and most important, I would like to thank my dear wife Dr. Shaimaa Ali Selim, and our little sweethearts Mohamed and Maiar for their love, encouragement, understanding and reminding me what is important in life. For these years, my wife has continuously supported me and taken care of our children, and they always bring me so much happiness and enjoyment, which are the driving force for my work, in particular, when I meet with difficulties. I always remember the love and concerns from my father and mother, my sisters and brothers and their full support to my study and work. For these years, my late father-in-law, my mother-in-law, brothers-in-law, and sister-in-law have all shown great interest in my dissertation work and offered hearty support, being an encouragement and spur for my work. Here, I would like to express my heartfelt gratitude and thanks to them.

This work was financially supported by CIMO Finland, Ympäristöstipendirahasto EKOKEM, Niemi Foundation, OLVI-säätiö, The Finnish Cultural Foundation, August Johannes ja Aino Tiuran Maatalouden Tutkimussäätiö and Department of Agricultural Sciences, University of Helsinki. Also, Helsingin Seudun Ympäristöpalvelut–kuntayhtymä HSY, Jätevedenpuhdistusosasto, Lahti Aqua, and Envor Biotech Oy are acknowledged for providing the sludge.

Helsinki, Finland, 2014 *Mahmoud Fathy Mahmoud Mohammady Seleiman*

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