Czech University of Life Sciences Prague

Faculty of Agrobiology, Food and Natural Resources

Department of Agroenvironmental Chemistry and Plant Nutrition

The Effect of Processed Sewage Sludge on the Growth and Accumulation of Nutrients and Pharmaceuticals in Maize Tissues in the Field Experiment

Master´s thesis

Author: Nang Sreynet
Natural Resources and Environment

Supervisor: prof. Ing. Pavel Tlustoš, CSc
Co-supervisor: Ing. Filip Mercl, PhD

Declaration

I hereby declare that I have authored this master's thesis titled "The Effect of Processed Sewage Sludge on the Growth and Accumulation of Nutrients and Pharmaceuticals in Maize Tissues in the Field Experiment" independently under the guidance of my supervisor. Furthermore, I confirm that all information sources I quoted are cited and acknowledged in the reference at the end of the thesis. Being an author of this master's thesis, I further proclaim that I have not infringed the copyrights of third persons in connection with its creation.

Prague, April 26, 2021 Nang Sreynet

Acknowledgements

Firstly, I would like to express my greatest gratitude to my research supervisors, prof. Ing. Pavel Tlustoš, CSc and Ing. Filip Mercl, PhD., for their precious guidance, enthusiastic encouragement and always generously contributing fruitful advice for this research work. I would like to thank all technicians of the laboratory of the Department of Agroenvironmental Chemistry and Plant Nutrition for their assistance during this study. My grateful thanks also extend my gratitude to brother Thai Saven for his support for statistical data analysis and Sister EK Sreykuoch for the consulting.

Secondly, I would like to enormously thank the Czech Government for providing scholarship to Cambodian students to study in the Czech Republic. This scholarship faithfully helped many Cambodian students to fortify their abilities, knowledge and perceptions as well. To me, this scholarship is like a golden opportunity that I obtained.

Last but not less, I would like to thank all my family members and friends. Nobody has been more important to me in the pursuit of this study than my mum and dad. I would like to thank them; their love and guidance are always with me wherever I go, whatever I pursue, no matter how big it is. I also wish to thank Brem for the love, support and motivation.

The Effect of Processed Sewage Sludge on the Growth and Accumulation of Nutrients and Pharmaceuticals in Maize Tissues in the Field Experiment

Summary

The rapid increasing of world population, urbanization, and industrialization resulting in significant increase of wastewater treatment plants and large quantities of sewage sludge production. Land application is generally considered as the most economical and beneficial way of sewage sludge disposal. Sewage sludge is nutrient-rich organic materials which can supply additional nutrients to soil and plants such as N, P , and in lesser extend K as well as other nutrients. Sewage sludge or biosolids were applied as fertilizer after drying and torrefaction (300 $^{\circ}$C). Precise field experiment on Cambisol was set up in 8 treatments, each in 4 replications. Maize was grown on the soils with biosolids amended in two rates, control treatments with N or NPK application were also set up. During the vegetation period, maize aboveground biomass was harvested and analyzed for the concentration of nutrients N, P, K and pharmaceuticals. There were 44 pharmaceutical compounds detected in dried biosolids, however, after torrefaction the total pharmaceuticals contents in biosolids were reduced by 92.2 to 99.5%. In all treatments, pharmaceuticals were found below the detection limit in maize tissues at every harvest period. The accumulation of N, P, K were found with the highest concentration in maize tissues grown on the soil amended with dried biosolids compared to torrefied ones. However, except K ,there were no statistically significant differences of N and P accumulation among the treatments,. Based on our found results, soil amended with biosolid potentially increased the maize yields as well.

This study showed that torrefaction had the ability to remove pharmaceuticals in high amounts, minimize further uptake of pharmaceuticals by plants, and increase aboveground biomass production. Nonetheless, similar to the use of the commercial fertilizers, biosolids could serve as an acceptable source of plant nutrients.

Keywords: Sewage sludge, thermal treatment, field experiment, maize

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1. Introduction

Wastewater is produced worldwide by anthropogenic activities, and commonly treated in municipal wastewater treatment plants (WWTPs) utilizing different techniques. Throughout the biological treatment of wastewater using activated sludge process, huge amounts of sewage sludge are unavoidably generated as byproduct (Wu et al., 2012). The actual rapid increasing world's population, urbanization and industrialization result in significant increase of wastewater treatment plants and hence, large quantities of sewage sludge production, leading to a need of appropriate disposal (Kelessidis & Stasinakis, 2012).

Disposing the sewage sludge in agricultural fields is the most common alternative application due to its rich sources of organic matter and nutrients with particular emphasis on nitrogen (N) and phosphorus (P), which are essential for plant growth and development (Razaq et al., 2017). Unfortunately, besides nutrients and organic matter rich materials, sewage sludge also contains high levels of toxic compounds such as heavy metals and organic contaminants. These compounds are significantly considered to have a potential harmful effect on soil, vegetation, animal and human. According to the principal of sewage sludge directive 86/278/EEC, the use of untreated sewage sludge in agriculture is prohibited, unless it is undergoes treated to meet a certain regulatory criteria such as biological, chemical, heat-term or other any appropriate process to minimize its harmful effect resulting from its utilization (Fytili & Zabaniotou, 2008).

Due to its efficiency in the volume of waste reduction and toxicity, the development of thermochemical conversion approaches such as pyrolysis has received significant attention by many researchers. Sewage sludge pyrolysis is a thermal cracking process, involves heating of sewage sludge in an oxygen-free atmosphere and consequently converts solid organic matter into gas, bio-oil and biochar, which is usually used as soil amendment in agricultural land (Barry et al., 2019). Majority of pollutants, however, such as heavy metals and pharmaceuticals originally contained in the sewage sludge still concentrated in biochar (Lu et al., 2016).

A major public concern regarding agricultural applications of treated sewage sludge or biosolids is the introduction of pharmaceuticals and other contaminants into food-chain via plant uptake, translocation and accumulation in plant tissues. Human exposure to contamination of

food by these pharmaceuticals compounds was likely to be low through daily consumption of crops grown in treated sewage sludge but chronic or long-term exposure may pose potential health risks to humans (Alenzi et al., 2021; Liu & Wong, 2013).

2. Scientific Hypothesis and Objectives

2.1. Hypothesis

- The accumulation of pharmaceuticals in plant tissues of maize is low.
- The accumulation of pharmaceuticals in sludge can differ if thermal treatment is made.
- Pharmaceuticals in sludge cannot affect the content of nutrients in plant tissues, if the rates of sludge are realistic.

2.2. Objectives

The aim of this study is to investigate the effect of thermal treated sewage sludge on the growth as well as the accumulation of nutrients and pharmaceuticals in maize tissues in field experiment. The main objectives of this research are:

- To determine the accumulation of nutrients N, P, K and pharmaceuticals in plant tissues of maize (biomass and grains).
- To determine the accumulation of pharmaceuticals in proceeded sewage sludge by thermal treatment compared to non-treated sewage sludge.
- To evaluate the effect of pharmaceuticals in sewage sludge on the content of nutrients in plant tissues.

3. Literature Review

3.1. Sewage Sludge Production

Sewage sludge is commonly referred to a byproduct obtained from the municipal wastewater treatment facilities. Wastewater treatment is the process of eliminating the contaminants and cleansing solid particles from the wastewater before releasing it into the water bodies such as seas, lakes, and rivers (Demirbas et al., 2017). This process contributed to

improvement of aqueous environment due to the implementation of the Urban Waste Water Treatment Directive (91/271/EEC), which was adopted by European Commission in 1991. Wastewater usually comes from domestic effluent (kitchen, bathing, toilet), industrial effluent, water discharge from institutional, commercial and establishments, including hospital, agriculture, stormwater, as well as from urban runoff (Mateo-sagasta et al., 2017). As suspended material from wastewater treatment, the sewage sludge is constituted by a complex and heterogeneous mixture of microorganisms, organic matter such as hydrocarbons, amino acids, proteins, fats, humic substances together with undigested organics of lignin and cellulose (residues from fecal matter, oil, plants, paper) with a wide variety of inorganic matter (Folgueras et al., 2013).

Figure 1 shows the various sources of wastewater, which normally discharge from domestic households, industrials, hospitals, agriculture, stormwater and urban runoff. The wastewater is collected and treated in WWTPs. After treatment processes, treated wastewater is released into the aquatic environment and the resulting sewage sludge is subsequently treated and processed for final disposal or reuse.

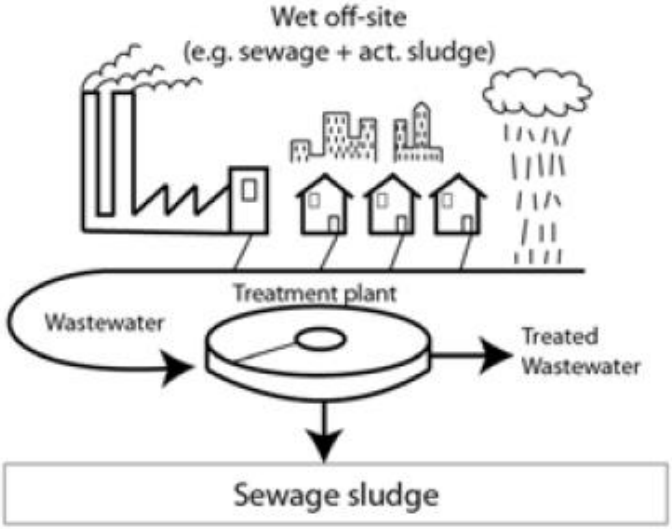


Figure 1 Source of wastewater and sewage sludge
(Mateo-sagasta et al., 2017)

Along with rapid increase of world population, industrialization, and urbanization resulted in considerable increase quantities of sewage sludge production in recent years. In the last decade, regarding to the implementation of Urban Waste Water Treatment Directive (91/271/EEC) introduced EU countries to improve water quality led to increase number of

wastewater treatment plants (WWTPs) and thus, in sludge production (Kelessidis & Stasinakis, 2012; Folgueras et al., 2013). As reported by the EU commission, more than 10 million tons of dry solid (DS) matter of sewage sludge annual production is estimated as produced by 26 EU Member States (Bianchini et al, 2016).

Figure 2 demonstrates the list of selected countries in the EU with the amount of sewage sludge production. Corresponding to the population, it is possible to assume that Germany is the highest sewage sludge producer, followed by the United Kingdom, and France. The sludge production of these three countries is 1000 – 25000 (10^3 ton DS/year). In contrast, countries with the lowest value of production are Slovakia, Estonia, and Latvia; the production is 25 – 50 (10^3 ton DS/year). Almost 75% of the EU sewage sludge is generated from five countries including Germany, United Kingdom, France, Spain, and Italy (Kacprzak et al., 2017).

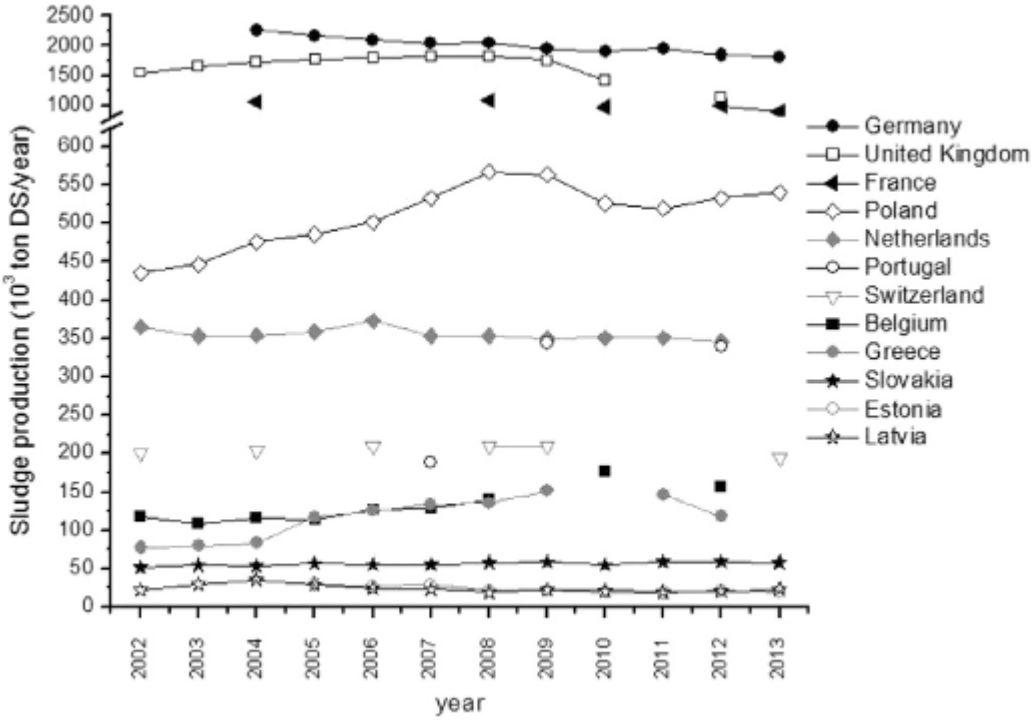


Figure 2 Production of sewage sludge in some countries of EU (Kacprzak et al., 2017)

3.1.1. Basic technological steps for sewage sludge production

As the residues originated from the process of wastewater, sludge production strongly depends on wastewater treatment systems designed for the treating as well as the separating of

liquid and solid phases. The liquid waste is being discharged into aqueous environment, while solid waste is stepped for further treatment and final disposal (Fytili & Zabaniotou, 2008). Basically, sewage sludge can be composed of primary sludge, which derived from the sedimentation or suspended solids of raw materials in a primary settlement tank and the secondary sludge, also known as activated sludge is made up of excess biomass due to microbiological activities. The resulting sludge is undergoing further treatment for final disposal, land application, or reuse in agriculture (Demirbas et al., 2017).

Figure 3 shows the simplified process of wastewater treatment and generated types of sludge in WWTPs. Firstly, entering raw wastewater is screened to remove the larger suspended or floating solids in the grid chamber of preliminary treatment. Then, water is discharged to the primary treatment stage, where most of the settleable solids are removed from the wastewater by simple gravity sedimentation. Therefore, primary treatment steps consist of settling tanks, floatation or clarifier tanks, which send separated solid (primary sludge) to digest units and liquid to following microbiological treatment units in the secondary treatment stage. Secondary sludge or biological sludge is the resulting from the uses of microbial activities, under varying growth conditions, to biochemical decompose organic compounds in the waste which proceed from primary treatment. According to each stage, the sludge will produce different characteristics of biosolids due to the origin of wastewater (Youcai & Ziyang 2017; Lakatos, 2018).

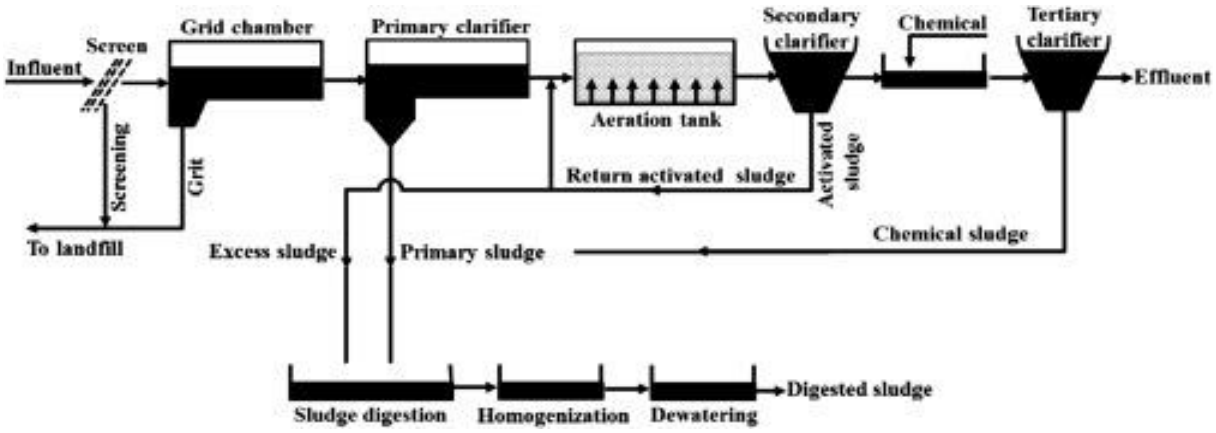


Figure 3 Sewage sludge generation process in wastewater treatment plants (Youcai & Ziyang, 2017)

3.2. Sewage Sludge Composition

The composition of wastewater contains approximately more than 95% of water and the resting part includes organic and inorganic compounds, suspended solids and dissolved materials, along with microorganisms. According to these mixing compositions, thus wastewater needs to be treated. To know the properties and components of the sewage sludge are comprehendible in the terms of mass and volume, this fundamental term is necessary in order to understand the correlation between the solid level and water content in the sludge. The water content and solid level may impact on the mechanical properties and management processes as well as the final disposal of the sewage sludge (Sperling, 2007).

Table 1 shows the connection between water content, dry solid content and the mechanical properties in most forms of sludge.

Table 1 Relation between water, dry solids content and mechanical properties of sewage sludge

Water content (%)	Dry solids content (%)	Mechanical properties of sewage sludge
100 – 75	0 – 25	fluid sludge
75 – 65	25 – 35	semi-solid cake
65 – 40	25 – 60	hard solid
40 – 15	60 – 85	sludge in granules
15 - 0	85 - 100	sludge disintegration into a fine powder

(Sperling, 2007)

Sewage sludge is determined as a heterogeneous substance comprising a mixture of many components including pathogenic organisms, pollutants, mineral nutrients, carbohydrates, proteins, fats, cellulose and lignin as well as other varieties of inorganic matter. In general, these kinds of components can be categorized into different six groups (Shao et al., 2010; Zaker et al., 2019):

1) Nontoxic organic carbon compounds, mostly from biological origin (60 % in dry basis)
2) Components containing of nitrogen and phosphorus
3) Toxic inorganic pollutants comprise mainly of poisonous elements, heavy metals such as Cadmium, Lead, Copper, Zinc, Nickel, Mercury and Chromium, which restrict the application of sewage sludge for agriculture purpose; and inorganic compounds refer to compounds that containing calcium, magnesium and silicates

4) Organic contaminants identified in it includes dioxins and furans, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), brominated fire retardants (BFRs), organochlorine pesticide, phenols and their derivative
5) Microbiological pollutants or pathogenic organisms such as virus, bacteria, protozoa, parasitic nematodes and fungi.
6) Pharmaceuticals and personal care products (PPCPs) together with water.

Normally, the major composition of sludge comprises on average 50 – 70% matters (OMs) and 30 – 50% mineral components (contains 1 – 4% of organic carbon). As already mentioned above, the present of these various contents and properties in the sludge is based on the original wastewater source and the method of the sewage sludge treatment in the facilities as shown in table 2 (Kacprzak et al., 2017).

Table 2 shows the basic chemical composition of untreated, digested primary sludge and secondary sludge from municipal wastewater process.

Table 2 Basic characteristics of municipal sewage sludge

Parameter	**Type of sludge**		
	Untreated primary sludge	Digested primary sludge	Secondary sludge
Total dry solids (TS, %)	2 -8	6 - 12	0.8 – 1.2
Volatile solids (% of TS)	60 – 80	30 – 60	59 – 88
Grease and fats (% of TS)	7 – 35	N/A	5 – 12
Protein (% TS)	20 – 30	15 – 20	32 – 41
Cellulose (% of TS)	8 - 15	8 – 15	7 – 9.7
Nitrogen (N, % of TS)	1.5 – 4	1.6 – 6	2.8 – 11
Phosphorus(P_2O_5,% of TS)	0.8 – 2.8	1.5 – 4	2.4 – 5
Potassium (K_2O, % of TS)	0 – 1	0 – 3	0.5 – 0.7
pH	5 – 8	6.5 – 7.5	6.5 - 8

⁎TS: total dry solid

(Kacprzak et al., 2017)

3.2.1. Nutrients content in sewage sludge

Most of the nutrients in wastewater as well as in the generated sewage sludge come from human excreta. The excretion of nutrients per capita particularly depends on diets, especially protein consumption which differ with countries, culture and wealth status. Nevertheless,

phosphorus presence in wastewater does not come only from human excreta but also comes from detergents used for laundry and dish washing (Mateo-sagasta et al., 2017).

Table 3 provides average values of nutrients production in human excreta, the results showing that most of nutrients are in urine.

Table 3 Typical nutrients production in human excreta (kg/cap/year)

Nutrient	In urine (500 l/year)	In feces (50 l/year)	Total
Nitrogen (N)	4.0	0.5	4.5
Phosphorus (P)	0.4	0.2	0.4
Carbon (C)	2.9	8.8	11.7

(Mateo-sagasta et al., 2017)

Sewage sludge has potential fertilizing properties and can be used to enrich agricultural soils due to contain of plant nutrients, with particular emphasis on nitrogen (N), phosphorus (P) and organic matter content (Fytili & Zabaniotou, 2008). The nutrients and organic matter are two principal elements that make the spreading of this kind of waste on ground surface suitable as a fertilizer or as an organic soil improver, routinely after meeting hygienic standard treatment or composting (Poykio et al., 2019). On the average, one ton of dry sewage sludge can yield 200 Kg of organic matter, 6 Kg of nitrogen, 8 Kg of phosphorus, and approximately 10 Kg of other different soluble salts (Iticescu et al., 2018). The nutrient contents of the sewage sludge vary considerably in the ranges of < 1 to 176 g/Kg for nitrogen, < 1 to 143 g/Kg for phosphorus and 0.2 to 26.4 g/Kg for total potassium. Nutrient concentrations in the sewage sludge would not be expected to change that much over time, different from trace element concentrations that could be declined (Pierzynski, 2015). Based on the results from a research of sewage sludge composition and characteristics, which produced by Jinan Guangda Sewage Plant in China, showed that the sludge contains large amounts of organic matter, N, P, K and other nutrients. In the dry matter of sewage sludge, there is 13.8 – 17.9 % organic matter, 16 – 31.8 g/Kg of total nitrogen, 6.8 –13.1 g/Kg of total phosphorus and 2.2–3.1 g/Kg total potassium. For available nitrogen, phosphorus, and potassium were respectively: 2.14 g/Kg, 0.17 g/Kg and 0.34 g/Kg (Tao et al., 2012).

Among macronutrients, nitrogen is one of essential nutrients for plant growth and development. The considerable availability of the nitrogen in sewage sludge is primarily derived from the protein in the source material, which results from the activity of microorganisms

(mainly bacteria) used for water purification. These microorganisms contain huge amounts of organic macromolecules that are linked by amide bonds or peptides (Djandja et al., 2020). The nitrogen in sludge becomes available for the processes of nitrification, denitrification, immobilization, volatilization and mineralization in the soil nitrogen cycle beyond the sewage sludge has been applied (Pierzynski, 2015). The total proportion of nitrogen can vary between < 0. 1 to maximum 18 %; while levels of mineral nitrogen can rise up to 6.7%.

The presence of phosphorus in the sewage sludge, existing in both organic and inorganic forms, but inorganic form is generally predominant. Similar to organic nitrogen, organic phosphorus typically must undergo mineralization in the soil before the phosphorus is available for plant uptake. The phosphorus content in sewage sludge as dry weight can range from 0.1 to 14% combined with significant amounts of other nutrients (Zaker et al., 2019).

As has been reported in many researches, sewage sludge is, however, considered to be a poor source of plant available potassium with respect to nitrogen and phosphorus when evaluating sludge as a fertilizer material. Potassium is a common soluble constituent in the sludge and when relatively high potassium concentrations >10 g/Kg are found in the sewage sludge, this usually reflects the sewage sludge with a low solids content which has been dried down before the analysis. Anyways, the potassium in sewage sludge is principally assumed to be a 100% available for uptake by plant (Pierzynski, 2015).

In table 4 shows the total content of macronutrient, nitrogen, phosphorus and potassium contained in the dry sewage sludge.

Table 4 Total nutrient contents of N, P, K in the sewage sludge from different WWTPs

Reference	**Nutrient contents (mg/Kg dry matter)**		
	N	P	K
(Poykio et al., 2019)	39800	20600	1810
(Tao et al., 2012)	22000	6800	2300
(Singh & Agrawal, 2010)	-	716.7	208.96
(Martínez et al., 2003)	17600	3700	5000
(Guoqing et al., 2019)	13600	17900	2300
(Zittel et al., 2020)	26900	3600	54600
(Černe et al., 2019)	3300	7100	1100
(Demirbas et al., 2017)	4500 – 4900	2200 – 3000	1200 – 1600

3.2.2. Heavy metals content in the sewage sludge

Sewage sludge is considered as organic waste and a good source of plant nutrients such as nitrogen, phosphorus, calcium, magnesium, and iron along with other organic constituents (Poykio et al., 2019). Meanwhile, due to the physical and chemical processes that are involved in wastewater sludge treatment, the sewage sludge may tend to accumulate heavy metals, many of which are classified as high level toxic present in the wastewater. The amount of heavy metals and organic contaminants are mostly originated by industrial activities (Singh & Agrawal, 2010). Heavy metals such as Pb, Cr, Zn, Cu, Cd, Ni and Hg are crucial elements which restrict and need to meet a certain regulatory criteria for sewage sludge utilization in agricultural land. These heavy metals are distinguished for their capability accumulation in human tissues and biomagnification through the food-chain, which can pose both environmental and human health risk. The mobility of heavy metals and their bioavailability related to plant toxicity is strongly based on the binding patterns or specific chemical forms which are found in the sewage sludge (Fytili & Zabaniotou, 2008). However, during the treatment process, approximately 50 – 80% of heavy metals content existing in wastewater is fixed directly into the sludge, and this is why some specific metals present high concentration in the sludge (Agrafioti et al., 2013).

Table 5 and 6 are shown the various average concentrations of heavy metals found in different sewage sludge analyzed during pyrolysis researches, which focus on minimizing any potential health risk and to improve the sewage sludge characteristics associated with its disposal of produced biochar into the agricultural land, landfilling, and incineration plant. Based on these studies, it can be proved that the sewage sludge composition and heavy metals can fluctuate considerably depending on its different wastewater treatment plants and the origination (Barry et al., 2019).

In general, wastewater from residential areas contains low heavy metals content compared to the discharge of industrial wastewater into the urban wastewater treatment plants. Anyway, the mixing of both domestic and industrial wastewater would drastically increase some specific heavy metals content in the sewage sludge (Lu et al., 2016).

Table 5 shows the average concentration of potentially toxic elements in different sewage sludge samples. According to the result in the table, it can be assumed the content of heavy metals sequence as follows: Zn > Pb > Cu > Cd > Cr >Ni

Table 5 Concentration of potentially toxic elements in the sewage sludge

Reference	Element (mg/Kg dry matter)					
	Zn	Pb	Cd	Cu	Ni	Cr
(Poykio et al., 2019)	350	10.0	0.50	370	14.0	29.0
(Werle et al., 2017)	544.01	26.44	-	37.05	8.04	24.10
(Lu et al., 2016)	735 ± 14	3740 ± 27	169 ± 4.3	169 ± 4.3	72.4 ± 2.8	100 ± 2.0
(Tao &Wu, 2012)	360	36	< 1	55	51	60
(Agrafioti et al., 2013)	-	91.23	0.78	176.51	22.86	23.86
(Inguanzo.,Pis, 2002)	1707	95	-	179	18	32
(Singh et al., 2010)	785.3±16	60 ± 5.7	154 ± 2.5	317.7±1.92	47.17±0.32	35.5 ± 0.76
(Jin et al., 2016)	2579±106	9511 ±2.5	-	1217 ± 29	121.1± 6.23	449.2 ±25

Table 6 shows the comparison of heavy metals contents from different sources of biomass materials accompanying the sewage sludge, paper sludge, wheat straw, beech wood as well as recovered fuel. The different content of heavy metals is principally connected to the origin of the sludge (Raheem et al., 2018).

Table 6 Potentially toxic elements contents in the sludge from different biomass materials

Feedstock	Heavy metal (mg/Kg dry matter)							
	Cd	Cr	Cu	Hg	Ni	Pb	Zn	As
WAS	< 1-3410	10-990 000	80-2300	2.7	2-179	13-465	101-49000	3-230
Paper sludge	< 0.4	110	310	1000	-	160	470	8
Paper sludge	350	100	450	-	480	480	170	-
Wheat straw	1.0	25	0.06	6	-	-	-	0.18
Beech wood	1.0	2.5	43	0.12	-	33	15	3.5
Recovered fuel	24	1020	2800	-	209	1100	-	37

* WAS: waste activated sludge (Raheem et al., 2018)

3.2.3. Pharmaceutical products content in sewage sludge

Besides heavy metals, another important group of pollutants which is present widely in sewage sludge are pharmaceuticals and personal care products (Kodešová et al., 2019). In reality, also like other toxic compounds, pharmaceuticals are leaching into the environment and discharge into the wastewater through human activities. These pharmaceuticals compose a mixture heterogeneous group with different structures, function and properties. Diverse group of medicines, cosmetics, bouquets, clean-up products and natural or synthesized hormones are

considered as emerging pollutants (EPs) which have received the most interest and have been the topic of the most depth research during the 1990s. One of the critical issues is that wastewater treatment processes are not capable of eliminating various kinds of pharmaceutical products for the reason that they were designed to remove organic matter and nutrients. As a result, these emerging contaminants are accumulated in the sewage sludge having adverse effects on their receiving environment (Lemus & Serna, 2020).

The primary source of PPCPs entering into the environment through the byproducts that are released from wastewater treatment plants. As reported by different countries such as USA, Spain, Finland, United Kingdom, and Japan, the existing of pharmaceuticals in WWTPs, mainly in the level of ng/l to μg/l and in the sewage sludge ng/g to μg/g of dry weight., Information about environmental presence of PPCPs and their fate in the sewage sludge attracted great attention (Liu & Wong, 2013). The result of studies in some locations in China and other countries focused on the content and fate of PPCPs in wastewater treatment plants, including sewage and sludge are shown in table 7 and 8. More details about the sources, pathway, uptake and accumulation of pharmaceutical products are described in subchapter 3.5.

Table 7 Content of PPCPs in the wastewater from different countries

Location	**Chemical**	**Concentration (ng/l)**	**Media**
Guangzhou	Antibiotics	1730–7910	Sewage influent
Hong Kong	Antibiotics	3.2–1718	Sewage influent
Wuhan	Hormones	4.8–82.4	Sewage influent
Guangzhou	Synthetic musks	500 - 33540	Sewage effluent of a cosmetic plant
Beijing	Pharmaceuticals	2.2–320	Sewage effluent
Shanghai	Carbamazepine	230–1110	Sewage influent and effluent
Japan	Synthetic musks	280 – 1400	Sewage influent
S. Korea	Pharmaceuticals	ND – 11239	Sewage influent
U.S.	Pharmaceuticals	330 – 43800	Sewage influent
Portugal	Hormones	103 − 2484	Sewage influent
Sweden	-	ND – 1340	Sewage effluent
Finland	Antibiotics	ND − 4230	Sewage influent
Canada	Hormones	2.4 – 78	Sewage influent
Norway	Triclosan	380 – 430	Sewage influent
U.K.	Antimicrobial agent	27 – 65381	Sewage influent

⁎ND: not detected

(Liu & Wong, 2013)

Table 8 provides the concentration of some pharmaceuticals and personal care products which are detected in the dry sewage sludge from different WWTPs in China.

Table 8 The concentration of PPCPs found in the sewage sludge in China

PPCPs	Concentration (ng/g of dry weight)
Antibiotics	ND – 21000
Azole antifungal drug	ND-1442
Hormones	1.6–372
Antimicrobial agents	200.1–5088.2
Polycyclic musks	700–17000
Sunscreen UV filters	ND-24700
Other pharmaceuticals	1.7–33.7

*ND: not detected (Liu & Wong, 2013)

3.3. Pyrolysis

Pyrolysis is the thermal cracking process in which organic matter is decomposed by high temperatures varying in between 300 and 1000 °C in an oxygen deficient environment and consequently transforms the solid organic matter into three basic products: a non-condensable gas, a condensable vapour bio-oil, and biochar as a solid product (Barry et al., 2019). The non-condensable gas fraction contains mainly carbon monoxide, carbon dioxide, methane and hydrogen, which is normally combusted to provide heat for the pyrolysis process. A liquid fraction, this condensable stream mainly consists of tar or oil; it typically contains substances such as acetic acid, methanol and acetone that are used as a fuel. The last fraction is solid product, biochar, mostly consisting of pure carbon combined with a little amount of inert materials. There are several potential applications of biochar including use as a soil amendment or fertilizer, as an absorbent or replacement for carbon black as well as a carbon neutral fuel. The varying proportion of these three productions strongly depends on several parameters such as temperature, pressure, heating rate, residence time together with characteristics of effluent (Fytili & Zabaniotou, 2008, Barry et al., 2019).

The pyrolysis has significant advantages over other methods; this technique appears to be less risky for the environment contamination compared to conventional methods such as combustion or incineration. The production from pyrolysis can be used as fuels or a feedstock, which is beneficial for petrochemicals and other applications (Karayildirim et al., 2006).

Pyrolysis is more favorable as the process condition and is different from the gasification. Gasification mainly used high temperature (usually above 700°C) coverts organic substances into syngas, using oxygen between 20 and 40% for the total required combustion process, whereas pyrolysis is typically focused on producing char, gas and liquid, called bio-oil which used as fuel (Fonts et al., 2012).

Figure 4 shows the scheme of low-temperature pyrolysis. The process is heated to moderate temperatures ranging from 400 – 500°C converted usually about 50% of biomass into biochar and it can be returned to soil. At this temperature, biomass undergoes an exothermic process and transforms pyrolyzed biomass into energy (Lehmann, 2007).

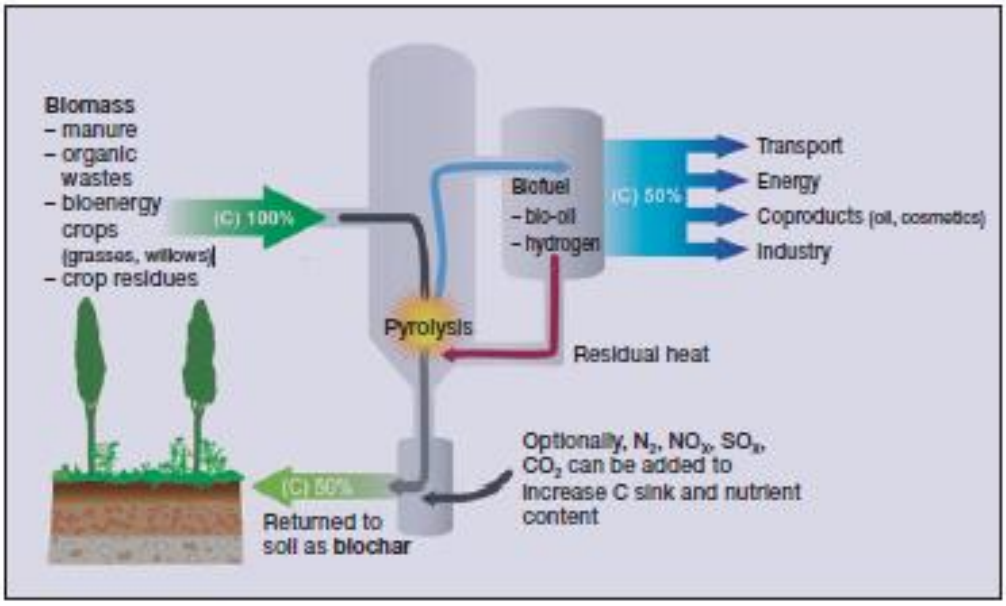


Figure 4 Concept of low-temperature pyrolysis
(Lehmann, 2007)

3.3.1. Pyrolysis conditions

The main conditions for pyrolysis process technologies are temperature, pressure, heating rate, residence time and particle size of the sludge. Due to its different properties and characteristics of effluent, the pyrolysis may be categorized as slow and fast processes based on operating conditions as shown in table 9 (Zaker et al., 2019).

a. Slow pyrolysis

This process condition is carried out at lower temperatures ranging between 300 – 700 °C with the heating rate around 0.1 to 1 °C/s, and 5 to 30 minutes of residence time (Raheem et al.,

2018). The slow pyrolysis operating process allows the formation of immense magnitudes of bio-oil and solid biochar, which are considered as realistic energy (Zaker et al., 2019).

b. Fast pyrolysis

Fast pyrolysis is a high-temperature reaction range from 550 to 1250 °C. This operating process has several important features such as providing shorter residence time, 0.5 to 20 s with higher heating rate, 10 to 300°C/s (Zaker et al., 2019). Due to its higher heating rate, the fast pyrolysis is encouraged to produce higher yield of bio-oil product up to 80 wt% on dry feed (Radiah et al., 2020).

Table 9 shows some main parameters for fast and slow pyrolysis processes.

Table 9 The range of main parameters for pyrolysis processes condition

Parameter	**Process condition**	
	Slow pyrolysis	Fast pyrolysis
Temperature (°C)	300 – 700	550 – 1250
Pressure (MPa)	0.1	0.1
Heating rate (°C/s)	0.1 – 1	10 – 300
Residence time (s)	300 – 500	0.5 – 20
Particle size (mm)	5 – 50	< 1

(Zaker et al., 2019)

3.3.2. Biochar

Biochar is the solid pyrolysis residue obtained from the thermal decomposition of organic matter. It has attracted great attention due to rich carbon content and could be applied into the soil as a fertilizing agent and soil improver with the potential to enhance the soil nutrients. There are two aspects that make biochar precious for this application: its high stability against decay and its superior ability to retain nutrients as compared to other soil organic matter forms (Lehman, 2007). In addition, biochar produced from the pyrolysis of sewage sludge provides valuable contents of macronutrients such as N, P, K and organic matter which are really essential for plant uptake as well as promote the soil microbial activity (Frišták et al.,2018). However, the majority of pollutants such as heavy metals (except the volatile elements Hg and Cd) and some pharmaceuticals and personal care products originally contained in the sewage sludge are still concentrated in biochar (Lu et al., 2016).

3.3.3. Pyrolysis of sewage sludge

Direct utilization of sewage sludge in agricultural fields is regularly discouraged with reference to toxic compounds and other contaminants. Therefore, methods for sewage sludge conversion and modification to increase the quality of sludge, decrease the amount of contaminants and heavy metals mobility are required (Frišták et al., 2018). Due to its efficiency in volume of waste reduction as well as toxicity, pyrolysis of sewage sludge has received significant attention by many researchers in recent years. Sewage sludge pyrolysis is an endothermic reaction involving heating of sludge in oxygen–free atmosphere, relatively at high temperature (300 – 1000°C) during the process (Lehmann & Joseph, 2015; Barry et al., 2019). After dewatered, dried and stored, raw sewage sludge is pyrolyzed at a specific temperature, heating rate and residence time in a pyrolysis reactor (muffle furnace). To maintain an oxygen-free atmosphere and uniform heating condition during the process, nitrogen is used to supply a system as flush gas. As the results from the decomposition, the pyrolysis of sewage sludge consequently converts solid organic matter in the sludge into gas, bio-oil and sewage sludge-derived biochar (Agrafioti et al., 2013; Frišták et al., 2018).

Normally, biochar produced from sewage sludge is defined as a carbon-rich product with potential benefits as soil amendment or fertilizer (Rajec et al., 2016). The application of sewage sludge derived biochar into soil can raise soil carbon sequestration, decrease atmospheric CO_2 concentrations, improve the quality of soil physico-chemical also provides valuable content and availability of nutrients such as N, P, K and organic matter, especially increase in plant yield (Frišták et al.,2018). Together with the availability of nutritive species, the stabilization and presence of heavy metals and organic contaminants from sewage sludge biochar is a major concern due to their potential high concentration in the biochar product (Barry et al., 2019).

3.3.4. Sewage sludge use in agriculture

Nowadays, the main ways to dispose of sewage sludge can be classified into three categories: agricultural reuse, landfilling and incineration. Disposal of the final sewage sludge in agriculture soil (name as a biosolid) is the most common alternative application, consisting of spreading the materials on the ground surface, disturbed land and forest land. This biosolids application is associated with the development of crops and improves the soil properties such as structure, water infiltration and water retention capacity. Due to their organic matter contents,

hence increasing soil cation exchange capacity (CEC) which promote more potassium holding in the soil (Metcalf & Eddy, 2003; Sperling, 2007).

The sewage sludge directive 86/278/EEC was adopted by European Commission in 1986 aimed to encourage the use of sewage sludge in agricultural land. Therefore, it regulates its application in a way that any potential harmful effect on soil, vegetation, animals and human beings is prevented. Based on this directive principle, the use of untreated sewage sludge in agriculture is prohibited, unless it undergoes treatment to meet a certain regulatory criteria. Furthermore, the term treated sludge is referred to the sewage sludge which has undergone chemical, biological, heat term and long term storage or any other appropriate process, hence to minimize its harmful effects and the health risks resulting from its utilization (Fytili & Zabaniotou, 2008).

The resulting from nitrification and denitrification phase in the wastewater treatment process, bringing the sewage sludge rich in nutrient elements such as nitrogen, phosphorus, potassium and organic matter. This phenomenon gives sludge a unique ability, since these contents of elements are very essential for plant growth and consequently increase plants productivity (Fytili & Zabaniotou, 2008; Razaq et al., 2017). Regarding the agricultural use of the sewage sludge as a potential fertilizing agent and soil improver, the negative aspects also appears due to the majority of harmful pollutants (heavy metals and pharmaceuticals) are still remained in the sewage sludge even though it undergone the treated processes (Singh & Agrawal, 2010; Lu et al., 2016).

According to the sewage sludge directive (86/278/EEC), all the EU member states have to set a limit value of toxic elements and other substances into their own regulations, corresponding to the utilization of sewage sludge in agriculture. Before applying the sewage sludge as a soil conditioner, there are various properties including organic matter, toxic elements, pathogens and nutrients like nitrogen, phosphorus, and potassium content needs to be evaluated (Mininni, et al., 2015).

Table 10 shows the limit values of potentially toxic elements in the sewage sludge regulated by some EU member states. Based on values in the table, it can be observed that Netherland is the only country that imposing more stringent limit values compared to others. In

contrast, Spain has the highest limit values and these limits are close to those of the sludge directive (Mininni, et al., 2015).

Table 10 Limit values of potentially toxic elements for sewage sludge use in agriculture

Countries	Elements (mg/Kg dry matter of sewage sludge)					
	Zn	Pb	Cd	Cu	Hg	Ni
Directive 86/278/EEC	2500-4000	750-120	20-40	1000-1750	16-25	300-400
Upper Austria	1500	300	10	500	10	100
Belgium	900	200	6	375	5	50
Czech Republic	2500	200	5	500	4	100
France	3000	1200	20	1000	10	200
Germany	1500	100	2	600	1.4	60
Italy	2500	750	20	1000	10	300
Netherland	300	100	1.25	75	0.75	30
Poland	2500	500	10	800	5	100
Spain	4000	1200	40	1750	25	400

(Mininni, et al., 2015)

3.4. Pharmaceuticals in the Environment

Emerging Pollutants (EPs) have been raising significant concern and regulatory attention in these recent years due to their persistent residues input and potential hazard to the environment and human health. Lately, the EPs have been found in terrestrial and aquatic environments including groundwater, surface water even in drinking water (Mzukisi et al., 2018). The EPs consist of a huge and diverse group of organic compounds which includes pharmaceuticals and integrates daily personal care products such as lotion, sunscreen, toothpaste, soap, fragrance and makeup, which are broadly used with large amounts all over the world (Liu & Wong, 2013). The manufacturing and increasing of pharmaceuticals and personal care products consumption have left an effect on the environment over time. As reported by the United Kingdom, approximate 70 pharmaceuticals have been found in environmental waters, while over 200 different pharmaceuticals have been reported in river waters globally (Alenzi et al., 2021).

3.4.1. Sources of pharmaceuticals in the environment

Pharmaceuticals can be introduced into the environment from various anthropogenic activities such as releasing waste from domestic, hospital, agriculture, industrial and direct

disposal pharmaceuticals from manufacturing into the water resources (Al-Farsi et al., 2017). Along with human consumption, pharmaceuticals are normally excreted and washed out of the body into the sewage and afterward reach wastewater treatment plants (WWTPs), where they cannot be fully eliminated. Pharmaceuticals may be absorbed into the sewage sludge and then transferred into the environment through disposing of the sludge on agricultural land, land application, dump in the aquatic environment etc. Although, the concentration of these pharmaceuticals are usually found at very low in the environment and improbable to effect human health shortly, but these compound could cause chronic exposure damage to aquatic organisms, animals as well as human through bioaccumulation and food-chain (Alenzi et al., 2021; Liu & Wong, 2013).

Currently, more than 4000 pharmaceuticals are being used, while the total global consumption of antibiotics is estimated around 100 000 – 200 000 tons and approximately 15 000 tons of antibiotics are released annually into the European environment alone (Bartrons & Peñuelas, 2017). The contaminants emerging concern of PPCPs are mostly found in the environment as shown in table 11.

Table 11 Emerging PPCPs contaminants commonly found in plants and environment

Family of contaminants	Pharmaceuticals
Analgesics and anti-inflammatories	Codine, diclofenac,fenoprofen, ibuprofe, ketoprofen, ketorolac, paracetamol, phenylbutazone, naproxen, and clofibric acid
Antidiabetics	Metformin
Antiestrogenics	Tamoxifen
Antiepileptics	Carbamazepine, 4-aminoantipyrine, codeine, diclofenac
Antiseptics	Chlorophene and Triclosan
Antiprotozoals	Quinacrine dihydrochloride
Diuretics	Furosemide, hydrochlorothiazide, iotalamic acid amidotrizoic acid, and diatrizoate,
Lipid regulators	Acebutolol, atenolol, bezafibrate, and fenofibric acid
Psychostimulants	Caffeine, and paraxanthine
Antidepressants and psychiatric drugs	Fluoxetin, diazepan,

Human antibiotics and veterinary	Azithromycin, chlortetracycline, ciprofloxacin, doxycycline, clarithromycin, methronidazole, norfloxacin, floxacin, roxithromycin, sulfapyridine, tetracycline, trimethoprim, and tylosin
β-Blockers	Celiprolol, propanolol, metoprolol, timolol, and sotalol
X-ray and contrast media	Iopromide, iohexol, diatrizoate iopamidol,
Cosmetics and personal-care products	Benzophenone, triclosan, triclocarban galaxolide, N, N-diethyltoluamide, and tonalide
Surfactants	Perfluorooctanoic acid (PFOA),and tergitol
Phytosanitary products	Clofibric acid

(Bartrons & Peñuelas, 2017)

3.4.2. Carbamazepine

Among the pharmaceutical compounds, carbamazepine is an antiepileptic drug extensively used for the treatment of epilepsy, neuropathic pain, bipolar disorder, trigeminal neuralgia, acute mania and other neurological therapies (Shenker et al., 2011). Although carbamazepine is effective in controlling seizures and further progression of epilepsy, it may also raise numerous worrying side effects. Thus, the presence of carbamazepine and its metabolites in the environment has been raising concern due to the potential ecological and human health risk associated with exposure to this kind of compound. As a result of its persistence, carbamazepine is the most frequently detected in the wastewater and biosolids among other pharmaceutical compounds at relatively high concentration (Bai et al., 2021; Paz et al., 2016).

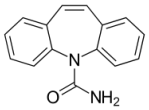
Carbamazepine has low acute toxic effect, with a predicted no-effect concentration of 0.42 µg/l according to ecotoxicology studies suggestion, but its long-term effect or chronic exposure from biomagnification through the food-chain required attention (Shenker et al., 2011). Most carbamazepine metabolites are more persistent and toxic than the parental compounds. For example, carbamazepine -10,11- epoxide (LC_{50}, 0.20 mg/Kg) is dramatically more toxic than carbamazepine (LC_{50}, 1.10 mg/Kg) (Ibe et al., 2018).

The annual consumption of carbamazepine is estimated to be approximately 1014 tons all over the world (Zhang et al., 2008). Carbamazepine can be absorbed and concentrated in aquatic

organisms and plants leading to potential adverse effects. Recently, there are a number of studies illustrated that carbamazepine was frequently found at levels of ng/g in the vegetables sold commonly in the market of many countries including Israel, China, Spain, and the United States (Li et al., 2018).

Table 12 demonstrates the chemical and physical properties of carbamazepine compounds.

Table 12 Structure and properties of Carbamazepine compound

Compound (application)	Solubility* (mg/l, °C)	Chemical formula	Structure	Molecular weight	pK_a	log K_{ow}	Half-life in soil (day)
Carbamazepine (anticonvulsant)	17.66	$C_{15}H_{12}N_2O$		236.27	2.3	2.45	495

* Calculated values using U.S. Environmental Protection Agency API Suite V.4.10

(Wu et al., 2012)

3.5. Partway of Pharmaceuticals Uptake by Plants

Treated sewage sludge (usually termed biosolids) is recent commonly reused worldwide, especially in land application and may be used as fertilizer on agricultural land due to its rich sources of nutrients, minerals, and organic matter for plant growth. As mentioned, PPCPs are frequently found in the influential wastewater treatment plants, thus the sewage sludge generated from municipal wastewater treatment plants can be a principal sink for many PPCPs compounds (Wu et al., 2010). Biosolids application is considered as a major pathway that introduces PPCPs into the terrestrial environment. Similarly, animal manure which may consist of veterinary medicine, spreading on arable land could also be a source of introduction of PPCPs into the soil environment, appearing in the concentration level of µg/Kg (Wu et al., 2015). As a result of agricultural application of biosolids and animal manure, a group of emerging contaminants known as PPCPs could also be transferred and accumulated in plants at concentration ng/Kg to µg /Kg (Bartrons & Peñuelas, 2017).

In addition, pharmaceutical compounds can lead to biochemical transformation of nitrogen and carbon in the soil, producing direct effect on plant growth by changing nutrient availability, root growth and development as well as plant productivity. The presence of pharmaceuticals in the soil causes accumulation in plant tissues and has harmful effects on the physiological traits of the plant. In turn, pharmaceuticals accumulated in the plants can be

introduced into human food, as agriculture serves as an important route for the food chain. By knowing the amount of pharmaceuticals and personal care products in biosolids and manure before apply them into the agricultural fields is thus crucial to avoid any possible issues of toxicity for plants, their microbiota and animals together with humans (Fytili & Zabaniotou, 2008).

Figure 5 shows the source and fate of PPCPs presence in the environment, which is predominantly associated with human activities and the discharge of wastewater from municipal, industrial plants, and agriculture for treating wastewater. The PPCPs in reused irrigation water, biosolids and animal manure are applied to soils where these compounds can affect soil microbiome, taken up and accumulated as well as metabolized by plants (Bartrons & Peñuelas, 2017).

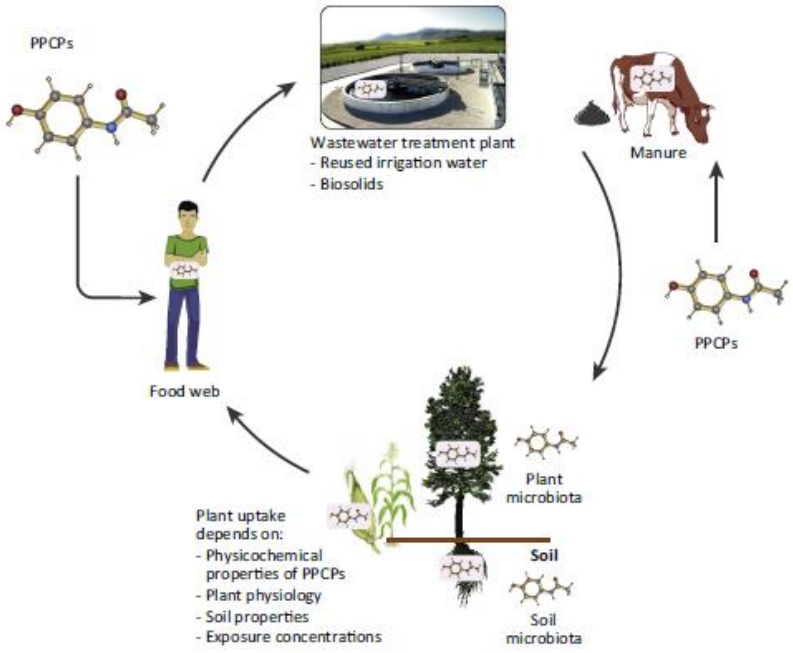


Figure 5 The main source of PPCPs in the environment and plants related to the human activities (Bartrons & Peñuelas, 2017)

3.5.1. Uptake and translocation of pharmaceuticals by plants

The uptake of pharmaceuticals by plants has increased significant attention in recent years. The growth of vegetables or plants in contaminated soil, composed of biosolids or soil fertilized with sewage sludge could result in the uptake and translocation of pharmaceuticals by plants (Mzukisi et al., 2018). Huge amounts of pharmaceuticals have been detected in various

species and plant tissues, along with highly concentrated variables ranging from no detection to 487 µg/Kg (Bartrons & Peñuelas, 2017; Reddy et al., 2018).

The physicochemical properties of pharmaceuticals such as water solubility, hydrophobicity, octanol-water partition coefficient, extremely influence the uptake, translocation and accumulation of pharmaceuticals in plants (Al-Farsi et al., 2017). However, the driving mechanism for uptake, translocation and bioaccumulation of pharmaceutical compounds in plants remains limited and not well understood (Miller et al., 2015). Plants uptake pharmaceuticals through the roots and transport them to aerial tissues. Pharmaceuticals taken up by plant root via mass flow or diffusion of dissolved compounds. Neutral compounds diffused through the root-cell membrane with a partition coefficient quite similar to which form water into the octanol, and ionizable compounds across the roots by combined with the diffusion of the neutral fraction together with electrostatic interactions of the ion fraction. Furthermore, aerial tissues take up pharmaceuticals through deposition from aerosols and volatilized compounds, which contact directly with irrigation or soil amended materials and translocation from the root tissues as shown in figure 6 (Miller et al., 2016; Bartrons & Peñuelas, 2017).

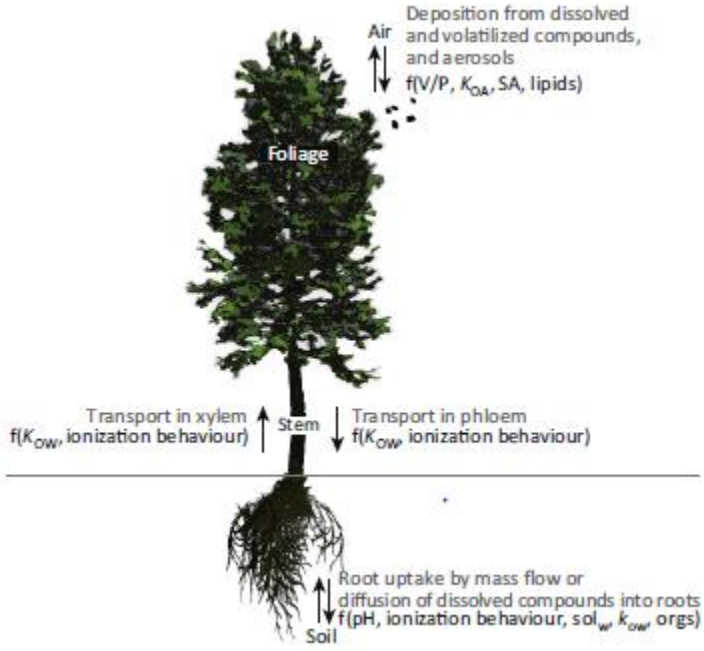


Figure 6 Principal pathways for uptake of pharmaceuticals by plants. The major factors affecting each pathway are: f (function), V/P (vapour particle), K_{OA} (octanol-air partition coefficient), SA (plant surface area), lipid, K_{OW} (octanol-water partition coefficient), sol_W (water solubility) and org_s (organic content of the soil)

(Bartrons & Peñuelas, 2017)

The hydrophobicity of pharmaceutical compounds is normally used for interpretation of uptake of organic compounds by plant roots, while the most hydrophilic compounds in equilibrium with the water will move directly to the xylem, from which nonionic pharmaceutical compounds accumulate in leaves, transported mainly in the direction of the transpiration stream. The ionic pharmaceuticals, which are repelled by the negatively charged anion cell walls and cytosol, may be caught up in the phloem and can accumulate more in the fruit as well as grain (Goldstein et al., 2014; Wu et al., 2015).

When treated sewage sludge or biosolids applied in agricultural fields, carbamazepine appeared to be frequently found in various plant tissues, meaning that carbamazepine has high bioavailability in soil and it is relatively easy to transfer from soil to plants. Once taken up by plants, carbamazepine gets translocate and accumulate to different compartments of plants which results in their detection in roots, stem, leaves and edible parts such as fruits and grains (Wu et al., 2015).

Table 13 shows the different concentrations of carbamazepine accumulated in various parts of plants grown in biosolids amended. In the shoots of all plants, carbamazepine had the highest concentrations among other parts, meaning that translocation potential from root to above ground tissues for carbamazepine is the highest. However, the concentrations of carbamazepine in the fruits of pepper and tomato were detected relatively low (Wu et al., 2012).

Table 13 Concentration of carbamazepine detected in various parts of plants

Plants	**Concentration (ng/g dry weight)**	
	Roots	shoot
Pepper	3.34 ± 1.20	23.37 ± 7.34
Collard	1.62 ± 0.37	8.28 ± 4.00
Lettuce	1.66 ± 0.36	7.42 ± 1.51
Radish	1.12 ± 0.50	3.42 ± 1.03
Tomato	1.06 ± 0.14	4.16 ± 0.39

(Wu et al., 2012)

According to Wu et al. (2012) study, the concentrations of carbamazepine in soils applied with treated sewage sludge were determined before plants were seeded and after harvest.

Table 14 shows the concentration of carbamazepine in soils applied with biosolids for planting different types of plants. After harvest, the concentration of carbamazepine dropped

39.5% and 21.5% respectively, in the soils planting tomato and radish. For other treatments, no decreasing trend was observed. This indicates that carbamazepine compound is very persistent in the soil (Wu et al., 2012).

Table 14 Concentration of carbamazepine in soils with biosolids application

Soil for planting	Concentration (ng/g dry weight)	
	Pre-planting period	Post-harvest period
Pepper	89.3 ± 8.95	60.5 ± 31.8
Collard	132 ± 30.7	109 ± 17.6
Lettuce	103 ± 25.4	98.3 ± 7.95
Radish	115 ± 9.54*	90.3 ±12.0*
Tomato	160 ± 12.0*	96.8 ± 4.36*
Plant-free control	94.7 ± 13.7	90.0 ± 3.76

* Significant difference at 95% confidence interval.

Exposure to pharmaceuticals may affect plant growth and development, either as a result of direct damage to the plants by inhibition of root elongation, decreased photosynthetic pigments, reduced number and size of mature leaves as well as decreased plant production (González et al., 2018). However, human exposure to pharmaceuticals was likely to be low through daily consumption of vegetables or crops grown in treated sewage sludge or biosolids amended but long-term exposure may pose potential high risk and threaten human health.

4. Material and Methods

4.1. Sewage Sludge Collection

In this study the sewage sludge feedstock for the preparation of biosolids originated from a municipal wastewater treatment plant (WWTP) located in the Czech Republic. The construction capacity of the WWTP is 29 000 population equivalent (PE), and $Fe_2(SO_4)_3$ is used for P precipitation. The used sewage sludge was anaerobically stabilized (mesophilic conventional anaerobic digestion) and dewatered by decanter centrifuge (dry matter content at collection was 24 wt. %) at WWTP. Afterward the collection, the sewage sludge was entirely homogenized by mixing and air-dried in thin layers at 105 °C until constant mass. The content of potentially toxic elements, polycyclic aromatic (PAHs) hydrocarbon and polychlorinated biphenyl (PCBs) in dried sewage sludge passed the Czech legislative limit values for reuse sewage sludge on agricultural land (Public Notice No. 437/2016, 2016). The main chemical characteristics and pharmaceuticals

content of materials used as amendments are shown in table 15 and 16. Dried sewage sludge was milled to a fine powder and passed through a 1 mm stainless sieve prior to application or pyrolysis, and was denoted as SS.

Table 15 shows the chemical characteristics of the dried and torrefied sludge materials applied as amendments in the field experiment in this study. Based on the values, it can be observed that sewage sludge contains a relatively high amount of nitrogen; this is very ordinary to sludge for soil amendment. In both biosolids materials, the content of nitrogen in dried sludge was higher than torrefied sludge, in contrast, the content of phosphorus and potassium in dried sludge were lower than torrefied sludge.

Table 15 Main chemical characteristics of biosolids amendment

Material	N [%]	P [%]	K [%]	C [%]	H [%]	S [%]
SS	4.76 ± 0.03	2.58 ± 0.34	0.31 ± 0.08	30.72 ± 0.10	4.61 ± 0.35	1.26 ± 0.02
TS	4.09 ± 0.01	3.66 ± 0.21	0.52 ± 0.05	30.46 ± 0.01	3.27 ± 0.03	1.02 ± 0.01

Values shown represent the arithmetic mean (n = 3) ± standard deviation. Dried biosolids are denoted as SS. Pyrolysed or torrefied biosolids samples are denoted as TS.

Table 16 Indicates the content of pharmaceuticals detected in dried and torrefied biosolids materials used in the field experiment.

Table 16 Content of pharmaceuticals detected in biosolids materials

Compound	Drug class	Dried sludge (µg/Kg dw)	Torrefied sludge (300°C, 3h)(µg/Kg dw)
Acebutolol	Beta blockers	7 ± 1	<MDL
Amantadine	Antivirotics	23 ± 2	< MDL
Amisulpride	Antipychotics	40 ± 4	<MDL
Amitriptyline	Antidepressants	39 ± 7	<MDL
Amlodipine	Calcium Channel blockers	173 ± 31	<MDL
Amorolfine	Antibacterials/antifungals	9 ± 2	<MDL
Atenolol	Beta blockers	<MQL	<MDL
Azithromycin	macrolide antibiotics	213 ± 64	<MDL
Bisoprolol	Beta blockers	28 ± 3	<MDL
Carbamazepine	Anti-epileptics and metabolites	62 ± 10	<MDL
Cetirizine	Antihistamines	86 ± 20	<MDL
Chlorprothixene	Antipsychotics	16 ± 4	<MDL
Ciprofloxacin	Antibiotics	644 ± 82	<MDL
Citalopram	Antidepressants	103 ± 27	<MDL
Climbazole	Antifungals	52 ± 16	<MDL
Clindamycin	Antibiotics	27 ± 3	<MDL
Clomipramine	Antidepressants	9 ± 2	<MDL

Diclofenac	Non-steroidal anti-inflammatory	59 ± 16	<MDL
Diphenhydramine	Antithistamines	19 ± 7	<MDL
Dothiepin	Antidepressants	64 ± 13	<MDL
Doxycycline	Antibiotics	109 ± 15	<MDL
Fenofibrate	Lipid regulators	42 ± 12	<MDL
Fluoxetine	Antidepressants	11 ± 3	<MDL
Gabapentin	Anti-epileptics and metabolites	66 ± 9	<MDL
Indomethacin	NSAIDs	<MQL	<MDL
Irbesartan	Antihypertensives	27 ± 3	<MDL
Lidocaine	Anesthetics	7 ± 1	<MDL
Memantine	Anti-dementia agents	9 ± 1	<MDL
Metoclopramide	Antiemetics	9 ± 2	<MDL
N-desmethyltramadol	Analgesics and metabolites	7 ± 1	<MDL
Norfloxacin	Antibiotics	165 ± 18	<MDL
O-desmethyl Venlafaxine	Antidepressants	70 ± 7	<MDL
Oxytetracycline	Antibacterials	<MQL	<MDL
Propafenone	Antiarrhythmics	568 ± 108	<MDL
Ranitidine	Antihistamines	3 ± 1	<MDL
Sertraline	Antidepressants	83 ± 23	<MDL
Sitagliptin	Antidiabetics	68 ± 16	<MDL
Solifenacin	Anticholinergics	105 ± 26	<MDL
Sulfapyridine	Veterinary	51 ± 4	<MDL
Sulpiride	Antipsychotics	6 ± 1	<MDL
Telmisartan	ARBs	4987 ± 1572	14 ± 2
Tetracycline	Antibiotics	12 ± 3	<MDL
Tiapride	Atypical antipsychotic	12 ±1	<MDL
Tramadol	Analgesics and metabolites	20 ± 2	<MDL
Trospium	Antimuscarinics	75 ± 12	<MDL
Venlafaxine	Antidepressants	32 ± 5	<MDL
Varapamil	Calcium Channel blockers	42 ± 9	<MDL

Values shown represent the arithmetic mean (*n = 3)* ± standard deviation; ARBs: angiotensin receptor blockers; NSAIDs: nonsteroidal anti-inflammatory drugs; < MDL: concentration below method detection limit; < MQL: concentration below method quantification limit but higher than MDL.

4.2. Pyrolysis

Pyrolysis of samples was performed in an inert atmosphere using laboratory tube furnace (GHA 12/600, Carbolite Gero Ltd., Hope, UK). The SS sample of known weight placed in ceramic holders was introduced into a cylindrical tube, made of quartz previously connected to the stream of nitrogen. The sample was kept in the operating furnace for 30 min to maintain an oxygen-free atmosphere during the process. Nitrogen (99.99%) was supplied into the system at a flow rate of 100 l/h giving calculated cold linear flow velocity for the empty reactor tube of 1.9

cm/s. Once the reactor was filled with nitrogen, it was inserted into the preheated furnace. Subsequently, the SS was pyrolysed for 3 hours at temperature of 300 ˚C. The resulting treated material was denoted as TS (torrefied sludge). Torrefaction is a mild form of pyrolysis, generally at temperatures of up to 350 ˚C and it may serve a promising, low-cost technology that provide sterilization and destruction of pharmaceutical compounds whereas keeping the recyclability of phosphorus at a high level. The scheme of the fixed-bed pyrolysis reactor used is shown in figure 7 (Mercl et al., 2020).

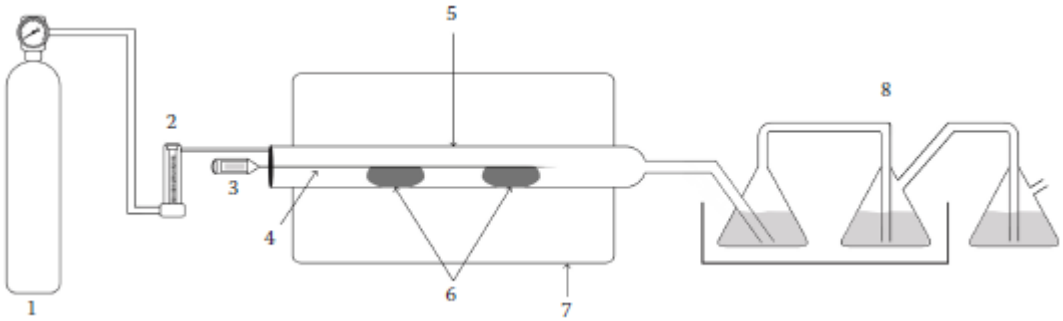


Figure 7 Schematic of the laboratory pyrolysis equipment. 1 – the source of nitrogen; 2 – gas flow meter; 3 – thermometer; 4 – thermometer probe; 5 – quartz tube; 6 – ceramic sample holders; 7 – electric furnace; 8 – volatiles collecting system

4.3. Experimental Design

Maize plants were selected to study uptake of nutrients and pharmaceuticals. The field experiments were carried out in the summer of 2020 located at the experimental station close to the city of Humpolec, Czech Republic. The characteristic of the site is given in table 17. Eight treatments were set there with four replications. This resulted in a total of 32 plots; the size of each plot is 18 m^2. Maize seeds were sown in the plot without any fertilizer application as control, treatment with the application of mineral nitrogen (N-1= 655 and N-2= 1309 g/plot), treatment with the application of NPK fertilizers (1309, 410, 720 g N, P, K/plot), treatment with untreated sludge (SS-1= 7563 and SS-2= 22689 g/plot) and treatment with thermal treated sludge (TS-1= 8802 and TS-2= 26406 g/plot). All treatments were set in two rates of nutrients according to the amount of nitrogen applied as shown in table 18. The rows were 75 cm far between each other. The schematic representation of the maize grown at the field experiment is shown in the figure 8.

Harvesting: The first set of samples was collected 60 days after the sowing. The second set of samples was harvested 110 days after sowing. The final samples of biomass as well grain

yield of maize were collected after 170 days. All samples were washed with demineralized water, air-dried, weighed, milled into fine powder and prepared for the analyses of nutrients and pharmaceuticals.

Table 17 Characteristics of experimental field. (Kulhánek et al., 2014)

Characteristics	**Experimental soil**
Latitude	49°33'15"N
Longitude	15°21'02"E
Altitude (m a.s.l.)	525
Mean yearly temperature (°C)	7.0
Mean yearly rainfall (mm)	665
Soil type	Cambisol
Soil sort	sandy loam
pH[1]	5.1
P (mg/kg)[2]	77 (± 10)
K (mg/kg)[2]	238 (± 47)
Ca (mg/kg)[2]	1625 (± 187)
Mg (mg/kg)[2]	112 (± 14)

[1] Estimated in 0.01 mol/L $CaCl_2$, 1:10 w/v; [2]Average basic data estimated using Mehlich in archive samples (1996)

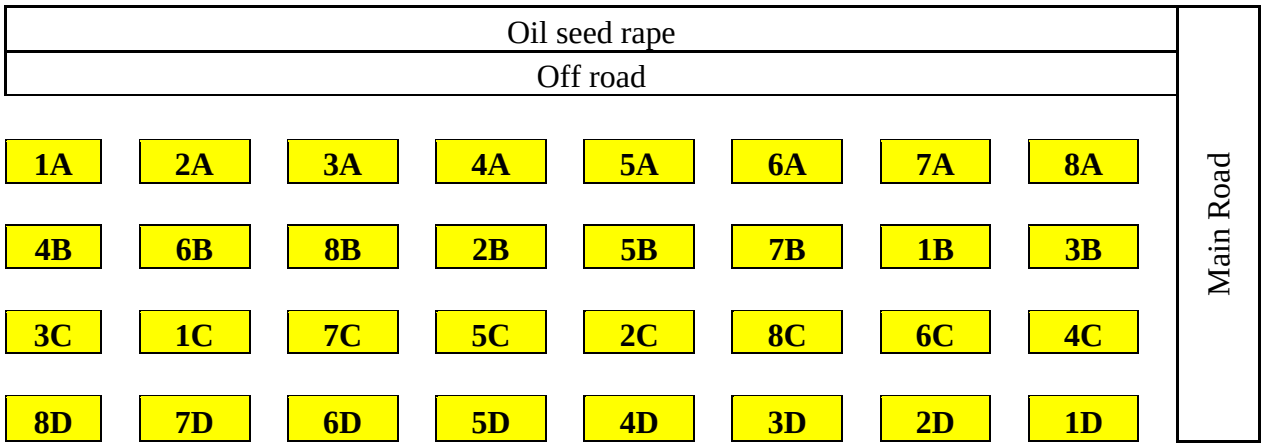


Figure 8 Schematic representation of the maize grown at the experimental site and description of individual treatments. Treatment: 1) control, 2) N-1 (100 Kg N/ha), 3) N-2 (200 Kg N/ha), 4) NPK (200, 50, 200 Kg N, P, K/ha), 5) SS-1 (200 Kg N/ha), 6) SS-2 (600 Kg N/ha), 7) TS-1 (200 Kg N/ha), 8) TS-2 (600 Kg N/ha)

Table 18 shows the differences in rates of fertilizer, dried and torrefied biosolids applied in individual treatments at the experiment site.

Table 18 The differences rate of fertilizer, dried and torrefied biosolids

Treatment	N (g/plot)	P (g/plot)	K (g/plot)
1. Control	0	0	0
2. N-1	655	0	0
3. N-2	1309	0	0
4. NPK	1309	410	720
5. SS-1	7563	1951	234
6. SS-2	22689	5854	703
7. TS-1	8802	3222	458
8. TS-2	26406	9665	1373

4.4. Biomass Digestion and Nutrients Analysis

For the digestion, 0.3 g of dry sample (maize biomass or grain) was digested with 8 ml of concentrated HNO_3 (65% v/v; Analytika), 2 ml of H_2O_2 (30% v/v; Analytika) and demineralized water to reach a final volume of 20 ml in an Ethos 1 microwave-assisted wet-digestion system (MLS, Leutkirch, Germany) at temperature 180°C for 55 min. Nutrient concentrations of P and K were then determined by inductive couple plasma-optical emission spectrometry (ICP-OES; Agilent 720, Agilent Technologies Inc., Santa Clara, CA). The standard reference materials used were 1573a Tomato Leaves (NIST, Gaithersburg, USA) and IAEA-V-8 Rye Flour (IAEA, Seibersdorf, Austria). For the determination of total N in the biomass sample, a CHNS Vario MACRO cube analyser was used (Elementar Analysensysteme GmbH, Hanau, Germany).

4.5. Pharmaceuticals Extraction

For the extraction of pharmaceuticals, 0.1g of dry sample (maize biomass) in a Falcon test tube was extracted with 6 ml of MeOH-water solution (50/50, v/v). The water solution (pH 2.5) consisted of 0.1% Na_2EDTA and 0.5% of HCOOH. The extraction was done by sonication of closed and sealed test tubes for 15 min at temperature 50 °C. Supernatant was collected after centrifugation at 4500 rpm for 10 min at temperature 20 °C. This procedure was repeated one more time, from the two extractions resulting in a total of 12 ml of supernatant. An aliquot of 1 ml was taken from the extract and transferred into Eppendorf for protein precipitation by adding

100 µl of 5-sulfosalicylic acid (485 mg/ml in Milli-Q), vortexing and put the Eppendof into the ice for 10 min, after that centrifugation at maximum 14000 rpm for 10 min at 20 °C. Finally, the protein-free supernatant was filtered through a syringe filter (regenerated cellulose, RC, 0.22 µm pore size) into an amber glass vial for following analysis by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

4.6. LC-MS/MS Analysis

Analyte separation of measured compounds was conducted using the UHPLC system (Agilent 1290 Infinity II, Agilent Technologies, USA) which consisted of coupled to a triple quadrupole mass spectrometer (6495B, Agilent). Compounds were separated in a reversed-phase C18 column (Poroshell 120, EC-C18, 3.0 ×100 mm, 2.7 µm particle size, Agilent) fitted with a pre-filter (Poroshell 120, EC-18, 3 mm, Agilent) and in-line pre filter (0.3 µm, Agilent) using 0.5 mM NH_4F and 0.005% HCOOH in water (A) and MeOH (B) as mobile phases at a flow rate of 0.4 m/l. Gradient elution started at 5% B and was ramped to 100% over 13 min and maintained for 8 min. The injection volume was 2 µl and column temperature was maintained at 40 °C. The mass spectrometer was equipped with an electrospray ionization (ESI) source and operated in a positive ionization mode (ESI+) with the following ion source parameters were: 2500 V capillary voltage, the source gas temperature 105 °C, the nebulizer flow 13 l/min, the sheath gas 12 l/min at 350 °C, respectively, nebulizer pressure 25 psi. Funnel parameters were respectively 110 V and 60 V on the high-pressure funnel and the low-pressure funnel. Nitrogen (99.99%) was used as the nebulizing, desolvation, and collision gas. A quantitative analysis of pharmaceutical compounds was operated in a dynamic multiple reactions monitoring (dMRM) mode.

4.7. Identification, Quantification, and Analytical Characteristics

Identification individual analyte was based on the presence of two transitions, qualification and confirmation MRM transitions and a match of retention time with the reference standard. Quantification of individual analytes was performed using a nine-point calibration curve ranging from 5 – 10 000 pg/ml prepared prior to each run by serial dilution of mixed stock solution (600 ng/ml in MeOH-0.02% EDTA solution) using the extraction solution. Each point of the calibration was made freshly in triplicate and measured at the beginning, in the middle and at the end of each run as well. The mixed stock solution was prepared prior to each run from the

mixed standard with methanol (4 μg/ml in MeOH, stored at - 20 °C). The mixed standard was prepared by mixing stock solution and was used for spikes before and after the extraction to obtain the matrix suppression (MS) and analyte recoveries. Recoveries were determined by analyzing spike samples. Considering the MS was estimated as the final concentration of individual analytes. MS was determined for each matrix, where the matrix was spiked in triplicate post-extraction at three concentration levels of 12, 120 and 600 ng/g. MS was calculated according to the equation: MS = ((Rsp.ex – Rex) × 100/Rstd) – 100, where Rps.ex is the response of the spike extract, and Rstd is the response of the standard solution used for spiking. A detail of method performance, such as the limit of detection (MDL) and the limit of qualification (MQL) is shown in table 16. To comply with quality-control procedures, each run included blanks, blank solvents, procedural blanks and control spiked samples.

All reagents standards were analytical standards purchased from Sigma Aldrich (Darmstadt, Germany) as 1.0 mg/ml or 0.1 mg/ml MeOH powder or solution. Only 10,11-dihydro-10-hydroxycarbamazepine was obtained in acetonitrile. Powdered compounds were dissolved in MeOH to 1.0 mg/ml concentration prior to use. MeOH, NH_4F, and HCOOH used for the preparation of mobile phase were LC-MS grade obtained from Honeywell (Seelze, Germany). The quality of Milli-Q water was 18.2 MΩ (Millipore, SAS, France; system equipped with LC-Pak polisher).

Table 19 shows the values of the method limit of detection and limit of qualification of compound in biomass and biosolids materials. Limit detection in biomass is 0.5 ng/g of dry weight for all pharmaceutical compounds, while 0.25 and 0.50 ng/g of dry weight is the lowest and the highest limit detection values for pharmaceutical compounds in dried and torrefied biosolids materials.

Table 19 Method of detection and quantification limits

Compound	**Biomass material** MDL/ MQL* (ng/g d.w.)	**Dried and torrefied biosolids** MDL/MQL** (ng/g d.w.)
10,11-Dihydro-10-Hydroxycarbamazepine	0.50	0.33
Acebutolol	0.50	0.35
Amantadine	0.50	0.34
Amisulpride	0.50	0.30

Amitriptyline	0.50	0.40
Amlodipine	0.50	0.30
Amorolfine	0.50	0.50
Atenolol	0.50	0.35
Benzocaine	0.50	0.34
Bisoprolol	0.50	0.30
Carbamazepine	0.50	0.40
Carisoprodol	0.50	0.33
Chlorprothixene	0.50	0.30
Cimetidine	0.50	0.30
Citalopram	0.50	0.30
Clindamycin	0.50	0.33
Clomipramine	0.50	0.40
Clozapine	0.50	0.33
Diltiazem	0.50	0.30
Diphenhydramine	0.50	0.40
Dosulepin	0.50	0.40
Ethenzamide	0.50	0.33
Fenofibrate	0.50	0.35
Fluoxetine	0.50	0.50
Furazolidone	0.50	0.34
Gabapentin	0.50	0.34
Indometacin	0.50	0.33
Irbesartan	0.50	0.30
Ketoprofen	0.50	0.30
Levetiracetam	0.50	0.35
Lidocaine	0.50	0.30
Memantine	0.50	0.33
Methocarbamol	0.50	0.25
Metoclopramide	0.50	0.35
Metoprolol	0.50	0.37
Mexiletine	0.50	0.33
Mirtazapine	0.50	0.33
Nalbuphine	0.50	0.33
Naloxone	0.50	0.33
Naproxen	0.50	0.31
N-desmethyltramadol	0.50	0.40
Nortriptyline	0.50	0.35
Oseltamivir	0.50	0.30
Paracetamol	0.50	0.25
Phenacetin	0.50	0.35
Pilocarpine	0.50	0.40
Praziquantel	0.50	0.30
Propafenone	0.50	0.25

Propranolol	0.50	0.34
Quinidine	0.50	0.33
Ranitidine	0.50	0.33
Salbutamol	0.50	0.30
Selegiline	0.50	0.30
Sertraline	0.50	0.35
Sitagliptin	0.50	0.33
Solifenacin	0.50	0.30
Sulfadoxine	0.50	0.30
Sulfamethoxazole	0.50	0.33
Sulfapyridine	0.50	0.40
Sulpiride	0.50	0.25
Terbutaline	0.50	0.35
Tramadol	0.50	0.35
Trazodone	0.50	0.40
Triclocarban	0.50	0.34
Trimethoprim	0.50	0.25
Varenicline	0.50	0.34
Venlafaxine	0.50	0.30
Verapamil	0.50	0.35
Yohimbine	0.50	0.38

MDL: method detection limit; MQL: method quantification limit; n.a.: not analysed due to low REC;* determined by spiking the blank-matrix samples before (spiked and left overnight at 4 °C, ensuring evaporation of the solvent) and after extraction at six increasing levels; ** due to the unavailability of blank-matrix sample, MDL and MQL were estimated as follows: MD(Q)L = (ER * ID(Q)L × 100)/REC where ER = V/m and ID(Q)L = 3.3(10)* Sd/b; V: volume of extractant; m: mass of sample; Sd: standard deviation of calibration function residuals; b:calibration function slope

4.8. Statistical and Data Analysis

Data was analyzed using Microsoft Excel 2010 and SPSS version 20 (IBM, SPSS, USA). The normality of the data was checked using the homogeneity of variances. One-way analysis of variance (ANOVA) was applied to examine differences between treatments in the individual parameters. Statistical significantly different (t-test; post-hoc Tukey's honest significant difference test, HSD) are shown at the 95.0% confidence level and the letter a, b and c in each group are used to indicate the significant difference ($p < 0.05$) among the treatments. Values below MQL were not considered in the analyses. The shoot to soil ratio (%) was calculated as the total amount of a given aboveground biomass (mol) divided by the total amount of pharmaceutical in soil applied at the form of dried biosolids prior to sowing (mol).

5. Results

5.1. Tested Biosolids

In this study, 69 different compounds belonging to 27 drug different classes were monitored. None reached a concentration above the qualification limit (MQL) in the experimental soils as shown in table 16. Out of 69 tested pharmaceutical compounds, 47 compounds were detected and 44 were qualified in the sample of biosolids. Overall concentrations of qualified pharmaceuticals in the dried biosolids ranged from 3 – 4 987 µg/Kg as shown in table 16. The highest concentrations were found for the angiotensin receptor blockers drug telmisartan (4987 µg/Kg) A concentration level of hundreds of micrograms per kilogram was determined for the antibiotics antiarrhythmic, the propafenone, the doxycycline, the norfloxacin, the calcium channel blocker amlodipine, the antidepressants citalopram and ciprofloxacin. The concentrations of the remaining compounds were in the range of tens to units of micrograms per kilogram.

The torrefaction treatment significantly reduced the contents of pharmaceuticals in the resulting materials as shown in table 16. Beside the angiotensin receptor blockers drug telmisartan compounds (14 µg/Kg), no pharmaceuticals were above detection limit in torrefied materials (TS).

5.2. Total Maize Aboveground Biomass Yield

Aboveground of maize biomass (biomass plus grain) was harvested during the maturity stage for analyzed total yield. The effect of fertilizer, dried and torrefied biosolids application on the production of total maize aboveground biomass differed between treatments. The total biomass and grain yield of maize were ton per hectare (t/ha) of dry weight.

Figure 9 presents the results of yield response of biomass to fertilizer and biosolids material application. The result showed no statistically significant differences among the treatments, but it was found that the production of total biomass differed in values. Total biomass yield ranged from 9.8 – 11.8 t/ha of dry weight. Biomass yields from all treatments were found higher than control. The highest yield was found on the SS-1 (11.8 t/ha) and N-1 (11.1 t/ha) treatments, followed by NPK, N-2, SS-2, TS-2 and TS-1.

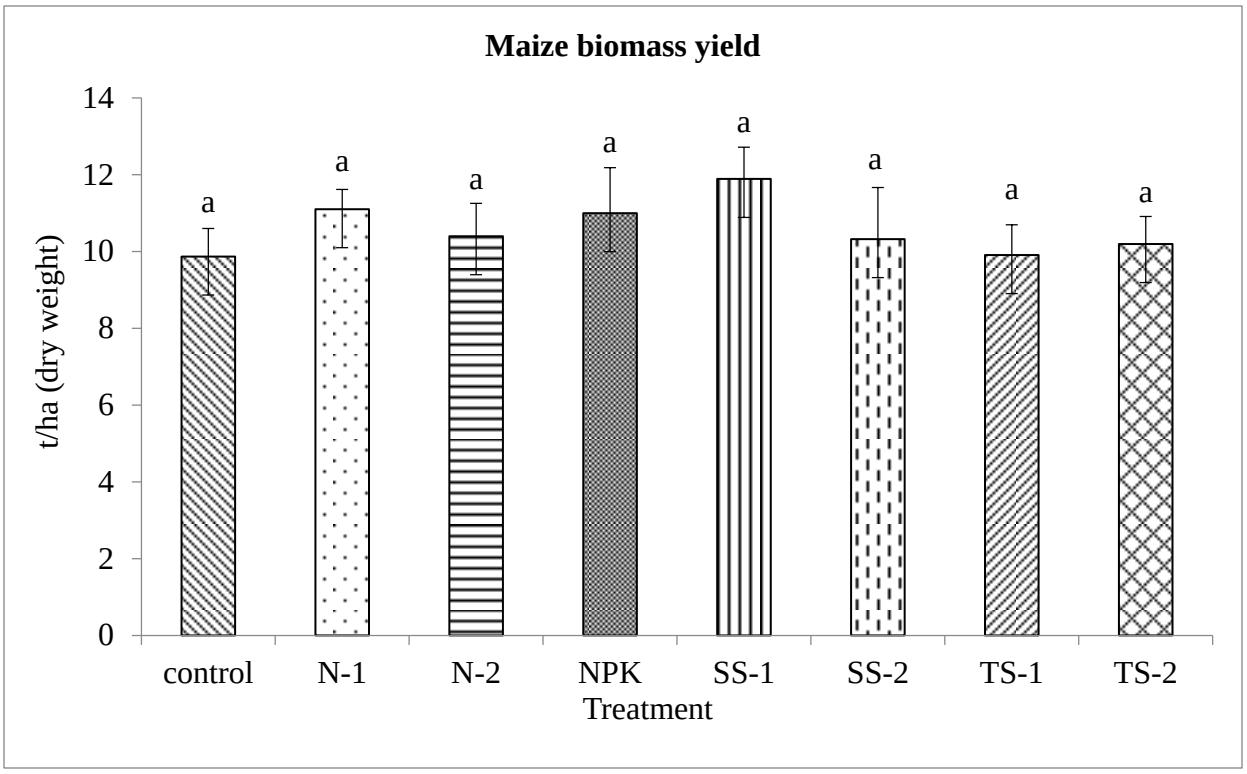


Figure 9 Yield of maize biomass grown on the soil of all treatments. Values shown represent the arithmetic mean (n=4); error bar indicate standard deviation; lowercase a above bars indicate statistical evaluation show no differences between the treatments on the level of 0.05

Figure 10 illustrates the total grain yield of maize for different treatments. The significant differences of total yield of maize grain were not found between the treatments. Total grain yield was obtained from 6.8 – 8.6 t/ha of dry weight. Among the treatments, the SS-1 (8.6 t/ha) and SS-2 (7.8 t/ha) amended with dried biosolids were the highest yield of maize grain in values. In contrast, the corresponding treatment on TS-1 (6.8 t/ha), torrefied biosolids led to the lowest grain production within the experiment as compared to control (7.5 t/ha).

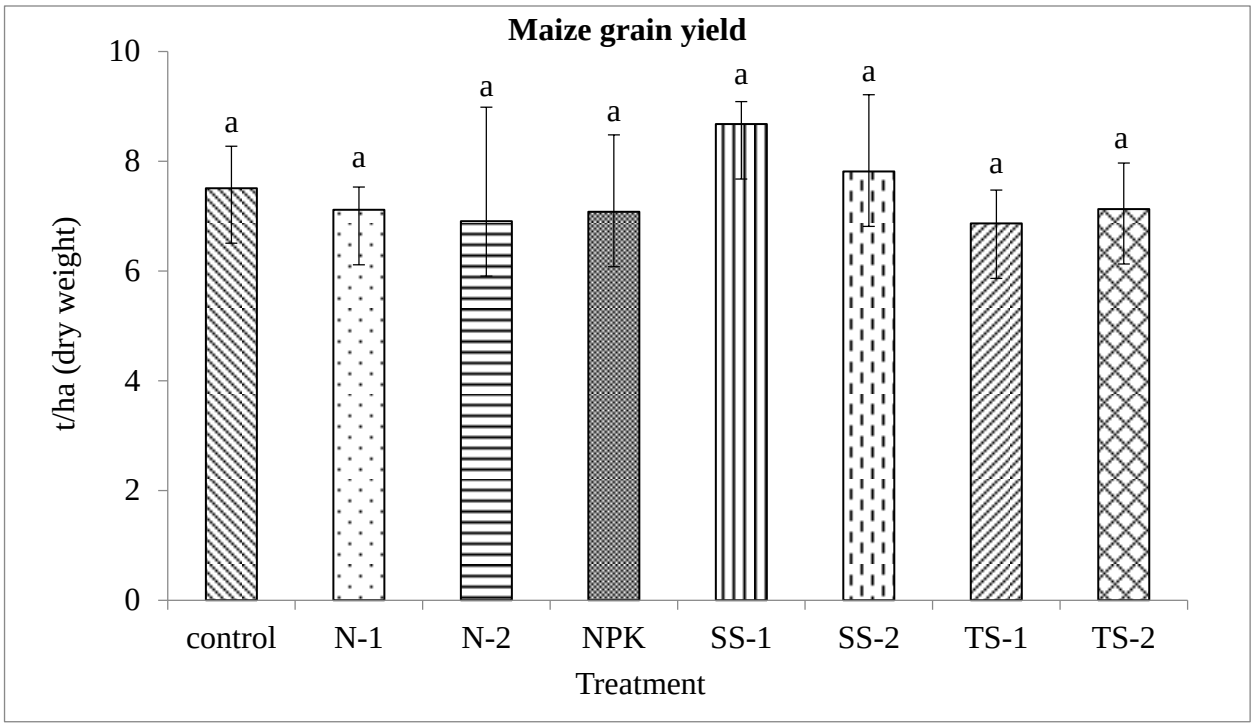


Figure 10 Yield of maize grain grown on the soil of all treatments. Values shown represent the arithmetic mean (n=4); error bar indicate standard deviation; lowercase a above bars indicate statistical evaluation show no differences on the level of 0.05

5.3. Content of Nutrients in Aboveground Biomass Maize

The maize aboveground biomass was analyzed in the term of total content of macronutrients, which emphasised on nitrogen, phosphorus and potassium. Analysis were made on maize aboveground biomass, separated for biomass without grain being called biomass and grain cobs being called grain. Biomass samples were analyzed from three harvesting periods at different stages, early growth, tasseling and maturity.

In table 20, the concentration of nutrients N, P, K in biomass from the first harvest showed some differences between the treatments. Based on statistical analyses, there were no significant differences of N concentration among all treatments. Compared to control which had no fertilizer application, treatment SS-2, N-2 and N-1 showed the highest concentration of N, 3.50, 3.33, and 3.32%, while the rest have lower concentrations ranging from 2.96 – 3.10% . The treatment which released the lowest of N was torrefied sludge - TS-2. From all treatments, the concentration of P in biomass ranging from 0.21 to 0.25% was found to have no significant differences. The highest P concentration in biomass was in SS-2, while TS-2 was the lowest in

values compared to control and other treatments. At the first harvest, K concentrations of biomass were found significantly differences among the treatments. The application of TS-1 led to significantly lower concentration of K in biomass (1.09%), while NPK application showed the highest release of K (3.49%) compared to control (3.19%).

Table 20 The difference in concentration of nutrients N, P, K in biomass from the first harvest

Treatment	First harvest		
	N (%)	P (%)	K (%)
Control	3.12 ± 0.52^{a}	0.22 ± 0.02^{a}	3.19 ± 0.28^{a}
N-1	3.32 ± 0.14^{a}	0.24 ± 0.01^{a}	3.12 ± 0.51^{a}
N-2	3.33 ± 0.21^{a}	0.22 ± 0.02^{a}	3.27 ± 0.37^{a}
NPK	3.10 ± 0.15^{a}	0.23 ± 0.01^{a}	3.49 ± 0.51^{a}
SS-1	3.10 ± 0.20^{a}	0.22 ± 0.02^{a}	3.08 ± 0.24^{a}
SS-2	3.50 ± 0.04^{a}	0.25 ± 0.01^{a}	2.37 ± 1.35^{ab}
TS-1	3.05 ± 0.09^{a}	0.22 ± 0.02a	1.09 ± 0.13^{b}
TS-2	2.96 ± 0.19^{a}	0.21 ± 0.02^{a}	2.80 ± 0.29^{a}
p-value	0.061^{ns}	0.243^{ns}	0.000^{***}

Values shown represent the arithmetic mean (n=4) ± standard deviation; differences letters indicates statistical significant differences (HSD test) between treatments; *, **, *** indicates the statistical significant difference at p-value < 0.05, 0.01, 0.000, respectively; ns: non-significant

Table 21 shows the macronutrients content N, P and K at the second harvest, from here the data indicated no significant differences of N content in biomass among the treatments. The highest concentrations of N in maize biomass were found in NPK (1.92%) and SS-1 (1.90%), followed by N-2 (1.76 %) and SS-2 (1.75 %). Compared to control, the lowest biomass concentration of N was determined on treatment amended by torrefied biosolids, TS-2 (1.38 %). Concentration of P in biomass remarkably no significant differences among the treatments, but it showed differences in values. The highest P concentrations were found in biomass with SS-1 (0.28%) and N-1 (0.27%) treatments, while biomass concentration of P remained the same in N-2, SS-2, TS-2 compared to control. At the second harvest, K concentrations show less significant differences between eight treatments compared to the first harvest. In this case concentration of K in biomass from NPK (1.80%) was the highest, while N-1(1.34%) was the lowest compared to control treatment (1.40%).

Table 21 The difference in concentration of nutrients N, P, K in biomass from the second harvest

Treatment	Second harvest		
	N (%)	P (%)	K (%)
Control	1.55 ± 0.18[a]	0.23 ± 0.02[a]	1.40 ± 0.15[ab]
N-1	1.60 ± 0.31[a]	0.27 ± 0.03[a]	1.34 ± 0.14[b]
N-2	1.76 ± 0.31[a]	0.23 ± 0.03[a]	1.54 ± 0.12[ab]
NPK	1.92 ± 0.17[a]	0.26 ± 0.01[a]	1.80 ± 0.14[a]
SS-1	1.90 ± 0.15[a]	0.28 ± 0.04[a]	1.67 ± 0.14[ab]
SS-2	1.75 ± 0.33[a]	0.23 ± 0.03[a]	1.52 ± 0.35[ab]
TS-1	1.44 ± 0.09[a]	0.24 ± 0.01[a]	1.56 ± 0.14[ab]
TS-2	1.38 ± 0.21[a]	0.23 ± 0.02[a]	1.55 ± 0.16[ab]
p-value	0.067[ns]	0.148[ns]	0.050[*]

Values shown represent the arithmetic mean (n=4) ± standard deviation; differences letters indicates statistical significant differences (HSD test) between treatments; *, **, *** indicates the statistical significant difference at p-value < 0.05, 0.01, 0.000, respectively; ns: non-significant

Table 22 indicates the different concentration of macronutrients N, P and K in maize biomass from the last harvest. The concentration of N in biomass ranged from 1.02 to 1.49 % had no significant differences among the treatments. TS-2 presented the highest value of N concentrations, 1.49%, while the application of N-2, NPK, SS-1 and TS-1 had lower concentration of N in biomass compared to control. The concentration of P in biomass was not significant differences among the treatments. The highest value of P concentration in biomass was found in SS-2 treatment (0.19 %), while the lowest value was with control (0.11%). The concentration of K in biomass, ranging from 1.02 to 1.58%, was found to have statistically significant differences among the treatments. The application of N, NPK, dried and torrefied biosolids with all rates were lower in content of K in biomass compared to control.

Table 22 The difference in concentration of nutrients N, P, K in biomass from the third harvest

Treatment	Third harvest		
	N (%)	P (%)	K (%)
Control	1.13 ± 0.14[a]	0.11 ± 0.01[a]	1.58 ± 0.29[a]
N-1	1.17 ± 0.12[a]	0.13 ± 0.02[a]	1.36 ± 0.20[ab]
N-2	1.08 ± 0.53[a]	0.15 ± 0.05[a]	1.16 ± 0.15[ab]
NPK	1.05 ± 0.12[a]	0.16 ± 0.02[a]	1.33 ± 1.59[ab]
SS-1	1.09 ± 0.19[a]	0.12 ± 0.03[a]	1.02 ± 0.10[b]
SS-2	1.31 ± 0.13[a]	0.19 ± 0.04[a]	1.37 ± 0.18[ab]
TS-1	1.02 ± 0.14[a]	0.15 ± 0.02[a]	1.20 ±0.05[ab]

TS-2	1.49 ± 0.25[a]	0.17 ± 0.04[a]	1.51 ± 0.24[a]
p-value	0.177 [ns]	0.890 [ns]	0.007[**]

Values shown represent the arithmetic mean (n=4) ± standard deviation; differences letters indicates statistical significant differences (HSD test) between treatments; *, **, *** indicates the statistical significant difference at p-value < 0.05, 0.01, 0.000, respectively; ns: non-significant

Table 23 are presented the values of different concentrations of N, P, K in harvested maize grain. The concentration of N in maize grain had no significant differences among the treatments. The application SS-2 (1.61%) and N-1 (1.60%) were the highest values of N concentration in maize grain, while torrefied biosolids (TS) were the lowest values. There were no significant differences of P concentration in maize grain among treatments. The content of P in maize grain of TS-2 contained the highest value of 0.47%. Grain concentration of P remained the same in N-2 (0.38%) compared to control. The application of SS-1, SS-2 and TS-1 had the lower values of P content compared to control. Concentration of K was found to have no significant differences in maize grain among the treatments. All the application of fertilizer, dried and torrefied biosolids contained higher K concentration in maize grain compared to control. The highest K concentration was found in TS-2 (0.56%) and N-1 (0.51%), while the lowest was found in control treatment (0.38%).

Table 23 The difference in concentration of nutrients N, P, K in maize grain

Treatment	**Grain**		
	N (%)	**P (%)**	**K (%)**
Control	1.43 ± 0.20[a]	0.38 ± 0.05[a]	0.38 ± 0.08[b]
N-1	1.60 ± 0.10[a]	0.45 ± 0.03[a]	0.51 ± 0.03[ab]
N-2	1.51 ± 0.20[a]	0.38 ± 0.03[a]	0.47 ± 0.03[ab]
NPK	1.51 ± 0.12[a]	0.39 ± 0.05[a]	0.47 ± 0.04[ab]
SS-1	1.46 ± 0.16[a]	0.35 ± 0.06[a]	0.40 ± 0.03[b]
SS-2	1.61 ± 0.11[a]	0.36 ± 0.02[a]	0.43 ± 0.03[ab]
TS-1	1.39 ± 0.12[a]	0.37 ± 0.07[a]	0.47 ± 0.06[ab]
TS-2	1.38 ± 0.12[a]	0.47 ± 0.13[a]	0.56 ± 0.11[a]
p-value	0.264[ns]	0.175[ns]	0.010[**]

Values shown represent the arithmetic mean (n=4) ± standard deviation; differences letters indicates statistical significant differences (HSD test) between treatments; *, **, *** indicates the statistical significant difference at p-value < 0.05, 0.01, 0.000, respectively; ns: non-significant

5.4. Uptake of Nutrients by Maize Aboveground Biomass

At physiological maturity maize aboveground biomass from each treatment of every replication were collected for N, P and K analysis. For all aboveground biomass, the application of dried or torrefied biosolids clearly resulted in highest uptake of nutrients N, P and K.

Figure 11 presents the result of N uptake by aboveground biomass of maize in different treatments. According to statistically analyzed, there were found no significant differences of N uptake among the treatments. The N uptake ranged from 197 to 263 Kg N/ha. At this maturity stage, compared to control the highest value of total N uptake at 263, 256 and 250 Kg N/ha of maize was determined in SS-2, SS-1 and TS-2, respectively, while the lowest uptake N was found on TS-1, 197 Kg N/ha.

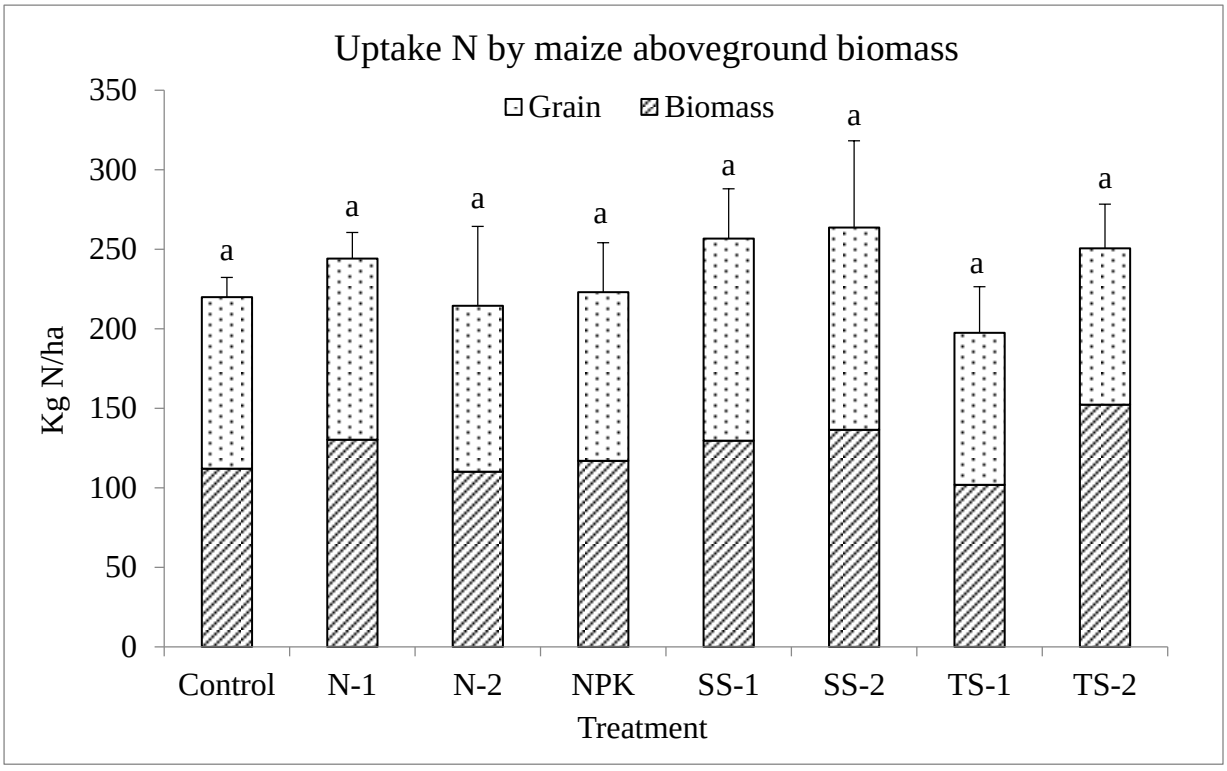


Figure 11 Uptake N by maize complete aboveground biomass from all treatments; Values shown represent the arithmetic mean (n=4) ± standard deviation; error bars indicate standard deviation; lowercase a above bars indicate statistical evaluation show no differences between the treatments on the level of 0.05

Figure 12 illustrates the uptake P by aboveground biomass of maize from each treatment. Based on the statistical analyses, there were no significant differences of P uptake by

aboveground biomass of maize were found between the treatments. However the values in the figure showed that all treatments applied with fertilizer, dried biosolids as well as torrefied biosolids material at different rates were obtained higher uptake of P by maize aboveground biomass as compared to control. Maize aboveground biomass uptake of P exhibited the trend TS-2 > SS-2 > N-1 > NPK > SS-1, N-2 > TS-1 > control. Highest value of P uptake by maize aboveground biomass was 51 Kg P/ha, while the lowest was 40 Kg P/ha.

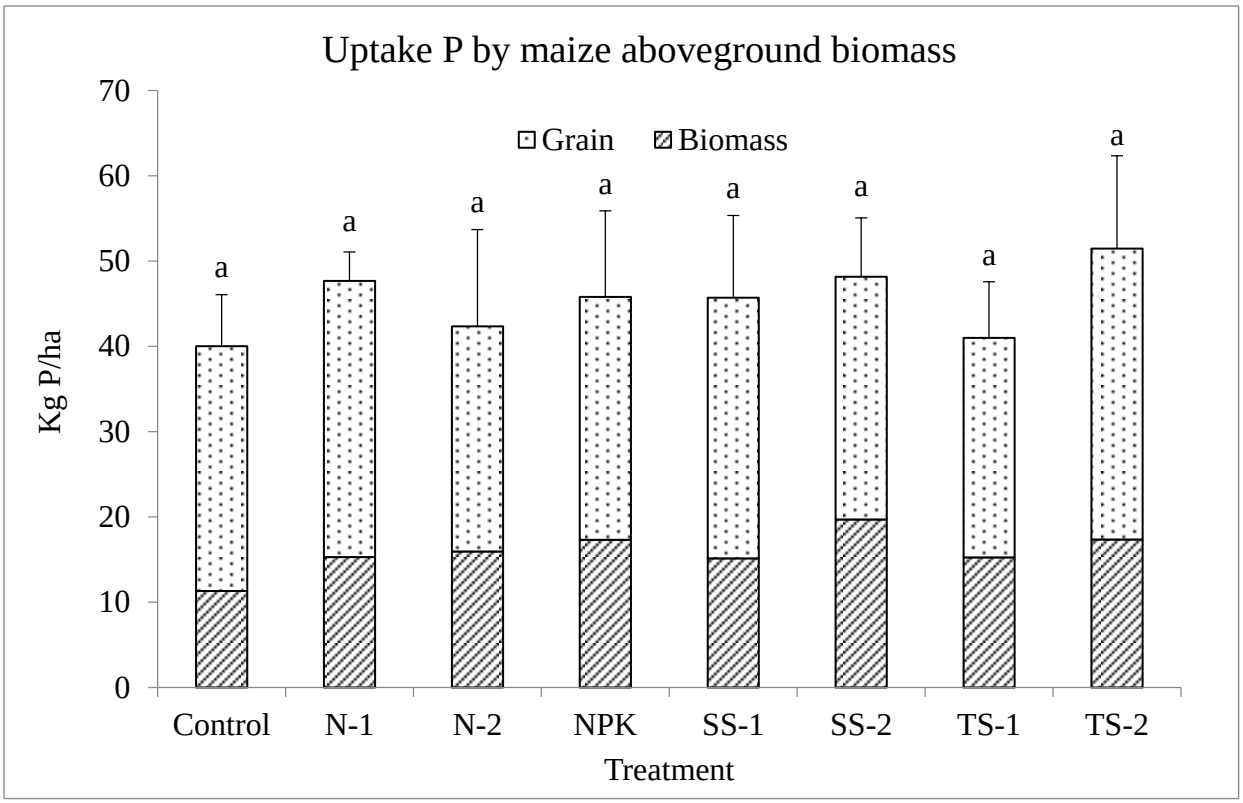


Figure 12 Uptake P by maize completed aboveground biomass from all treatments; Values shown represent the arithmetic mean (n=4) ± standard deviation; error bars indicate standard deviation; lowercase a above bars indicate statistical evaluation show no differences between the treatments on the level of 0.05

Figure 13 shows the aboveground biomass K uptake by maize affected by different application of fertilizer and biosolid material treatments. Statistical significant differences of K uptake among the treatments were not found. Based on the value in this figure, K uptake by maize aboveground ranged 151 – 193 Kg K/ha. There are only treatments TS-2 (193 Kg P/ha) and N-1 (188 Kg P/ha) were found to have higher value of K uptake, while the rest were obtained

at a lower value than control (185 Kg K/ha). The lowest value of K uptake was found TS-1 treatment

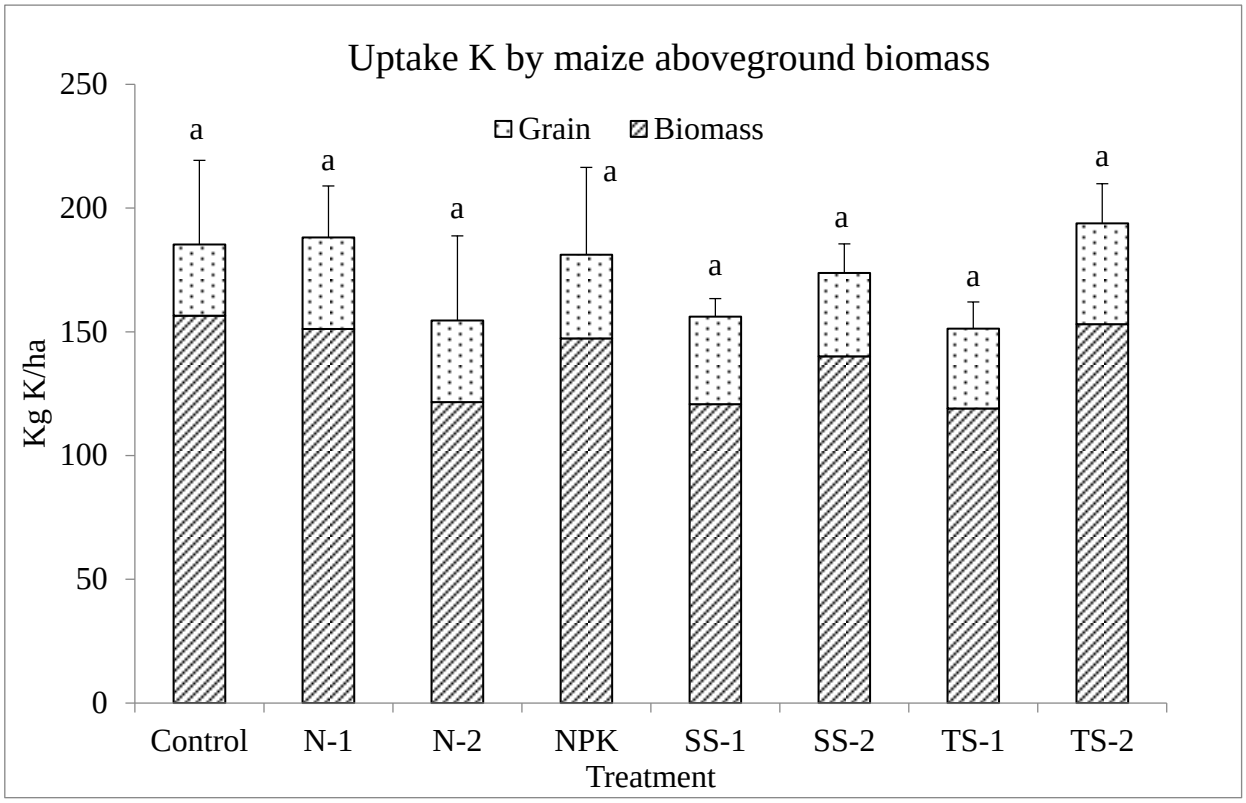


Figure 13 Uptake K by maize complete aboveground biomass from all treatments; Values shown represent the arithmetic mean (n=4) ± standard deviation; error bars indicate standard deviation; lowercase a above bars indicate statistical evaluation show no differences between the treatments on the level of 0.05

5.5. Concentration Changes of N, P, K Within the Vegetation Period

The time-course of the change in N, P, K concentration in dry matter of maize aboveground biomass are shown in figure 14, 15 and 16, respectively, for all treatments of the experiment. In this study, vegetation period of maize referred to the first (early growth), second (tasseling) and third harvest (maturity) of maize aboveground biomass.

Figure 14 illustrates the concentration changes of N within the vegetation period from all treatments. With the exception of the TS-2, N concentration of all treatments were declined steadily with increasing of age, whereas N concentration of T-2 was increased during the second and third harvest, from 1.39 – 1.50% N. Within the comparison, concentrations of N were highest for the early growth or first harvest of maize biomass.

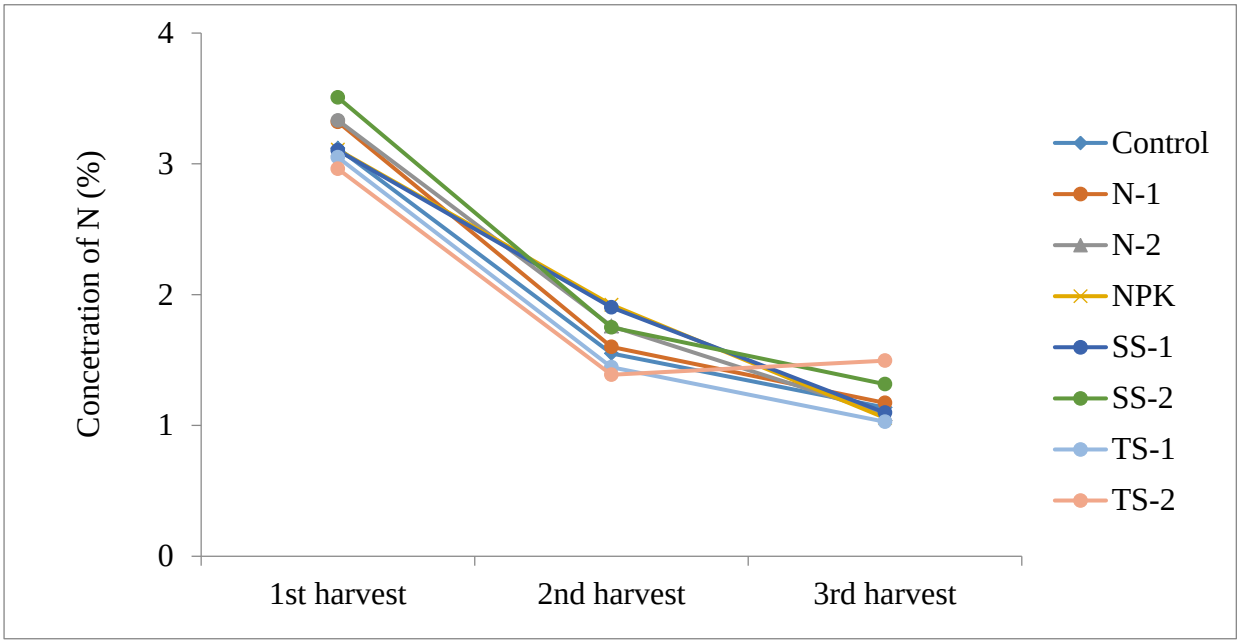


Figure 14 Concentration changes of N within vegetation period

In figure 15 shows the concentration changes of P within the vegetation period of maize. According to the graph, it can be observed that P concentration of all treatments except SS-2 were significantly increased during the second harvest or tasseling stage and sharply declined within the third harvest. In contrast, P concentration of SS-2 treatment was steadily reduced with increasing time. By comparison, concentrations of P were highest for the second harvest for these maize plants.

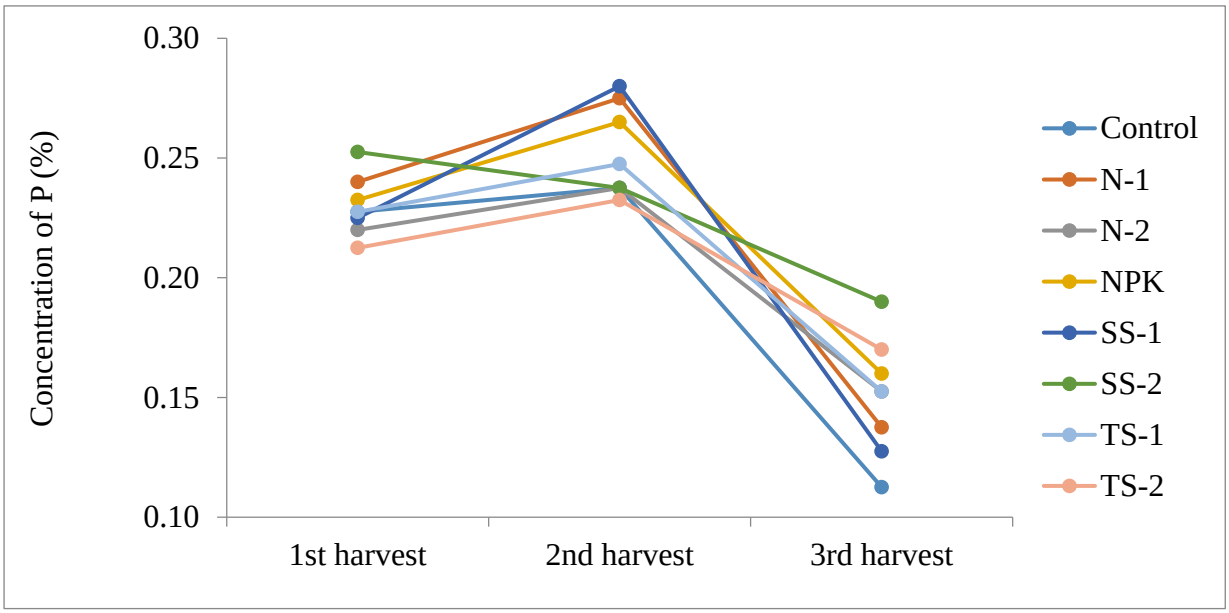


Figure 15 Concentration changes of P within vegetation period

Figure 16 presents the corresponding data for concentration changes of K within the vegetation period. The patterns of response were generally similar to concentration changes of N in figure 14, although K concentrations in biomass of all treatments tended to fluctuate somewhat within the period. With the exception of TS-1, K concentration from all treatments were significantly declined in accordance with period. In contraste, K concentration of TS-1 treatment was increased during the second harvest and slightly decreased at last harvest.

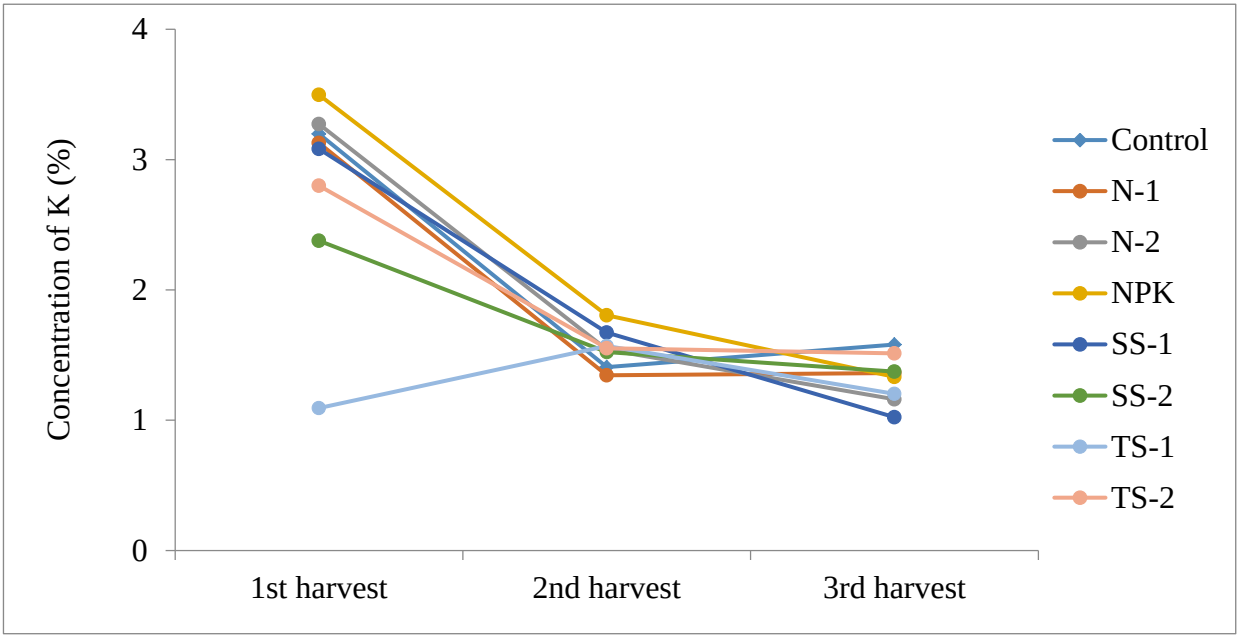


Figure 16 Concentration changes of K within vegetation period

5.6. Uptake Pharmaceuticals by Maize

As already mentioned in subchapter 4.3, there were 2 treatments with 8 replicates, resulted in a total of 16 plots were applied with different amount of dried biosolids (SS-1 = 7563 g/plot and SS-2 = 22689 g/plot) as well as torrefied biosolids materials (TS-1 = 8802 g/plot and TS-2 = 26406 g/plot). At the early growth, tasseling and maturity stages maize aboveground samples were harvested from these 16 plots as well as other treatments for pharmaceuticals analyses. According to material and method, to analyze pharmaceuticals in maize biomass, liquid chromatography-tandem mass spectrometry (LC-MS/MS) was conducted. As shown in table 14, the limit of detection was 0.5 ng/g of dry weight for biomass material. Any of the pharmaceuticals analyzed were detectable in the maize biomass but all were below the limit of detection at every harvested biomass material.

6. Discussion

6.1. Tested Biosolids

In this study, torrefaction of biosolids at temperature of 300°C for 3 hours was applied. It was noteworthy that the tested biosolids contained very low concentration of pharmaceuticals which meets the legislative criteria for land application. Based on the results in table 15, the contents of pharmaceuticals in dried biosolids ranged from 3 to 4987 µg/Kg of dry weight. In torrefied biosolids all pharmaceuticals were below the detection limit, except the ARBs telminsartan (14 µg/Kg d.w). Mercl et al. (2021), Moško et al. (2021), and Gao et al. (2020) reported that thermal treatment of biosolids before their utilization is the practice of choice to reduce the risk of soil contamination by application of biosolids. Torrefaction had a significant effect on the removal of pharmaceutical compounds and none of them was detected in torrefied sludge. The results revealed that the torrefaction temperature as 300 °C was sufficient to remove all pharmaceuticals below the detection limit (Moško et al. 2021).

Biosolids can be effectively recycled and applied as soil amendments for agricultural crops because it is a valuable source of organic matter and nutrients, especially phosphorus and nitrogen which favor for yield formation of crops (Razaq et al., 2017, Lu et al., 2012). The results in table 14 showed that dried biosolids contained 4.76% nitrogen. In contrast, torrefied biosolids contained slightly lower nitrogen (4.09%). Lower total nitrogen can be related with the potential loss mineral nitrogen due to thermal oxidation (Dad et al. 2019). In dried biosolids there were 2.58% of phosphorus and 0.31% potassium; while it was higher in torrefied biosolids, 3.66% and 0.52%, respectively. Mackay et al. (2017), Bridle & Pritchard (2004), Lu et al. (2012) also found that the contents of phosphorus and potassium were higher after torrefaction. They added that thermally converted biosolids can increase P and K concentration compared with dried biosolids due to a decrease in the concentration of carbon and other elements (Mackay et al. 2017; Yue et al. 2017).

6.2. The Effects of Biosolids on the Maize Aboveground Biomass Yield

Biosolids can serve as an acceptable source of plant nutrients the same as the use of commercial fertilizers for increasing crop yields (Pierzynski, 2015). The use of biosolids as a soil

amendment brings a numerous of positive impacts for crop growth as well as increased biomass yields (Vaccari et al., 2015; Ding et al., 2016; Wang et al., 2019). Kocsis et al. (2020), also supported that the use of biosolids significantly increased the maize yields.

In our experiment, we also found that the application of dried biosolids (SS-1) provided the highest maize biomass (11.8 t/ha) and grain yield (8.6 t/ha) in comparison to control and other treatments. In contrast, the lowest yield of aboveground biomass (9.9 t/ha biomass, 6.8 t/ha grain) was found on the torrefied biosolids (TS-1) application. Ilie et al. (2018) and Cornelissen et al., (2013) found that crop yields were higher when applied to biosolids compared to mineral fertilizer. Other studies illustrated that torrefaction of biosolids does not lower the acid or bicarbonate soluble phosphorus content; therefore, higher biomass and grain yield of maize grown on acidic soil amended by dried biosolids can be contribute to the higher availability of phosphorus and to some extent also nitrogen (Mercl et al. 2021). Due to torrefaction, however, the mobilization of nutrients decreased or inhibited; thus, the total yields of maize obtained from torrefied biosolids application were low. Roberts et al. 2017, Adhikari et al., 2019, and Huang et al., 2017) confirmed that increasing pyrolysis temperature of biosolids can decrease the plant-availability of phosphorus in biochar.

6.3. Content of Nutrients in Aboveground Biomass Maize

The effects of fertilizer, dried biosolids, and torrefied biosolids application on the nutrients concentration of maize aboveground biomass were shown in subchapter 5.3. Maize aboveground biomass was sampled at vegetation period (early growth, tasseling, and maturity stage) in all treatments. Biomass and grains from maturity stage harvest were considered as aboveground biomass in this study. Both the first and second harvest, the accumulation of N and P in biomass was highest in the maize grown on the soil amended with dried biosolids, which nutrients were easily available for plant uptake. However, the accumulation of N and P was the least in biomass grown on torrefied biosolids. Several studies confirmed that N and P thermal-stable or locked up in organic compounds were released slowly throughout the vegetation period and it thus nourished the plants at a slow rate over a long period (Arduini et al., 2018; Tejada et al., 2016). On the other hand, the concentration of K in biomass was higher in NPK application in both first and second harvest. Thus, the uptake of K was affected by NPK fertilizer applied to the soil.

At the last harvest, the highest level accumulation of N was found in the biomass grown on the torrefied biosolids (TS-2). This can be observed because at the maturity period, N in the thermally treated sewage sludge was relatively easily released and available taken up by plant. Arduini et al. (2018) also proved that N remobilization was calculated during maturity and grain filling. The contents of P were detected with the highest level in dried biosolids (SS-2). According to Corrêa (2004), mostly P availability from biosolids was greatest during the first sixteen weeks as a result of organic matter mineralization. In biomass grown on control treatment was found accumulated highest of K even though there was no fertilizer application. Kulhánek et al., 2014 stated that the soil was relatively rich in K in the same study site. Thus, it may be the influence factor. In contrast, the application of fertilizer showed no effect of accumulation rate, except the TS-2. The accumulation of N in maize grain was found higher in SS-2, which N-availability in dried biosolids was relatively easily taken up and accumulated in the grain. For the highest accumulated of P and K in the grain was detected in the plant grown on TS-2, which demonstrated in table 15, the material content of P and K were high in torrefied biosolids.

6.4. Uptake Nutrients by Maize Aboveground Biomass

N, P and K uptake by biomass and grain of maize at final harvest in all treatments are shown in figure 11, 12, and 13 respectively. The results demonstrated that the uptake of N found no significant differences among the groups, even though the different rates of fertilizer were applied in the soil. Based on the value, the highest uptake N by maize biomass and grain was found on the soil amended by dried biosolids (both SS-1 and SS-2), while the lowest uptake was TS-1 compared to the control. N was firmly bound under high temperature of torrefaction; hence, the immediate effect was relatively low. Furthermore, the mobilization of N in torrefied biosolids was even decreased compared to sewage sludge or dried biosolids which N was quite easily available (Geng et al., 2017; Zheng et al., 2016; Kirchmann et al., 2017).

All treatments with the application of fertilizer, dried biosolids, and torrefied biosolids were found significantly higher uptake of P compared to control. Among the treatments, the highest P uptake was detected by the maize grown on the soil amended with torrefied biosolids (TS-2). The results proved that P availability in torrefied biosolids was also affected by the increase of temperature on biosolids. Silveira et al. (2013); Bridle & Pritchard (2004) confirmed that torrefaction was an effective means to recover the valuable phosphorus present in the sewage

sludge. According to the figure 13, the highest uptake K by maize aboveground biomass was found on the TS-2, N-1 NPK, especially even in and the control but K uptake by maize was relatively at a remarkable rate. The results may be affected by the soil of the field experiment site which is relatively rich in K available (Kulhánek et al. 2014).

6.5. Concentration Changes of N, P, K Within Vegetation Period

As mentioned in the result, nutrients accumulation of N, P and K from all treatments were different at each growth stage in the biomass dry matter. The highest N concentration found during the early growth stage in all treatments. At this period, N is needed for plant's growth and development. Sufficient N at this period will support the stem elongation. At the tasseling stage, N concentration found declined rapidly in response to maintain dry matter and protein production but not compromising crop stability and maturity. The lowest concentration of N always observed in the maturity to ensure good protein level and to balance P. A comparison among all stages found that the younger the biomass, the higher the concentrations of N was found in all treatments. Other studies on change in nutrient concentration with time confirmed that N concentration in wheat biomass was higher at early growth stage and decreasing until harvest (Burns 1992; Gerdner 2014).

P concentration increased rapidly from the early growth to tasseling to promote strong leaf growth and build up strong crop canopy. From the tasseling to the maturity stage, P concentration dropped compared to the previous stage. This was in accordance with Payman et al. (2017) who found that P concentration hastily increases from active tilling to panicle initiation and it continues to increase at a decreasing rate to flowering. Moreover, concentration of P was observed lower than the previous stage from flowering to maturity. In contrary, Gerdner (2014) stated that the concentration of P in leaf biomass decreased with the increasing time period.

The highest concentration of K was found at the early growth stage. This trend was similar to N concentration because K is needed to promote strong leaf growth as well as build up strong crop canopy. During the tasseling or flowering stage, K is needed for protein synthesis and to maximize growth and yield at maturity. From the tasseling to maturity stage, K concentration was slightly decreased (Gerdner 2014).

6.6. Uptake of Pharmaceuticals by Maize

Sewage sludge residue is frequently used as a soil amendment, and may contain various organic contaminants including pharmaceuticals. These contaminants may eventually be taken up by plants (Wu et al, 2012; Kodešová et al., 2019; Bartrons & Peñuelas, 2017). There were no signs of biosolids-borne pharmaceuticals uptake when biosolids were torrefied or pyrolyzed (Mercl et al. 2021; Moško et al. 2021). Torrefaction (300°C) of biosolids significantly reduced the content of pharmaceuticals in resulting material (TS) as shown in table 14. Thus, our torrefied biosolids were safe for the soil application. Based on the result described in subchapter 5.6, no pharmaceutical compounds in maize tissues were detected in all treatments because these compounds in torrefied biosolids were below the detection limit and less in dried biosolids (table 14). Mercl et al. (2021) and Kodešová et al. (2019), on the other hand, reported that the uptake was limited because of soil properties (pH and CEC) which can influence the sorption of the pharmaceuticals in soil. Wu et al. (2012) also indicated that most pharmaceutical compounds tended to persist in the soil more than taken up by plants.

7. Conclusions

Biosolids are best known for their potential fertilizing properties and commonly used to improve agricultural soils by enriching it with slow release organic matter – preferable to synthetic fertilizer. Meanwhile, biosolids also contain organic contaminants, which are significantly considered to have a potential harmful effect on soil, vegetation, animals and humans. After drying and torrefaction (300°C), dried biosolids contained 4.76% of nitrogen, 2.58% phosphorus and 0.31% potassium, while torrefied biosolids contained of 4.09% nitrogen, 3.36% phosphorus and 0.52% of potassium, respectively. Biosolids were amended with the Cambisol soil at the field experiment close to Humpolec city. Maize was grown on amended soil to study uptake nutrients and pharmaceuticals.

The results of this study found that maize grown on the soil amended with dried biosolids at the rate of 200 Kg N/ha provided the highest of both biomass (11.8 t/ha) and grain yield (8.6 t/ha). Anyway, the statistically significant differences of total aboveground biomass yield were

not found among all treatments. Dried biosolids amendments at the rate of 200 and 600 Kg N/ha, resulted in the highest concentration of nitrogen and phosphorus in maize tissues from both the first and second harvest. The highest accumulation of nitrogen, phosphorus and potassium from the last harvest of biomass and grain was detected in torrefied biosolids at the higher rate. However, with the exception of K, there were no statistically significant differences between the accumulation of N and P in maize tissues. The application of dried biosolids at the higher rate, led to the highest uptake of nitrogen by maize 263 Kg N/ha, while the application of torrefied at the same rate led to the highest uptake of 51 Kg P/ha and 193 Kg K/ha, respectively.

Compared to dried biosolids, all pharmaceutical compounds in the torrefied biosolids were found below the detection limit after the pyrolysis temperature of 300°C was applied. Based on the results of this study, any detectable pharmaceuticals in maize tissues were not found above the detection limit (0.5 ng g^{-1} dry weight). Thus, the torrefaction (300°C) had ability to remove the pharmaceuticals from biosolids and to reduce the uptake of biosolids-borne pharmaceuticals by maize as well as had the greatest direct fertilizing effect. Therefore, thermally-treated biosolids would be suitable for long-term application on agricultural land because this asset increases plant growth and yield by providing nitrogen, phosphorus, potassium, and other essential nutrients. Furthermore, thermal-treated biosolids can minimize the risk of pharmaceuticals transfer from soil to plants.

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