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Comprehensive evaluation of sewage sludge and sewage sludge char soil amendment impact on the industrial hemp growth performance and heavy metal accumulation

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ABSTRACT

Industrial hemp (*Cannabis sativa L.*) has emerged as a potential multipiurpose crop: fibre crop, energy crop, and phytoextractor of pollutants from the soil. However, the multipurpise grwoth of hemp was restricted as its cultivation in EU was legalized only in the past two decades. Most scientific researches of sewage sludge (SS) describe it at relatively low application levels for cultivation of different crops. Such lack of information requires a wide range of practices for fertilizing hemp with high level of sewage sludge or sewage sludge char (SSCh) doses. At this moment there are no guidelines for sewage sludge char fertilization of energetic plants, depending on soil type. The aim of the study was to analyze and compare SS and SSCh soil application effect on industrial hemp (*Cannabis sativa L.*) growth performance, and heavy metals accumulation to determine optimum SS or SSCh application rates for the growth of hemp. The experimental design consisted of four treatments: 1–25; 2–50; 3–100; 4–200 of sewage sludge and sewage sludge char Mg ha^{-1} , and control soil (clay loam). It was determined that hemp ability to accumulate heavy metals depending on fertilisation intensity of sewage sludge and sewage sludge char decreased in all parts. Heavy metal distribution of hemp was selective; therefore their contents were decreasing in the following order: roots > stems > leaves. The study has confirmed the nutritional value of SS and SSCh. The potential of using the sewage sludge and its char as a source of organic matter for improvement of clay loam soil and a reasonable production of bioenergy crop like hemp without the use of inorganic fertilizers was shown.

1. Introduction

Fossil fuel reserves are becoming depleted and the need for increasing the share of renewable energy is increasing. The energy crops have high potential to increase the share of renewable energy. Many crop species are multi-purpose, i.e. they can be used to produce more than one type of bioenergy, for example oil and solid biofuel [\(Tuck](#page-11-0) [et al., 2006\)](#page-11-0), they can be grown as industrial crop or for seeds. The use of energy crops is highly promoted in EU as it may decrease greenhouse gas emissions and mitigate climate change ([Banja et al., 2019](#page-10-0)).

Industrial hemp (*Cannabis sativa L.*) has emerged as a potential energy crop having numerous advantages: high land use efficiency and biomass content, other factors such as low feed-stock cost, good weed suppression, low nutrients requirement, no/zero pesticide demand and improvement of soil health [\(Lehmann et al., 2011;](#page-11-1) [Li et al., 2010](#page-11-2); [Prade](#page-11-3) [et al., 2011\)](#page-11-3). It can be effectively grown in diverse climates and can be used in organic crop rotation ([Barberá et al., 2011;](#page-10-1) [Kreuger et al.,](#page-11-4) [2011\)](#page-11-4). Because of these properties, hemp is valuable crop for the biobased economy. European Union produces about 29 % hemp of total world production [\(Barberá et al., 2011](#page-10-1)). The cultivation of hemp is increasing in EU reaching more than 33,000 ha in 2016 (Carus, 2017). Different climate change models under SRES scenarios have showed that the distribution of hemp in Europe in the 21st century is expected to move northwards, and hemp cultivation would disappear from Southern Europe and shift to Northern Europe by the 2050s and 2080s ([Tuck et al., 2006](#page-11-0)).

It is recommended to use industrial or energy plant species not only for energy purposes but also for the phytoextraction of pollutants (such as heavy metals, organic pollutants) from the soil (Bielińska, 2016). Many authors confirm that it is possible to use energy plants (grasses)

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for the phytoremediation of soils polluted with heavy metals ([Barbosa](#page-10-2) [et al., 2015](#page-10-2); [Shrestha et al., 2019;](#page-11-5) [Van Ginneken et al., 2007](#page-11-6)).

Despite the positive properties of industrial hemp, its cultivation was legalized in EU countries only in the 1990s or even later, i.e., in Lithuania the cultivation of hemp (*Cannabis sativa L.*) was legalized in 2014. Thus, the cultivation of industrial hemp for energy purposes is a relatively new agricultural branch that requires knowledge about the optimal fertilization rates to get as much biomass as possible [\(Prade,](#page-11-7) [2011\)](#page-11-7). The energy value of the biomass obtained is the main parameter of energy plants, which depends on plant density, environmental conditions (soil properties), cultivation pattern, etc. The biomass of energy plants could be increased with fertilizers application or sewage sludge/ biosolids amendments ([Schröder et al., 2018\)](#page-11-8).

Sewage sludge (SS) is a source of many valuable nutrients (especially N and P), trace elements (S, Mg, Ca), but also contains potentially toxic compounds such as heavy metals, persistent organic pollutants posing a risk to humans and environment. The application of SS for energy plants cultivation may be good and cheap alternative to the fertilizer's application, could help to improve soil quality, reduce sewage sludge disposal in landfills and herewith greenhouse emissions from landfills and contribute to climate change mitigation [\(Schröder](#page-11-8) [et al., 2018](#page-11-8)). Hemp is an excellent soil phytoremediation agent because it extracts heavy metals ([Mihoc et al., 2012](#page-11-9)).

At very heavily contaminated and denuded sites, such as former mine areas, particularly where there are surface leachates of heavy metals and unconsolidated soils and wastes, biochar may be useful to restrict the wider impact of contamination beyond site boundaries ([Moreno-Jiménez, 2016\)](#page-11-10). However, most crop plants cannot survive on soils heavily contaminated with heavy metals ([Mihoc et al., 2012](#page-11-9)). Therefore, more extensive research is needed to determine the optimum dosage of SS in order to have high energy plants yield and do not pose risk to soil biota.

Most of research has focused only on SS application impact and sewage sludge char (SSCh) application was less studied. Sewage sludge char is the most appropriate pyrolysis product, which was also preferable from point view of energy balance [\(Wang et al., 2012](#page-11-11)). The energy implications of biochar production were discussed recently by [Callegari](#page-11-12) [and Petr Hlavinek, 2018](#page-11-12) and [Capodaglio et al., 2016.](#page-11-13) Sewage sludge char physico-chemical properties and afterwards impact to soil biota is driven by pyrolysis process temperature and feedstock (sewage sludge) properties [\(Callegari and Capodaglio, 2018;](#page-11-14) [Hossain et al., 2011](#page-11-15); [Lehmann et al., 2011\)](#page-11-1). Physico-chemical characteristics of SSCh; in addition to microstructures based on their pores (specific areas), heavy metal content and leaching potential, has an important benefits to use it as fertilizer in energy crop production. Heavy metals accumulation, particularly As, Cd, Cr, Cu, Pb, Ni, Se and Zn and, is of specific concern as far as agricultural activities are concerned, and is also one of the principal reasons for the existing limitations on continuing sludge agricultural disposal practices ([Callegari and Capodaglio, 2018](#page-11-14)). More detailed analysis of biochar after microwave pyrolysis of sewage sludge was recently discussed by [Racek et al., 2019](#page-11-16).

Heavy metal solubility and bioavailability in a Mediterranean agricultural soil after amended with raw SS or its biochar and found that the leaching of Cu, Ni and Zn was lower in the soil treated with biochar than in the soil treated directly with SS, and plant availability of Ni, Zn, Cd and Pb has also been reduced in the biochar-amended soil when compared with the SS-amended soil [\(Méndez et al., 2012\)](#page-11-17). However no information is available on difference in heavy metal and trace elements bioconcentration when biochar was produced at different temperatures ([Praspaliauskas et al., 2018;](#page-11-18) [Song et al., 2014](#page-11-19)). In some studies of sewage sludge (SS) (Bielińska, 2016) or sewage sludge char [\(Finnan and Burke,](#page-11-20) [2013](#page-11-20)) has been applied at relatively low levels following recommendations for productive arable crops. The studies of fertilization with municipal sewage sludge ([Borkowska and Molas, 2013](#page-11-21); [Kołodziej et al.,](#page-11-22) [2015](#page-11-22)) provided evidence that the best effects on the growth and development of plants are exerted by nitrogen, phosphorus, and potassium.

The sewage sludge used for fertilizing energy crops is a perfect source of these elements, particularly of nitrogen and phosphorus [\(Kołodziej et al.,](#page-11-22) [2015](#page-11-22)). However, sewage sludge contains too little potassium to meet the needs of energy crops, therefore it should be applied in mineral form after a prior evaluation of the soil resources of this component. This lack of information has led to a wide range of practices for fertilizing hemp with high level of SS and SSCh char doses.

However, sewage sludge contains too little potassium to meet the needs of energy crops, therefore it should be applied in mineral form after a prior evaluation of the soil resources of this component ([Kirchmann et al., 2017\)](#page-11-23). This lack of information has led to a wide range of practices for fertilizing hemp with high level of SS and SSCh doses. The effects of plant fertilization with sewage sludge and sewage sludge char have been extensively studied, there is insufficient information on the amount of heavy metals, alkaline earths and alkali metals being accumulated by the increasingly used energy crops that are increasingly used. There is not enough information on how heavy metals are distributed in different parts of the industrial hemp: roots, stems and, leaves at different fertilization intensities. It is important to take into account these factors, since the further use of biomass of energy plants, due to the increased concentration of elements, causes problems related to ash melting in the boilers.

Taking into account the above considerations, our aim was to analyze and compare SS and SSCh application effect on industrial hemp (*Cannabis sativa L.*) growth performance, and heavy metals accumulation to determine optimum SS or SSCh application rates for the growth of hemp.

2. Materials and methods

2.1. Soil, municipal sewage sludge and sewage sludge char

Clay loam soil was selected due to reason that this type of soil is predominant in Lithuania and covers about 21 % of the total area. The soil pH_{KCl} was of 7.20 \pm 0.04, the content of available phosphorus, potassium and magnesium was at a low level.

Sewage sludge was collected from the local municipal wastewater treatment plant. Char was prepared using also this sewage sludge at selected condition. More details are presented in paper of Praspaliauskas, Pedišius and Striūgas, 2018 [Table 1.](#page-2-0)

Sewage sludge and sewage sludge char was mixed with a mixture of field top-soil, perlite and fine sand (5:3:2, by volume).

2.2. Conditions of the experiment

The experimental design consisted of four treatments: 1–25; 2–50; 3–100; 4–200 of sewage sludge and sewage sludge char Mg ha−1, and control soil (clay loam). All the treatments were executed in three replicates. Industrial hemp (*Cannabis sativa L.*) seeds were sown in plastic pots at the density of 65 plants m-2. Plants were grown for four months in closed-top 10 m^3 -growth chambers under the following conditions: a day/night air temperature 21/14 °C, the relative air humidity (RH) of 50/60 %, a light level of \sim 270 μmol m⁻² s⁻¹ photosynthetically active radiation (PAR) and a day length of 14 h.

The following endpoints were measured to evaluate the growth of industrial hemp: plant height, root length, stem, leaf and root dry biomass. After harvesting all hemp parts were separated (roots, stems, leaves) and oven-dried (60 °C). Roots were washed with deionized water, and then oven-dried and subjected to elemental analysis. At the end of the experiment, soil samples were collected, dried and the same elements as in industrial hemp were measured [\(Fig. 1\)](#page-2-1).

2.3. Element analysis

The obtained samples (soil, roots, stems and leaves) were mineralized at the same method for determination of the selected elements.

Table 1

Element concentrations (mg kg−1) in sewage sludge, sewage sludge char [\(Praspaliauskas et al., 2018\)](#page-11-18).

The samples were mineralised with 3 ml of concentrated nitric acid, 3 ml of hydrofluoric acid and 1 ml of hydrochloric acid at 800 W, 6 MPa, pRate: 50 kPa·s^{-1}). After the mineralization, the samples were flooded with 18 ml of boric acid (H_3BO_3 to avoid and eliminate fluoride toxicity) and again placed into a mineralizer for 1 h and 10 min (at 800 W, 6 MPa, pRate: 30 kPa·s⁻¹). The analysis of the solutions (including determination of Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, S, Sb, Ti, Zn, Ba and Be) prepared from the soil, roots, stems and leaves samples were performed using an ICP-OES (Perkin Elmer).

Determinations in each of the analyzed samples were carried out in three replications. For the data acquisition of the samples, a quantitative analysis mode was used. The scanning of each single sample was repeated three times to gather reasonably good results. During measurements, care was taken to avoid memory effect and therefore a wash-out time of 1 min was used.

2.4. Processing of the results

Bioconcentration (BCF) factor defined as a coefficient which shows what quantities of elements able to absorb to plant different parts from a soil. This factor defines the ability of individual elements to accumulate in certain parts, regardless of whether the fertilizer is used at high or low concentrations. The Bioconcentration factor can be used to evaluate the plant's phytoextraction efficiency and calculated according to Eq. [\(1\)](#page-2-2) and metal pollution occurs only in the active rooting zone, that is, top soil layer (0–20 cm) [\(Okieimen, 2011](#page-11-24)).

$$
ECF = \frac{E_p}{E} \tag{1}
$$

where, E_p —element concentration in the tissues of the plant (root, stems, leaf) (mg kg⁻¹), *E_s*— initial concentration of element in the soil $(mg kg^{-1})$.

The rates of sewage sludge or sewage sludge char derived from sludge required for gaining the highest biomass and for ensuring the maximum removal of hazardous substances (e.g. heavy metals) from a soil are determined based on this criterion. The higher the value of this coefficient, the greater amount of elements from the soil can be absorbed by the plant compared with its primary amount in the soil. In generally when a BCF \leq 1, it indicates that the plant can only absorb but not accumulate heavy metals; when a BCF > 1 , it shows that plant can accumulate metals ([Liu et al., 2009](#page-11-25)). According to [\(Pachura et al.,](#page-11-26) [2016\)](#page-11-26), the accumulation factor in the parts of the plant is divided into four groups:

Fig. 1. Simplified diagram of the experiment of element accumulation in industrial hemp parts.

- *BCF* < 0,01 the element is not accumulated in the plant;
- \bullet 0,01 < *BCF* < 0,1 low level of accumulation;
- \bullet 0,1 < *BCF* < 1,0 average level of accumulation,
- $BCF > 1$ high level of accumulation.

A one-way analysis of variance (ANOVA) was used to assess the sewage sludge and sewage sludge char application dose effect on estimated endpoints. Significant differences between treatments were determined by Student's test and $p < 0.05$ were considered to be significant. Regression and correlation analysis were used to assess the relationship between sewage sludge and sewage sludge char doses and plant response. All the statistical analysis was carried out using Statistica software.

3. Results

3.1. Soil properties after soil treatment with SS and SSCh

Heavy metal content in the soil did not exceed permissible values when SS and SSCh were used for reclamation. Exception was Zn which exceeds the limited value in highest fertilization intensity of SSCh, and chromium which concentrations in all soil types (even in the control) of this experiment were increased ([Table 2](#page-3-0)). Be, Cd and Pb was not found in untreated soil and in soil after treatment with SS and SSCh. The analysis of these elements will not be discussed further in this paper.

It has been observed that the soil in which the sewage sludge char was added retained the moisture longer than in the soil containing the sewage sludge or soil that was not fertilized. Studies show that pyrolysis char produced from sludge during pyrolysis is more resistant to leaching and retains moisture longer than raw sewage sludge or ash from direct combustion. ([Manara and Zabaniotou, 2012\)](#page-11-27).

The analysis of the elements showed that the concentrations of the heavy metals regulated by the soil for the studies were different, and some metals were higher than the background concentrations, probably due to the higher content of these metals in sludge applied to the soil. The higher background concentrations of heavy metals in the soil ([Table 2](#page-3-0)) were Cr and Ni, respectively 89.77 mg kg⁻¹ and 25.57 mg kg^{-1} , when the regulated rates for these types of soils are respectively below 44 mg kg⁻¹ and 18 mg kg⁻¹. The concentration of Cu in the selected soil was very low compared with the background concentration and was only 0.55 mg kg⁻¹, while in Pb, Cd and in soil was below the detection limit of the method.

Sewage sludge and sewage sludge char application dose had very significant effect on the heavy metals concentrations in the soil (ANOVA, $F_{SS} > 30.50$, $F_{SSCh} = 47.39$, $p < 0.01$) and metal concentrations in the soil increased along with sewage sludge and sewage sludge char application dose ($r^2 > 0.59$, $p < 0.05$) with the exception of Cr concentration after SS application [\(Table 2\)](#page-3-0). The highest increase in heavy metals concentrations after SS and SSCh application was for Cu and Zn, while the effect on the Cr, Ni and Ti was less pronounced. Regardless of the amount of heavy metals used with the agent used in the soil, it is important to periodically check the concentrations in order not to exceed the MRLs. It is also important to evaluate the leaching of heavy metals from the soil layers in order to avoid groundwater pollution [\(Libra et al., 2011\)](#page-11-28).

Concentrations of essential nutrients such as Ca, Mg, Fe and Mn significantly increased along with SS and SSCh application doses and the SSCh application resulted in higher nutrients level in the soil (t-test, p < 0.05). However, no increase in K concentration, major nutrient for plant growth ([Zörb et al., 2014\)](#page-12-0), was observed with the addition of sewage sludge or sludge char. The content of magnesium in the soil after the SS and SSCh application increased up to 1.30 and 1.47 times, respectively, compared with the background soil concentration, indicating that SS was not rich in Mg. The small difference in concentration of K and Mg before and after the treatment with SS and SSCH indicates that fertilization with SS and SSCh may be insufficient to meet

Elemental composition of soil without treatment and after treatment of sewage sludge and sewage sludge char (mg kg−1 d.m). Elemental composition of soil without treatment and after treatment of sewage sludge and sewage sludge char (mg kg^{-1} d.m.)

4

Table 2

the requirement for these micronutrients for plant growth and additional K and Mg supplementation could be required. No significant changes in K and Mg concentrations in soil were also recorded in France after 30 and 60 t ha⁻¹ sewage sludge application ([Bourioug et al.,](#page-11-29) [2015\)](#page-11-29). Since sewage sludge usually contains large amounts of phosphorous, soil amendment with SS and SSCh has led to a dramatic increase in P level in the soil. Significant accumulation of total and plantavailable P in soil after sewage sludge application was also reported in other studies ([Krogstad et al., 2005\)](#page-11-30).

The amount of phosphorus in the soil increased linearly with the dose of SS and SSCh added to the soil ($r^2 = 0.95$ and $r^2 = 0.92$, respectively, $p < 0.0001$ and was by 6.15–26.85 times higher than P level in control soil. Is it assumed that phosphorous present in sewage sludge could cover up to 20 % of phosphorous demand [\(Kominko et al.,](#page-11-31) [2018\)](#page-11-31). However, if the sewage sludge application as fertilizer is regulated on nitrogen and heavy metal content basis, P soil concentrations can exceed plant needs. P excess may result in surface or ground water pollution due to P surface runoff or leaching [\(Wang et al., 2020](#page-12-1)). The concentration of plant essential nutrient S, constituent of amino acids, proteins and coenzymes, was also significantly increased (7−63-fold) after SS and SSCh application.

3.2. Biometric parameters of industrial hemp

The total dry biomass of industrial hemp and other morphological parameters are presented in [Table 2](#page-3-0). Hemp yield depending on the treatment, varied within the following range: 4820–64 kg ha⁻¹. The data presented in the table show that SS and SSCh application had both positive and negative impact on the growth of hemp. The research showed that application of 25 Mg ha^{-1}of sewage sludge has led to an increase in hemp yield by 3.26 times, though SSCh had no positive impact. Further increase in application intensity resulted in significantly decreased aboveground and belowground biomass gain. Application of 50 Mg ha^{-1}of both sewage sludge or sewage sludge or sewage sludge char has resulted in two-fold lower aboveground biomass and the biomass of roots was more affected and it was by 64 % lower than that of control plants. Doses higher than 50 Mg ha⁻¹of sewage sludge or sewage sludge char caused a drastic decrease in hemp yield. 200 Mg ha−1of sewage sludge dose had lethal consequences for plants and further analysis using this rate of sewage sludge was not carried out. Industrial hemp grown in the soil amended with maximum rate of SS char survived, but biomass gain was negligible (2.1–5.9 % of control biomass ($p < 0.05$)). Root biomass was more negatively affected by the sewage sludge or SS char application than above ground biomass. In case of aboveground biomass growth, stems were more sensitive to negative SS and SSCh impact than leaves.

The study results indicate that fertilization with sewage sludge and fertilization with char exceeding 25 Mg ha^{-1} cannot be recommended. In this case, the need to carry out further research using sludge rates below 25 Mg ha⁻¹for fertilization arises, and to enrich char with additional microelements, such as potassium, nitrogen and phosphorus. Positive SS char application effects at low doses (up to 15 Mg ha⁻¹) were recorded in two cropping seasons of corn ([Melorose et al., 2015](#page-11-32)), tomatoes [\(Hossain et al., 2010](#page-11-33)) ([Table 3\)](#page-4-0).

The quantities of industrial hemp biomass obtained were significantly lower during this research compared with the results of other authors ([Kreuger et al., 2011;](#page-11-4) [Rice, 2008\)](#page-11-34). The comparison of biomass influence with the results of other research using sewage sludge for fertilization or char derived from sewage sludge is a conditional matter. Assessing biomass gain is not very objective due to a different chemical composition of sewage sludge and char. The main problem using sewage sludge and char for fertilization of energy plantations is that such sludge contains too little potassium that is insufficient for plant growth. For this reason, mineral potassium contained in fertilizers is used for soil fertilization ([Major, 2012](#page-11-35)). The soil selected was relatively poor because of its chemical properties. A seeding density is another important parameter, i.e. a wrongly selected seeding density leads to reduced biomass gain and its quality. Biomass gain of herm also depends on the environmental conditions and the cultivation model selected [\(Zörb et al., 2014](#page-12-0)).

In comparison of other morphological parameters such as the height of the stem or the length of the roots and the biomass, it can be seen that fertilization with sewage sludge and char derived from sludge had a different effect. Increase of amount of sewage sludge or char derived from sludge resulted for the most part in decline of the aforementioned parameters in the soil. Only a 25 Mg ha^{-1}rate of sewage sludge had positive effects compared with a blank control. The average increase in height of stems was 15 cm, the roots at this rate of fertilization were slightly shorter (around 3 cm), however biomass was about 130 g higher. All other fertilization rates had a negative effect on the height of industrial hemp's stem, the length of the roots and amount of biomass. The results of the research show that additional amount of these substances causes industrial hemp stress which is usually due to heavy metals.

A three times higher increase in biomass than in a blank control has been determined when a soil has been fertilized with sewage sludge at a rate of 25 Mg ha−1. Further increase in fertilization intensity resulted in significantly decreased biomass gain, and in the maximum fertilization rate no industrial hemp has grown up. The biggest biomass gain has been determined at 50 Mg ha⁻¹ rate using char derived from sewage sludge for fertilization. However, it was significantly lower compared with biomass gain when a fertilization rate of sewage sludge was 25 Mg ha⁻¹. Continued increase of fertilization intensity, same as sewage sludge, resulted in a significant decrease in biomass gain. Industrial hemp survived at the maximum rate of char, but biomass gain was absolutely insignificant. It is important to mention that compared with a blank control biomass gain was higher only when a sewage sludge rate was 25 Mg ha−1, while all other fertilizations have had a negative impact on biomass gain. It can be said that fertilization with sewage sludge and fertilization with char exceeding 25 Mg ha⁻¹is completely useless and unnecessary. In this case, the need to carry out further research using sludge rates below 25 Mg ha⁻¹ for fertilization arises, and to enrich char with additional microelements, such as potassium, nitrogen and phosphorus.

The height of the stem and the length of the roots responded to SS and SSCh application in the same manner as biomass did. Only a 25 Mg

Table 3

The effect of fertilization doses of sewage sludge and sewage sludge char on the biomass yields and plant biometrics.

	Heigh, cm	Root length, cm	Stem yield (d.m), kg/ha	Leaf yield $(d.m.), kg/ha$	Above ground biomass yield (d.m.), kg/ha	Root yield (d.m.), kg/ha
Control	91.4 ± 15	$33.2 + 6.9$	861.0 ± 287.8	616.6 ± 122.1	1477.6 ± 407.02	253.1 ± 73.2
25SS	$113.3 + 9.6$	32.7 ± 4.4	1802.1 ± 170.2	$3018.8 + 215.9$	$4820.9 + 186.9$	384.6 ± 6.5
50SS	45.4 ± 15.9	$21.1 + 7.7$	264.1 ± 150.56	548.1 ± 283.7	812.2 ± 434.3	92.1 ± 50.9
100SS	$25.0 + 9.7$	20.1 ± 8.7	110.0 ± 62.7	187.1 ± 139.6	292.9 ± 204.6	33.8 ± 19.9
25SSCh	53.6 ± 21.3	16.8 ± 5.8	518.3 ± 437.4	340.9 ± 191.8	859.1 ± 627.3	119.3 ± 96.8
50SSCh	46.0 ± 14.7	18.3 ± 5.8	$499.8 + 362.6$	$397.8 + 184.5$	$897.6 + 537.8$	91.6 ± 58.5
100 SSCh	$29.2 + 8.5$	14.5 ± 6.0	$103.1 + 87.9$	$187.9 + 109.5$	$291.0 + 197.2$	20.2 ± 14.6
200 SSCh	20.5 ± 1.66	11.0 ± 4.51	23.4 ± 1.1	64.2 ± 28.4	87.6 ± 27.9	5.4 ± 2.9

ha⁻¹ rate of sewage sludge had positive effects compared to control. All other SS and SSCh fertilization rates had a negative effect on stem height and root length, the length of industrial hemp. Our results are in line with other studies, showing a stimulatory effect on plant growth (height and biomass) at low SS doses and inhibition at high SS doses (Bielińska, 2016; [Kołodziej et al., 2015;](#page-11-22) [Ramírez et al., 2008](#page-11-36))

3.3. Elemental uptake by hemp parts

Heavy metal uptake by hemp parts, as a sum of the entire growth period (four months) are presented in [Figs. 4, 5 and 6.](#page-7-0) It is apparent from the present study that industrial hemp absorb a wide range of heavy metals in different parts, in different concentrations, with respect to different SS and SSCh amendments. Analysis of variance (ANOVA) has revealed that SS and SSCh amendment dose had a highly significant effect on the heavy metal, macro- and micronutrient concentrations in hemp tissues. Metal concentrations mostly were significantly $(p < 0.05)$ higher in roots than in stems and leaves with the exception the content of macro- and micronutrients. Variability of all analyzed metals in different parts of hemp may also be due to their compartmentalization and translocation in the vascular system [\(Fig. 2](#page-6-0)).

One major pattern was found: the largest amounts of Co, Cr, Cu, Ni and Fe were accumulated in the roots, while Zn and Mn were equally accumulated in all parts of the industrial hemp Co concentration decreased in all parts of the hemp with the amount of sewage sludge, though increased with sewage sludge char concentration. When hemp grew in SS char amended soil, Co concentration in leaves and stems increased with SSCh concentration $(r_{\text{stem}}^2 = 0.72, r_{\text{leaves}}^2 = 0.62,$ p < 0.05). Similar patterns of cobalt concentrations in stems and roots have been identified by [\(Wa Lwalaba et al., 2017](#page-11-37)) study.

Cr was found only in hemp roots, and our results are in line with other findings that Cr predominantly accumulates in the roots and only small part of it is translocated to the aboveground parts of plants ([Citterio et al., 2003\)](#page-11-38). As SS or SSCh had no significant effect on Cr concentrations in the soil ([Table 3\)](#page-4-0), no increase in Cr concentration was also found for hemp tissues. Moreover, Cr concentrations in hemp grown in SS and SSCh amended soil were lower than in the control plants (t-test, $p < 0.05$). The results of the study are confirmed by other studies [\(Khan et al., 2013](#page-11-39); [Dede and Ozdemir, 2016](#page-11-40)), whereas decrease in Cr was also observed with the intensification of fertilization. The use of sewage sludge char has resulted in a uniform decrease in Cr concentration regardless of fertilization. With minimal and maximum char rates in the soil, the Cr concentration decreases by about 49 % \pm 5 in the control variant. Decrease in heavy metals accumulation from SSCh amended soils partially could be explain by the increase in char sorbtive capacity leading to the immobilization of metals and lower bioavailability [\(Khan et al., 2013\)](#page-11-39).

Similar to Cr, Ni was found only in roots and stems of hemps grown in sewage sludge amended soil. Lowest Ni concentrations were detected in the root and stems of hemps grown in the treatment of 25 (SS) Mg ha−1, further increase in SS fertilization dose has led to an increase in Ni content up to 44 % (t-test, $p < 0.05$) compared with control. With the increase in the fertilization intensity, Ni stem content gradually increased from 0.1 mg kg^{-1} to 0.86 mg kg^{-1} . Similar pattern of Ni and Cr accumulation in *C. sativa* was observed by [Citterio et al., 2003](#page-11-38). Ni retention in roots might be explained by Ni-0-histidine complexes in the vacuolar Ni compartmentation [\(Richau et al., 2009](#page-11-41)).

The increase in Cu concentration in hemp was observed along with SS and SSCh application rates. In the case of sewage sludge, the copper concentration gradually increased from 291 % to 526 % in the roots and from 6% to 742 % in the stem compared with the control. The highest concentration of Cu in the leaves was recorded in the treatment with 25 (SS) Mg ha^{-1} (up to 556 % with respect to control), and further decreased with SS concentration (279 $%$ \pm 12 compared with control). In case of soil amendment with sewage sludge char, similar Cu content was determined in the roots and leaves, regardless the fertilization

intensity. Though Cu concentration in the stems increased gradually with increasing SSCh fertilization (r_{stems}^2 = 0.83, p < 0.05) reaching the maximum at 200 Mg ha^{-1}. Cu content in the stems of hemps grown in SSCh amended soil was up to 152 % higher than that in control plants. Relatively high stem Cu concentrations could be explained by micronutrients translocation via stems ([Page and Feller, 2015\)](#page-11-42).

Ti was mainly accumulated in the roots and the highest Ti level was detected in control plants. Analyzing SS and SSCh treatments it could be seen that higher SS and SSCh dose resulted in higher amount of Ti accumulated, however it remained below the control level. Despite that SS and SSCh application increased Ti content in the soil ($p < 0.05$, [Table 3](#page-4-0)), Ti in these matrices could of low bioavailability. The distribution of iron concentration in the hemp tissues exhibited the similar pattern as Ti. The highest accumulation of Fe was in roots and stems with leaves accumulated similar content ($p > 0.05$).

Accumulated Zn in hemps was rather equally distributed among the roots, stems and leaves. SS and SSCh in most cases resulted in higher Zn content in hemp tissues compared with those of control plants. Though some differences could be found. Zn concentrations in roots and leaves of hemps grown in SSCh amended soil were lower than those in roots and leaves of hemps grown in SS amended soil. The highest increase in Zn concentration was detected in stems, both for SS and SSCh fertilization (up to 14.3-fold increase). The study showed that Zn was mostly accumulated in all parts of the hemp comparing to other heavy metals. High Zn accumulation in different plants species was also shown in other studies ([Gong et al., 2018](#page-11-43); [Song et al., 2014\)](#page-11-19). The comparison of As, Cd, Cr, Cu, Mi and Zn accumulation from SS amended soil by energy plants (maize, oilseed rape and hemp) has revealed that Zn accumulation was the highest one [\(Seleiman et al., 2012](#page-11-44)). An analysis of the uptake of manganese by hemps showed that Mn added the soil with sewage sludge is mostly accumulated when the sewage sludge rate of 50 Mg ha^{-1}is applied.

The content of alkaline earth and alkali metals in energy crops is very important because of ash slagging during the combustion. Increased concentrations of alkaline earth and alkali metals, especially Ca, K, Mg and Na, form compounds with chlorine, phosphates, sulfates and other combustion products [\(Prade et al., 2011](#page-11-3); [Wang et al., 2018\)](#page-12-2) ([Fig 3\)](#page-7-1).

The concentration of Ba was lowest among studied alkaline earth and alkali metals ([Fig. 5](#page-8-0)). It was found that the highest Ba root concentration was in control plants and fertilization with SS or SSCh did not lead to the increase in its root content. The results of the study show that the accumulated Ba amounts in stems and leaves are significantly lower than in roots when using sewage sludge for fertilization. Though than sewage sludge char was used for fertilization, the difference among the Ba content in roots, stems and roots were somewhat smaller. Moreoever, Ba content in stems and leaves increases with SSCh fertilization rate ($r_{\text{stem}}^2 = 0.88$, $r_{\text{leaf}}^2 = 0.67$, p < 0.05).

Analysing sewage sludge and sewage sludge char suitability as an fertilizer and their capability to replace inorganic fertilizers, macronutrients tissue concentrations were assessed. SS and SSCh was a potential source of macronutriens essential for plants growth and resulted in an increses in macronutrients concentrations. Root, stem and leaf Ca concentrations in hemps grown in SS amended soil were similar and accumulated Ca was distributed evenly. While, in case of SSCh amended soil, Ca concentration was in the following order: leaf, stem, root. Additionaly, in most treatment doses, hemps grown in SSCh amended soil took up more Ca compared with those grown in SS amended soil. The increase of Ca concentration in hemps grown inSSCh amended soil was determined by increasing fertilisation of sewage sludge char ($r_{\text{root}}^2 = 0.58$, $r_{\text{stem}}^2 = 0.98$, $r_{\text{leaf}}^2 = 0.33$, p < 0.05). In hemp stems amount of Ca at minimum fertilization rate reached $8.7 g kg^{-1}$ and increased significantly up to 27.5 g kg⁻¹ at the maximum 200 Mg ha^{-1} dose. In leaves, the increase in Ca concentration with the increase in sludge char amounts was less pronounced, but total Ca leaf concentrations were higher than stem concentrations. Potassium

Fig. 2. Accumulated amounts of heavy metals in different parts of hemp (*C. sativa*) after treatment with SS an SSCh.

concentrations in different hemp parts have been distributed over a wide range. Potassium was predominately accumulated in the aboveground hemp tissues; stem and leaf concentrations were up to 2.9- and 4.1-fold, respectively, higher than root concentration. In the case of sewage sludge, potassium was absorbed at about 9.6 g kg $^{-1}$ ± 0.8 g kg $^{-1}$, regardless the fertilization intensity. This amount was by 51 % \pm 4% lower than for hemps grown in unfertilized control soil. After fertilisation with sewage sludge char hemp accumulated higher amount of potassium than fertilising with sewage sludge: hemp accumulated about 13.1 g kg⁻¹ ± 1.4 g kg⁻¹ of potassium in roots, though it was by 33.3 % \pm 7.2 % lower than in control plants.

The highest amounts of accumulated potassium were found in stems and leaves after the SSCh application in a range of doses from 50 Mg ha⁻¹to 200 Mg ha⁻¹. Greater K accumulation in plants can be considered as an 'insurance strategy' of the plant, enabling it to survive unfavorable growth conditions ([Zörb et al., 2014\)](#page-12-0). The largest increase in K concentration due to SS and SSCh application was characteristic for stems and K stem concentration increased up to 4.5 and 6.9 fold, respectively, compared with control plants. Stem K concetrations were linearly related with applied SS and SSCh dose $(r^2 = 0.94)$ _{stem}, p < 0.05). Sewage sludge is not balanced fertiliser in terms of plant nutrients, as it contains low amounts of K, but high amounts of P. Therefore mixing sewage sludge with ash containing higher content of K and lower of P is widely used practice for fertilization [\(Dimitriou](#page-11-45) [et al., 2006](#page-11-45)).

The amounts of magnesium in the different parts of hemp, independently on fertilization, were distributed in the same range in the roots and stems, but slightly higher concentrations were determined in the leaves. For both, SS and SSCh amended and non amended plants magnesium was accumulated at the level of 3.4 g kg⁻¹ ± 0.4 g kg⁻¹ in

Fig. 3. Accumulated amounts of alkaline earth and alkali metals in differnet parts of hemp after treatment of SS an SSCh.

the roots, $3.0 g kg^{-1} \pm 0.5 g kg^{-1}$ in the stems and $6.4 g kg^{-1} \pm 1.7$ in the leaves. Soil amendments with SS and SSCh did not lead to an increase in Mg content in the hemp roots with the exception of the highest SSCh dose when Mg concentgration was 1.63-fold higher than in control plants. Sodium in herbaceous plants usualy are determined in low concentrations and Na concentrations increase when herbaceous plants are aditionally fertilized [\(Jenkins et al., 1998\)](#page-11-46). Sodium in hemp tissues was distributed as follows root $>$ stem $>$ leaf. The highest sodium levels were detected in the roots of hemps grown in SS amended soil. Analysing Na tissue concentration, no clear dependence on the SS and SSCh dose could be detected. Summarizing the distribution of alkaline earth and alkali metal concentrations in different hemp parts, it can be seen that relatively more of these elements were absorbed when sludge pyrolysis char was used for fertilization.

As sewage sludge is rich in P, soil amendment with both, SS and SSCh, had a highly significant effect on the P content in hemp tissues (ANOVA, $p < 0.05$, data not shown). In control plants P was mainly pooled in the aboveground tissues, while in hemps grown in SS and SSCh amended soil, P was allocated nearly equally among roots stems and leaves. Significant increases in phosphorus concentrations in hemp tissues have been observed with the use of fertilization of sewage sludge and sewage sludge car. The highest increase in phosphorus content was

Fig. 4. Accumulated amounts of P and S in differnet parts of hemp after treatment of SS an SSCh.

Fig. 5. The influence of added dose of sewage sludge and sewage sludge char to heavy metal accumulation factor in different parts of hemp.

determined in the roots. In the treatments with sewage sludge, P root concentration has increased from 16 to 28 times, the use of SS char resulted in somewhat lower increase – from 10 to 20 times. After sewage sludge addition, the highest amounts of phosphorus in stems was accumulated at 50 Mg ha⁻¹, i.e., 5 times higher than in control. The effect of sewage sludge char application on the P stem concentrations was less pronounced and 5-fold increase in stem P concentration was detected only in the treatment with the highest 200 Mg ha^{-1} dose. Independently of the fertilizing agent and the fertilization intensity, it was determined that phosphorus was accumulated by leaf at about 3.6 g kg⁻¹ ± 0.59 g kg⁻¹.

The pattern of sulphur concentrations changes in hemp with SS and SSCh fertilization was similar to phosphorus. Tissue S concentration increased along with SS and SSCh dose ($r^2 > 0.63$, $p < 0.01$), though SSCh induced more pronounced increases in S content. Significantly

higher S concentrations were found in all hemp parts in all tested treatments compared with control ($p < 0.05$). Stem S concentration after SS and SSCh addition increase 20–44 times and 4–271 times, respectively. The highest amounts of sulphur were determined in roots and leaves in the treatment of 200 Mg ha⁻¹ of sewage sludge char. The roots accumulated 23.4 g kg⁻¹ ± 0.56 g kg⁻¹ and the leaves 26.9 g $kg^{-1} \pm 0.39 g kg^{-1}$.

3.4. Bioconcentration factor

Bioconcentration factor (BCF) is known as the soil to plant uptake factor. There were large differences between root, stem and leaf concentrations of all studied metals, which indicated an important restriction of the metal uptake by roots from soil and further internal transportation of metals into steam and leaves. Thus, BCF indicates that

Fig. 6. The influence of added dose of sewage sludge and sewage sludge char to alkaline earth and alkali metals accumulation factor in different parts of hemp.

heavy metals can accumulate in plant parts in very high amounts over time and accumulation is always greater in leaf rather than stems.

This indicator is particularly important in evaluation of potential soil remediation and the amount of accumulated element in biomass. Due to the presence of chemical elements such as Ca, Fe, K, Mg, Na, P the use of thermochemical processes in biomass faces the problems of slagging and low melting temperatures in incineration plants [\(Prade](#page-11-3) [et al., 2011;](#page-11-3) [Tuck et al., 2006\)](#page-11-0) Also part of certain elements such as Cl, S, Na, K, As, Cd, Hg, Pb, Zn are eliminated with gaseous products in the form of aerosols that cause significant impact to ambient air quality.

The study results show that the ability of hemp to accumulate heavy metals in the individual parts changes with increasing fertilization intensity ([Fig. 7\)](#page-10-3). A decrease in BCF of Co, Cu and Zn in both belowground and aboveground tissues could be seen with SS and SSCh dose.

Bioconcentration factors (BCF) of Co and Zn in most cases were < 1 , indicating that these elements were not easily taken up by the hemp. Cu was more efficiently extracted by the hemp roots from the soil amended with SS, though amendment with SSCh exacerbated Cu extraction form the soil. Translocation of both Zn and Cu from root to aerial parts were not very intensive. Decrease of BCF with heavy metals external concentration was observed in other studies indicating that at low external concentrations Zn and Cu are essential for plant metabolism and may be intensively absorbed and translocated ([Pachura et al., 2016](#page-11-26); [Žaltauskaitė and Šliumpaitė, 2013](#page-12-3)). Additionally, hemp has been uptake these metals more efficiently from the soil amend with SS. It may

be explained by that in sewage sludge char the mobility and bioavailability of heavy metals are lower than from origin sewage sludge. Analysis of nickel BCF showed that accumulation of nickel in the roots and stems increased with SS fertilization dose, though BCF were very low indicating very low hemp ability to take up Ni from the soil. The analysis of titanium BCF dependence on fertilization intensity shows obvious differences between roots, stems and leaves. This element was not accumulated in the stems and leaves (BCF \leq 0.01), whereas a low level of accumulation of this element has been determined in the roots. As the intensity of fertilization with sewage sludge or char derived from sludge increased, accumulation of Ti in the roots decreased ($r = -0.69$), while in the stems and leaves its accumulation increased. Same as in the case of titanium, the BCF of Fe were very low and the highest BCF was in the roots. However, it significantly decreased with the increasing intensity of fertilization ($r = -0.73$). A slight correlation ($r = 0.22$) between fertilization intensity and the accumulated amount of Fe has been determined in the stems and leaves. The results showed that a low Fe level it has been accumulated in the roots as fertilization with sewage sludge and char derived from sludge increased. Translocation of Fe was very low (BCF < 0.01) and only small Fe amounts were transported to aerial parts. Manganese BCF increased with SS and SSCh dose and higher uptake intensity was for SS treatments. Low heavy metal accumulation intensity was also shown for other energy crops such as *Miscanthus x gigantheus* and *Phalaris arundinacea,* though total accumulated metal content could be quite high if taking into

Fig. 7. The influence of added dose of sewage sludge and sewage sludge char to P and S accumulation factor in different parts of hemp.

consideration the biomass of these plants ([Fijalkowski et al., 2018](#page-11-47)). Moreover, total accumulated heavy metal amount by energy plants of high biomass could be even greater than that of hyperaccumulators ([Antonkiewicz et al., 2016](#page-10-4)). Research show that when sewage sludge or char derived from sludge are used after pyrolysis process, the increase of fertilization intensity has the greatest influence on Ni, Ti, Fe and Mn absorption. The pyrolysis of sewage sludge has decreased uptake and accumulation of Cu, Ni, Ti, Zn, Fe and Mn compared with that of sewage sludge and our results are in line with the results of other stu-dies ([Méndez et al., 2012](#page-11-17)). BCF of Ba, Ca, K, · increased with the intensified fertilization with sewage sludge and char derived from sewage sludge irrespective of hemp part.

A strong positive correlation between a fertilization dose and BCF of Ba, Ca, Mg and K has been determined for all elements, respectively $r = 0.79$, $r = 0.8$, $r = 0.68$ and $r = 0.55$. Other elements, such as Al, Si, P and S, may also affect the formation of pollutants during the biomass combustion process. For example, the sulphur content acts as a catalyst and increases the amount of chlorine compounds which can cause corrosion of boilers ([Obernberger et al., 2006](#page-11-48)). Consequently, it is also important to take into account the accumulation factors of these elements in energy plants (Fig. 9) in order to assess the compounds that are likely to occur during combustion. These elements and their compounds resulting from burning may cause problems of furnace, for example slagging, soiling and corrosion.

The accumulation of phosphorus and sulphur in hemp was significantly higher. A strong relation between fertilization intensity and the accumulated P in the parts of the hemp has been determined $(r > 0.6)$. The accumulation of sulphur in hemp was more intense form the soil fertilized with SSCh than with SS. Under the conditions of the increased fertilization the accumulation factor increases gradually, but not as intensively as phosphorus. A moderate correlation between fertilization intensity and increase of BCF has been determined. Our results agreed with other studies reporting SS to have a positive effect to nutrients such as P, S, K, Ca content in plants grown in SS amended soils ([Kołodziej et al., 2015;](#page-11-22) [Melo et al., 2018\)](#page-11-49).

4. Conclusions

The study has highlighted that different rates of sewage sludge char and sewage sludge modified the soil characteristics compared with the unamended soil:

• Accumulation of Co, Cr, Cu and Zn in all parts of the hemp decreased with amount of both sewage sludge and sewage char added to the soil. Accumulation of Ni was increasing significantly in the roots and stems when sewage sludge was used for fertilization. Accumulation of Ti and Fe in the roots decreased and increased in the stems and leaves with sewage sludge and sludge char doses. Manganese accumulation increased in all parts of the hemp when doses of sewage sludge are increased, whereas manganese accumulation did not change with sewage char dose The study has

shown that sewage sludge pyrolysis could be a potential measure in order to reduce side effects of sewage sludge application in agriculture as it may decrease heavy metals bioavailability, mobility and accumulation in plants.

- Hemp accumulation of alkaline earth and alkaline metals increased significantly with SS and SSCh fertilization intensity. Strong relationships between the accumulation factor of Ba, Ca, K and Mg and sewage sludge and sludge char doses were detected.
- Fertilization with SS and SSCh has led to hemp biomass yield improvement only at low SS and SSCh doses (25 Mg ha−1), higher doses had a negative effect to hemp grown and biomass increment. It can be argued that fertilization with sewage sludge and sewage sludge char in excess of 25 Mg ha^{-1} is completely useless and unnecessary. The study highlighted the needs to perform further research using sewage sludge doses below 25 Mg ha $^{-1}$ for fertilization, and to saturate sewage sludge char with additional macroelements, such as potassium.
- The study has confirmed the nutritional value of SS and SSCh. The potential of using the sewage sludge as a source of organic matter for improvement of clay loam soil and a reasonable production of bioenergy crop like hemp without the use of inorganic fertilizers was shown.

references

[Tuck et al. \(2011\).](#page-11-50)

CRediT authorship contribution statement

Marius Praspaliauskas: Conceptualization, Investigation, Data curation, Writing - original draft, Visualization. **Jūratė Žaltauskaitė:** Investigation, Formal analysis, Data curation, Writing - original draft, Visualization. **Nerijus Pedišius:** Resources, Data curation, Visualization. **Nerijus Striūgas:** Resources, Data curation, Visualization.

Declaration of Competing Interest

None.

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