Journal of Hazardous Materials 424 (2022) 127316

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Research Paper

Earthworm *Eisenia fetida* potential for sewage sludge amended soil valorization by heavy metal remediation and soil quality improvement

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ARTICLE INFO

Editor: Dr. G. Echevarria

Keywords:
Bioaccumulation
Epigeic earthworms
Soil fertility
Municipal sewage sludge
Vermiremediation

ABSTRACT

Sewage sludge reuse in agriculture is increasing, however it can be an important route for contaminants to enter the environment. The aim of this study was to evaluate earthworm *Eisenia fetida* capability to reduce heavy metal content in the sewage sludge (SS) amended soil and increase soil fertility in terms of soil nutrients content. Adult earthworms were introduced into aged SS amended soil (0–200 Mg ha^{-1}) and left for 65 days. Earthworms have stabilized soil pH and accelerated organic matter mineralization. The concentrations of most heavy metals during the vermiremediation sharply decreased, K and Mg decreased to a moderate extent, whereas Ca content has increased. The highest removal efficiency was detected for Ni, Co and Mn (> 80%), bioconcentration factors were as follows Zn > Co > Cu > Ni > Mn > Cr. The content of major nutrients (S, P) was substantially higher compared to the initial values. The most efficient remediation and soil quality improvement was achieved under the doses of 25–50 Mg ha^{-1}. Higher (≥ 100 Mg ha^{-1}) doses might restrict this technique application because of earthworm mortality and retarded growth. Overall, the study shows that vermiremediation might be a sustainable technique for ecological stabilization of SS amended soil and converting to usable for agricultural needs.

1. Introduction

Rapid industrialization and urbanization rise concern on soil contamination. It has been estimated that in EU there are ~342 thousand contaminated sites and most of it needs remediation (Van Liedekerke et al., 2014). Management of these contaminated sites, including remediation, is estimated to cost 6.53 billion Euros annually (Panagos et al., 2013). Heavy metals are the main contaminants contributing approximately 34.8% to soil contamination. Industry, transport, agricultural fertilizers and pesticides, household and industrial waste, wastewater and sewage sludge are the main sources of heavy metals in the environment.

Sewage sludge production is inevitably increasing worldwide, and sustainable sewage sludge disposal is of great concern. Agricultural or forestry reuse of sewage sludge (SS) is the dominant route of sewage sludge disposal in EU, in 2017 more than 50% of generated SS was used in agriculture or for land reclamation (Collivignarelli et al., 2019). However, in developing countries especially in those with extremely rapid industrialization and urbanization sewage sludge production and disposal has emerged as one of the major environmental challenge (Suanon et al., 2018). Agricultural SS reuse is highly encouraged because of high content of organic matter and nutrients (N, P) (Fytili and Zabaniotou, 2008). However, its application on land is strictly regulated and limited as it comprises different inorganic (heavy metals) and organic (such as PAH, PCB, PCDD, etc.) contaminants and pathogenic microorganisms. Along with positive influence of SS on soil quality and plant production (Singh and Agrawal, 2008; Wang et al., 2008), negative impact on environment was reported as well. Soil analysis after 16-years of repetitive sewage sludge application in Spain has shown that SS amendment had led to an increase in organic matter, nitrogen content, microbial activity, improvement of carbon and nitrogen mineralization processes and some enzymatic functions, but an increase in heavy metal, phenols and extractable nitrates was also recorded (Roig et al., 2012). Another study has also highlighted that long-term SS land application may pose a risk of phosphorus and copper leaching (Kidd et al., 2007). Minimal leaching of nitrogen and phosphorous was found in Sweden after SS application to willows and poplars (Dimitriou and Aronsson, 2011). Accumulation of nutrients in soils and increased nitrates concentrations in soil water were found in Brazil after two years cultivation of corn in SS fertilized plots (Breda et al., 2020). Elevated

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https://doi.org/10.1016/j.jhazmat.2021.127316
Received 31 March 2021; Received in revised form 9 September 2021; Accepted 19 September 2021

heavy metals (Cu, Hg, Pb, Zn) concentrations in superficial groundwater was found after SS application (5–20 t ha^{-1} y^{-1}) to willow plantations (Hasselgren, 1998). Most of studies emphasize heavy metal accumulation in soil and plants after SS application (Sharma et al., 2018; Wang et al., 2008), implying that the dose must be properly determined or the soil should be remediated after SS application.

Current technologies for heavy metals contaminated site remediation are chemical treatment, biological treatment, soil washing, soil flushing, vitrification, incineration, and landfilling. Recently bioremediation has become a popular and highly recommended technique as an alternative for traditional remediation technologies. Among bioremediation technologies, most studies have focused on phytoremediation, whereas vermiremediation, i.e., the use of earthworms and/or their products (vermicompost) for contaminants removal or degradation, could be considered as one of the most promising bioremediation options (Shi et al., 2020; Zeb et al., 2020). As earthworms constantly are exposed to various contaminants present in the soil, they are featured as able to survive under unfavourable conditions and known as early colonizers and ecosystem engineers (Eijsackers, 2010; Lavelle et al., 1997). Research in the field of soil remediation using earthworms started before 40 years, however the rapid development of this method started only in the last decade.

Vermiremediation has many benefits over traditional soil remediation approaches. It is an environmentally friendly, sustainable, low cost, easy to construct and maintain technology. Firstly, is that by aerating the soil, increasing the content of organic matter, nutrient concentration and biological activity, vermiremediation can significantly improve soil quality (Chaoui et al., 2003; Sinha et al., 2008). Earthworms contribute significantly to the content of organic matter, increase mineral nitrogen content in soil, enhance carbon sequestration (Singh et al., 2020; Zeb et al., 2020). There is also an environmental and economic benefit of this remediation technology. Vermiremediation has shown a potential to remediate sites contaminated with hydrocarbons in less than one year (during several cycles of growth) (Kuppusamy et al., 2017). Additionally, vermiremediation has an aesthetic benefit. During the cleaning process, environmental disturbance is very minimal (especially when applying in situ). Earthworms clean polluted areas without damaging topsoil, preserving and enhancing its productivity and fertility at the same time (Sanchez-Hernandez et al., 2019). Earthworms also minimize the amount of pathogens in soil due to the antibacterial properties of their coelomic fluid and use certain protozoa, bacteria and fungi as food sources (Balachandar et al., 2018; Huang et al., 2018; Sinha et al., 2010). Moreover, earthworms were shown to be efficient in soil remediation from broad range of contaminants: pesticides (Lin et al., 2019), hydrocarbons (Koolivand et al., 2020; Rodriguez-Campos et al., 2019), metals (Suthar, 2008; Wu et al., 2020b) and various mixtures (Lacalle et al., 2020).

As other biological treatment methods, vermiremediation has drawbacks too. It could be applied only in low or moderately polluted soils causing no significant lethal toxicity to earthworms. Soil contamination can induce diverse sublethal effects and disturb earthworm physiology, growth, behaviour and reproduction (Anderson et al., 2013; Spurgeon et al., 1994; Zaltauskaite and Miskelyte, 2018; Žaltauskaitė and Sodienė, 2014), thus remediation efficiency could also be impaired. Additionally, in situ vermiremediation is restricted to soil depths where earthworms are present (usually in the topsoil), depending on the ecological classes of the earthworm species used. Because of inadequate handling/control of the vermiremediation process, contaminants accumulated in the earthworms may also be transferred via the food chain posing a risk to other species. Finally, environmental conditions need to be considered as earthworms are sensitive to climate, seasonal conditions fluctuations which can impede earthworm survival, functioning and subsequent processes (Cheng et al., 2021; Svendsen et al., 2007).

Most of studies analysing earthworms' capability to clean soil from contaminants focus on single contaminant, only some analyse their capability to extract chemicals from soil contaminated with mixture of chemicals. Assessing earthworms' potential to remediate sewage sludge or wastes amended soils, generally only heavy metals are addressed, and, in most studies, only freshly amended soils are in investigated. Aging of contaminants was shown to affect chemicals bioavailability and toxicity to soil dwelling organisms (Diez-Ortiz et al., 2015; Smolders et al., 2009) subsequently influencing toxicants removal from the soil. Moreover, soil amendment with sewage sludge results in substantial increase not only in heavy metals, but other metals (such as Ca, Mg, K) and essential nutrients (P, S) as well. In our previous study we have found that industrial hemp (*Cannabis sativa* L) grown in sewage sludge amended soil have substantially bioaccumulated not only heavy metals but alkaline earth and alkali metals as well (Praspaliauskas et al., 2020). Therefore, the aim of this study was to evaluate earthworm *Eisenia fetida* capability to reduce heavy metal, alkali and alkaline earth metal content in the aged sewage sludge amended soil and to increase soil fertility in terms of soil nutrients. In addition, the earthworm life cycle parameters and their relationship with remediation efficiency were investigated.

2. Materials and methods

Clay loam was collected from uncontaminated site, sieved, and mixed with perlite and fine sand (5:3:2, by volume). Soil mixture was amended with sewage sludge (SS) collected from the municipal wastewater treatment plant (agglomeration of 10,000–20,000 PE). The following SS properties were determined: moisture – 9.84 ± 0.02%, ash – 34.57 ± 0.04%, C – 32.30 ± 1.26%, N – 4.23 ± 0.42%, S – 1.42 ± 0.04%, and P – 27.15 ± 1.00 g kg^{-1}. Detailed physico-chemical characteristics of the sewage sludge was presented in Praspaliauskas et al. (2018). The soil amendment with SS were as follows: 25, 50, 100 and 200 Mg ha^{-1}. All soil samples were watered with distilled water and left for 24 months for aging at 20 ± 1 °C. During the aging period, soils were aerated and periodically watered with distilled water. The aging of soil was performed to imitate the conditions of historically sewage sludge amended soil. All the treatments and control were executed in three replicates.

The selected adult (with well-developed clitellum) earthworms *Eisenia fetida* were acclimatized for 7 days. Aged soil samples were weighted (500 g) into plastic containers and ten washed and weighed (420 ± 116 mg) earthworms were added to each container. Soil samples were watered with distilled water to obtain the final required water content (45–50% of the maximum water holding capacity). All containers were placed in closed-top growth chambers at 20 ± 1 ºC, photoperiod 14 h/10 h (day/night)), the relative air humidity (RH) of 50–60%. The earthworms were kept in SS amended soil for 65 days. Water content in each container was checked weekly. The earthworms were weekly supplied with additional oatmeal (approximately 0.5 g per earthworm). The unconsumed food was removed prior to resupplying a new portion. Survival of earthworms was measured on a weekly basis by counting the survived earthworms in each container. The growth of earthworms was measured every week by weighing the surviving earthworms in each container. The earthworms were returned to the same test soil.

Soil samples for chemical analysis were taken before the earthworms were added to the containers and after the whole experiment. Soil pH_{KCl} was measured potentiometrically (inoLab 720, WTW). The method of Loss-on-ignition was used to determine soil organic matter (SOM) content.

After the experiment earthworms were removed from the soil, washed with deionized water and placed on moistened filter paper in Petri dishes for 48 h to void their gut content. After the depuration, the earthworms were sacrificed by deep freezing and dried at 60 °C until constant weight for further analysis. In a Milestone Ethos One closed vessel microwave system earthworms were mineralized with mixture of HNO_3 and H_2O_2. The soil samples were oven dried (at 60 °C), grounded and then digested in Milestone Ethos One closed vessel microwave system with mixture of HNO_3, HCl, HF and H_3BO_3. Concentrations of metals and non-metals, such as Ni, Co, Mn, Cu, Zn, Cr, K, S, P, Mg, Ca, were measured in soil and earthworms with PerkinElmer ® OptimaTM 8000 ICP-OES. C, N and S concentrations were measured using a Flash 2000 analyzer.

The contaminant accumulation in earthworms was estimated with bioconcentration factors (BCF). BCF was calculated as the ratio between contaminant concentration in the earthworm and concentration in the soil.

Chemicals' remediation efficiency (RE) was calculated as RE = $(c_i-c_f)/c_i \times 100$, where c_i and c_f are initial and final chemicals concentration in the soil, respectively.

The probability of earthworm survival (S(t)) was estimated using a surviving model (Jager et al., 2011):

$$S(t) = \exp^{-H(t)}, \tag{1}$$

where H(t) denotes the individual's cumulative hazard at time t. H was estimated by using the following equation:

$$H(t) = \lambda_0 t + \alpha c_w t, \tag{2}$$

where λ_0 is background hazard rate, c_w – sewage sludge concentration in the soil, α – slope parameter. The parameters of the model were estimated using a log-likelihood function.

The Statistica software was used to perform statistical analysis. In order to determine the sewage sludge concentration effect on the studied variables, one-way variance analysis (ANOVA) was performed. Two-way ANOVA was used to evaluate the SS dose and time of exposure interaction. Significant differences between the control and SS treated samples were determined by the Dunnett's test. The LSD test was used to determine any significant differences between the treatments and statistically significant differences at $p < 0.05$ were considered. Regression and correlation analysis were used to assess the relationship between sewage sludge doses, chemicals concentration in the soil, earthworms, and remediation efficiency.

3. Results

3.1. Earthworms' survival and growth

As vermiremediation efficiency highly depends on survival and growth of earthworms, earthworms' survival and growth was monitored during the whole experiment. Sewage sludge amendment dose had a significant effect on the earthworm survival after 9 weeks of exposure ($F = 4.97$, $p < 0.05$) (Fig. 1a). Significant reduction in earthworms' survival (by 55%) in the soil amended with 200 Mg ha^{-1} of sewage sludge was recorded from the first week of exposure (Dunnett, $p < 0.05$). After four weeks of exposure, the survival in the treatment of 200 Mg ha^{-1} has dropped to 10%. In the treatments with 25–50 Mg ha^{-1} no significant impact on survival of earthworms was observed and exposure to 100 Mg ha^{-1} has reduced survival by 43.33% at the end of the experiment. Fitted survival model has shown that SS had posed a significant risk of the death of the earthworms. The risk of death of the earthworms increased significantly with SS concentration in the soil and with time (model parameters: $\lambda_0 = 2.12 \pm 0.14$, $\alpha = 0.0045 \pm 0.0023$, $\chi^2 = 4.08$, $p < 0.05$).

Fresh earthworm weight varied significantly with time and SS concentration and their interaction (two-way ANOVA, time $F = 47.67$, $p < 0.001$, SS concentration $F = 60.82$, $p < 0.001$, time × SS concentration $F = 1.73$, $p = 0.017$) (Fig. 1b). The growth pattern of earthworms in the 25 Mg ha^{-1} treatment was very similar to that of control earthworms and the earthworm weight in this treatment at the end of vermiremediation experiment was by 17.8% higher than in the control. During the first week of vermiremediation process, temporary significant weight loss was recorded in the treatments with 50–200 Mg ha^{-1} ($p < 0.05$). In the treatments with 100–200 Mg ha^{-1} weight loss persisted until the 4th week and afterwards earthworms started to gain weight until the end of the experiment reaching up to 76.4–98.4% of control earthworm's weight.

3.2. Soil pH and organic matter content

Soil amendment with SS had a highly significant affect to soil organic matter content ($F = 46.21$, $p < 0.05$) and pH value ($F = 238.22$, $p < 0.05$). SS application resulted in decreased soil pH and increased SOM (Table 1) values and the relationships were significant ($R^2 = 0.76$ and $R^2 = 0.89$ for pH and SOM, respectively, $p < 0.05$). Soil pH after vermiremediation process was remarkably increased compared to initial

Table 1
Initial and final soil pH and SOM in different SS treatments.

Sewage sludge dose, Mg ha^{-1}	$pH_{initial}$	pH_{final}	SOM $content_{initial}$, (g/ kg^{-1} d.m.)	SOM $content_{final}$, (g/ kg^{-1} d.m.)
0	$6.75 \pm 0.02^{a*}$	$7.09 \pm 0.04^{a*}$	$40.24 \pm 1.15^{a*}$	$6.69 \pm 0.61^{a*}$
25	$6.60 \pm 0.04^{b*}$	$6.84 \pm 0.03^{b*}$	$61.24 \pm 1.47^{a*}$	$10.99 \pm 0.45\ ^{*b}$
50	$6.07 \pm 0.01^{c*}$	$6.85 \pm 0.01^{b*}$	100.07 ± 6.02^{b}	11.87 ± 1.01^{b}
100	$6.06 \pm 0.02^{c*}$	$6.25 \pm 0.02^{c*}$	$132.30 \pm 6.45^{c*}$	$19.51 \pm 1.13^{c*}$
200	$5.81 \pm 0.02^{d*}$	$6.34 \pm 0.02^{d*}$	174.05 ± 15.24^{d}	19.62 ± 0.32^{d}

Different letters indicate significant differences (LSD, $p < 0.05$) between treatments with different SS dose. * indicates significant differences between initial and final values

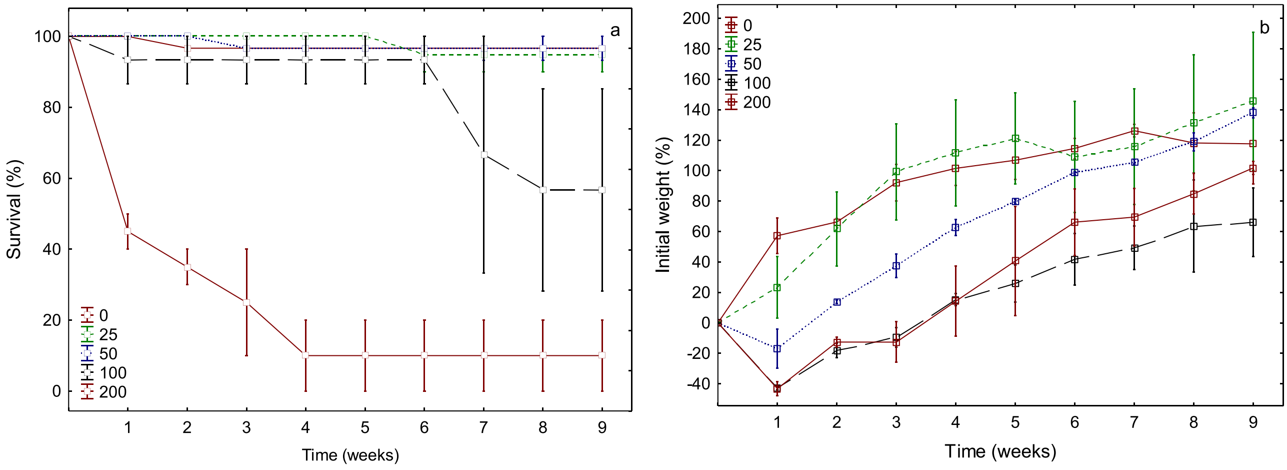

Fig. 1. Survival (a) and changes in the fresh weight (b) of earthworm *E. fetida* exposed to SS amended soil for nine weeks. Error bars represent standard errors (SE).

values, though remained significantly lower than that of control soil. During the vermiremediation SOM dramatically decreased (5.6–8.9-fold) and the magnitude of reduction increased with SS dose ($R^2 = 0.29$, $p = 0.04$).

3.3. Soil heavy metals and other chemicals concentrations

Analysis of the chemical elements revealed that the SS used in the present study did not exceed permitted limits of agriculture and forestry use (Council of European Communities, 1986). SS application had a significant effect to all analysed alkaline earth, alkali metals and

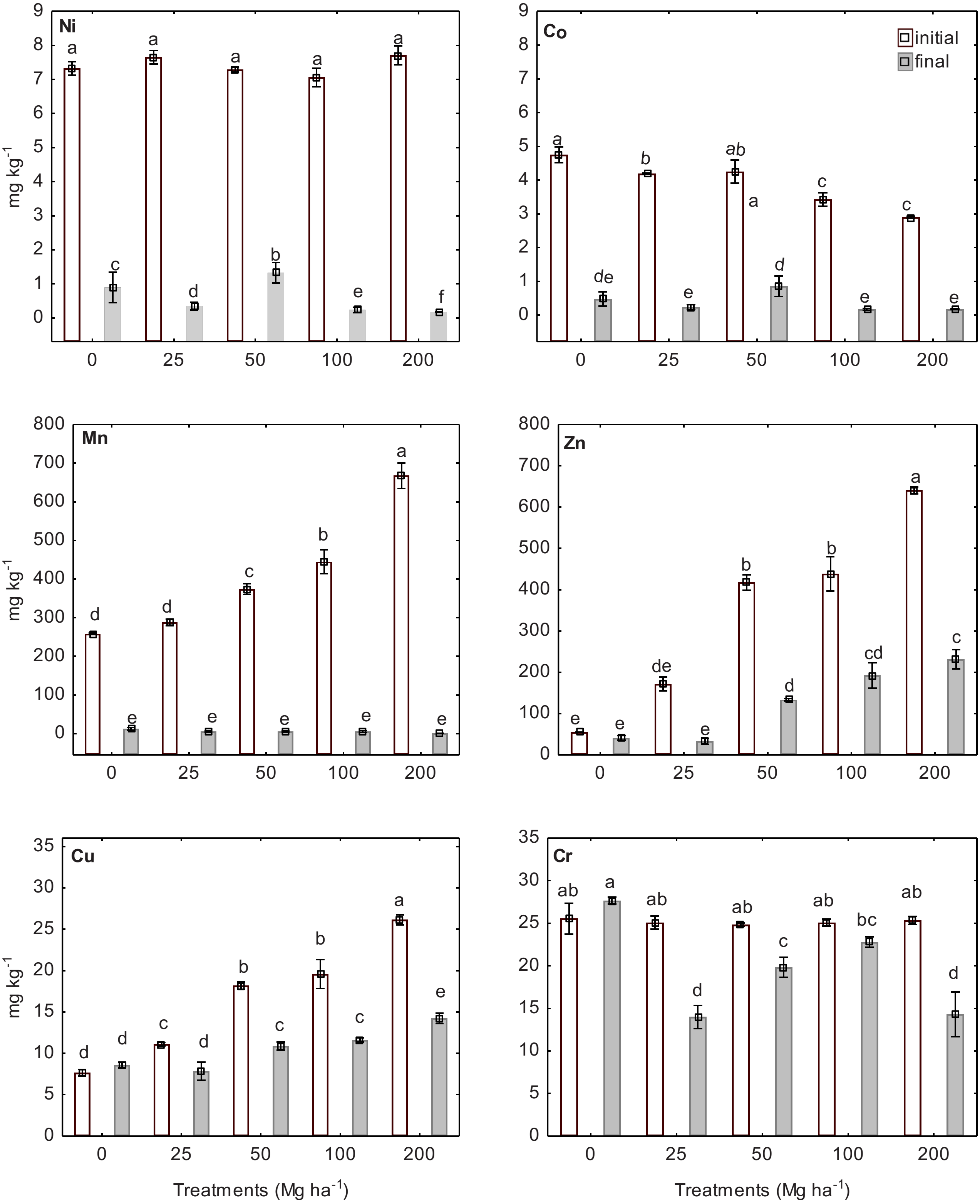

Fig. 2. Initial and final (after vermiremediation) heavy metals concentrations in different SS treatments. Error bars represent standard errors (SE). Different letters indicate significant difference ($p < 0.05$) among the treatments (LSD test).

non-metals concentrations in soil (One-way ANOVA, $p < 0.05$). In case of heavy metals only Ni and Cr levels did not change significantly after SS application (Fig. 2). Upon SS application the increase in heavy metals concentrations was in the range of 3.13–11.76-fold for Zn, 1.44–3.41-fold for Cu and 1.11–2.59-fold for Mn, whereas Co concentrations slightly decreased. Alkali and alkaline earth metals concentration changes in soil after fertilization with SS were less expressed (Fig. 3). K changes were in the range of 1.1–13.4%, Mg and Ca concentrations increased by 14.9–32.0% and 16.6–52.3%, respectively, after SS fertilization. Very sharp increase in P and S level in soil was observed after SS amendment and their concentrations significantly increased along with SS dose ($R^2 = 0.99$, $p < 0.001$) up to 10.8 and 27.7

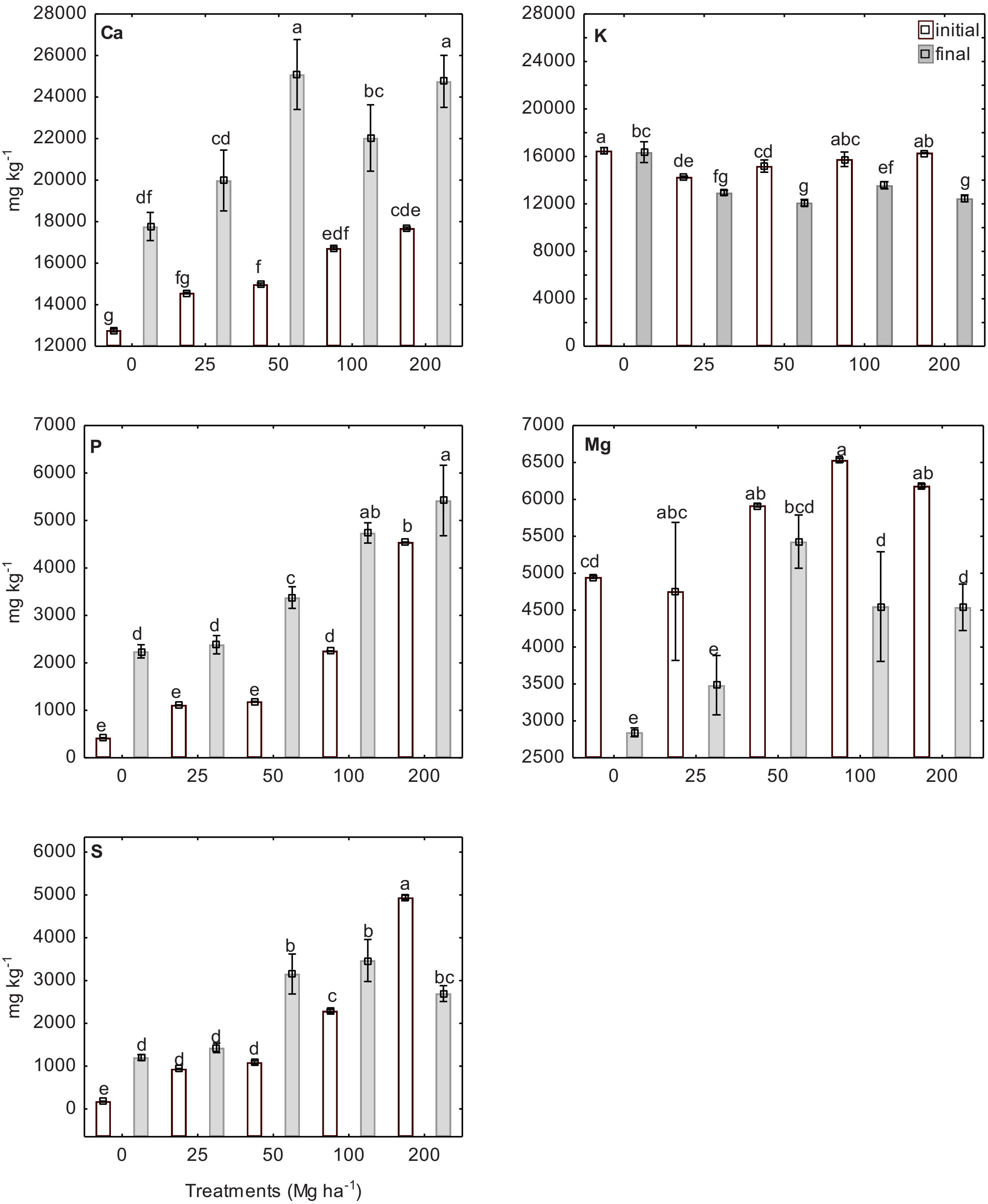

Fig. 3. Initial and final (after vermiremediation) Ca, K, Mg, P and S concentrations in different SS treatments. Error bars represent standard errors (SE). Different letters indicate significant difference ($p < 0.05$) among the treatments (LSD test).

times, respectively.

After the vermiremediation period, Ni, Co and Cr soil concentrations were below the control level, though no clear dependence on the SS treatment dose was observed. Cu and Zn concentrations in the treatment with the lowest 25 Mg ha^{-1} dose after the vermiremediation period was by 8.5% and 19.5%, respectively, lower than in the control ($p > 0.05$). Whereas in the treatments with 50–200 Mg ha^{-1}, their concentrations considerably exceeded that in the control (for Cu 26.8–65.7%, for Zn 3.3–5.7 times). K concentrations in the soil after the vermiremediation was significantly lower (11.7–21.2%) than in the control. No significant difference was recorded in Ca and Mg level in the treatment of 25 Mg ha^{-1}, but in the treatments with higher SS fertilization doses their concentrations remained significantly higher than in the control soil even after the vermiremediation. The concentrations of macronutrients S and P in the treatment with higher doses than 50 Mg ha^{-1} after vermiremediation remained above the control level ($p < 0.05$).

Ni, Co and Mn concentrations sharply reduced (more than 80%) at the end of vermiremediation period as compared to the initial concentrations (Fig. 4). Zn was removed at the efficiency in the range of 56.2–81.1%. Cu and Cr were remediated to a lesser extent and remediation efficiency variated in the range of 28.8–45.6% and 9.0–43.9%, respectively. Despite considerable remediation efficiency for studied heavy metals, no clear relationship with applied SS dose was detected except for Mn ($R^2 = 0.51$, $p < 0.01$). Regarding the macronutrients, it was noted that only K and Mg were removed from the SS amended soil during the experiment. K removal efficiency was between 9.3% and 23.5% and its relationship with SS concentration was nearly significant ($p = 0.059$). Mg removal was somewhat greater than that of K and reached up to 38.7%, however no relationship with SS dose was detected. P, S and Ca, contrary to other elements, have increased after the vermiremediation period. Concentrations of S have increased in the treatments with 25–100 Mg ha^{-1}, while a decline was observed in the treatment of the highest 200 Mg ha^{-1} SS concentration. An increase in final P concentrations in comparison with initials ones, were determined in all the treatments though the increment declined with SS dose ($R^2 = 0.61$, $p < 0.01$).

3.4. Bioaccumulation in earthworms

SS dose had a significant effect on heavy metal concentrations in the earthworms (ANOVA, $F > 11.62$, $p < 0.05$) except for Ni ($F = 3.80$, $p = 0.06$) (Fig. 5). The bioconcentrations of heavy metals could be ranked as follows Zn > Mn > Cu > Co > Ni > Cr. Most of heavy metals accumulation increased along with SS application rate (Zn, Cr: $R^2 = 0.76$, Cu: $R^2 = 0.74$, Mn: $R^2 = 0.55$, $p < 0.05$) and heavy metals soil concentration (Zn: $R^2 = 0.80$, Co: $R^2 = 0.72$, Mn: $R^2 = 0.55$, Cu: $R^2 = 0.54$, $p < 0.05$). Co concentrations in earthworms decreased with SS (Co: $R^2 = 0.72$) dose and no relationship was found for Ni accumulation.

BCFs of heavy metals were ranked as follows: Zn > Co > Cu > Ni > Mn > Cr (Fig. 5). Only for Zn and Co in the control and treatment with the lowest 25 Mg ha^{-1} SS dose BCFs were above 1. The BCFs of other heavy metals were in the range between 0.03 and 0.79. In most cases BCFs have shown a tendency to decrease with applied SS dose and heavy metal soil content. Only for Cr a significant ($p < 0.05$) increase in BCF with SS dose was found, however the BCFs for Cr were very low and reached only 0.12 in the treatment of 200 Mg ha^{-1}.

SS dose had no significant effect on the bioconcentrations of macronutrients K, Mg and P (ANOVA, $p > 0.05$) whereas Ca and S bioconcentrations increased with SS dose (Ca: $R^2 = 0.67$, S: $R^2 = 0.96$, $p < 0.05$) (Fig. 6). BCFs of macronutrients were in the order: S > P > K > Ca > Mg and values of BCFs of S and P always exceeded 1, indicating substantial accumulation. BCFs for Ca and Mg were low, in the range of 0.15–0.26 and 0.12–0.14, respectively, K was concentrated in the earthworms at the higher extent (BCFs 0.61–0.86). Phosphorous and potassium BCFs decreased along with SS dose, though only for P it was significant ($R^2 = 0.58$, $p < 0.05$). BCFs for Ca ($R^2 = 0.28$, $p = 0.06$) and Mg showed a tendency to increase with BCF dose.

Soil physical-chemical characteristics, metal properties and earthworm physiology are major factors determining earthworms' ability to take up and accumulate chemicals from the soil. Heavy metal bioconcentrations in the earthworms were inversely correlated with soil pH (except for Co) indicating that higher acidity has led to the formation of more bioavailable forms and higher uptake. Uptake of S and P were also inversely related with soil pH, though K bioconcentrations were not affected by soil pH ($p > 0.05$). Consequently, lower bioaccumulation at higher soil pH might result in lower remediation efficiency, though significant relationship was determined only for Cu and Mn (Cu: $r = -0.77$, Mn: $r = -0.74$, $p < 0.05$).

Heavy metals concentrations in earthworms positively correlated ($p < 0.05$) with soil OM with the exception of Co ($r = -0.88$, $p < 0.01$) and Ni ($r = 0.33$, $p = 0.29$). No significant relationship was found between K bioconcentrations and soil OM, while other macronutrients bioconcentrations were positively related with soil OM content ($p < 0.05$).

Uptake of heavy metals by earthworms is strongly influenced by the presence of other cations, especially Ca, in soil (Li et al., 2009), therefore we have investigated the relationship between heavy metal bioaccumulation and soil Ca content. The strongest positive correlation was found between Cu and Zn bioconcentrations and Ca soil content ($r = 0.88$, $p < 0.001$), followed by Cr ($r = 0.66$, $p = 0.013$), while in case of Ni and Mn the correlation was insignificant (Ni: $r = 0.25$, $p = 0.43$, Mn: $r = 0.53$, $p = 0.06$). Bioconcentrations of Co were

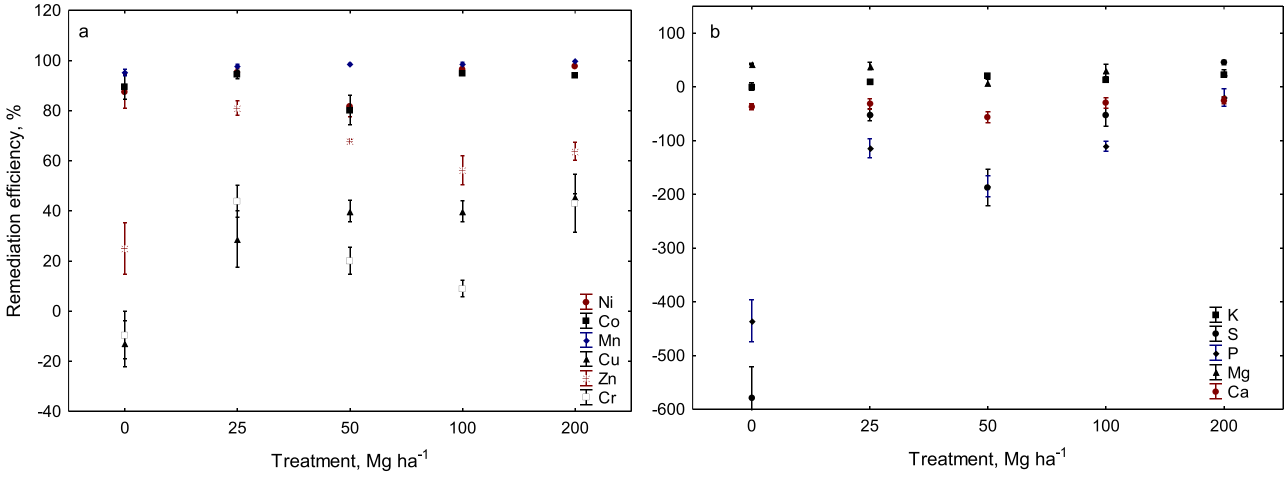

Fig. 4. Metal ((a) Ni, Co, Mn, Cu, Zn and Cr), (b) K, S, P, Mg and Ca) remediation efficiency in different SS treatments. Error bars represent standard errors (SE).

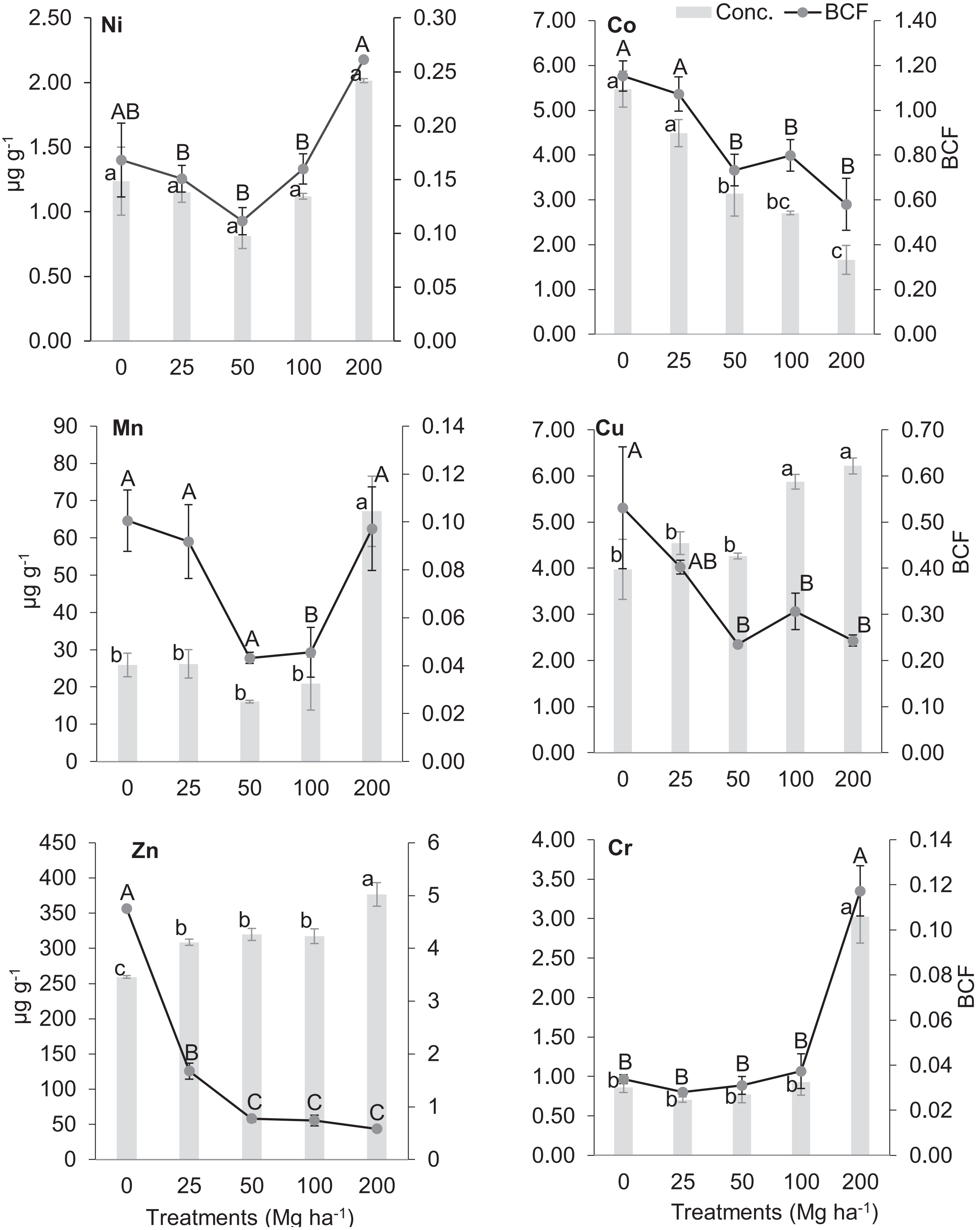

Fig. 5. Heavy metal earthworm concentrations and bioconcentration factors (BCF) in different SS treatments. Error bars represent standard errors (SE). Different letters indicate significant difference ($p < 0.05$) among the treatments: uppercase for BCF and lowercase for earthworm concentrations (LSD test).

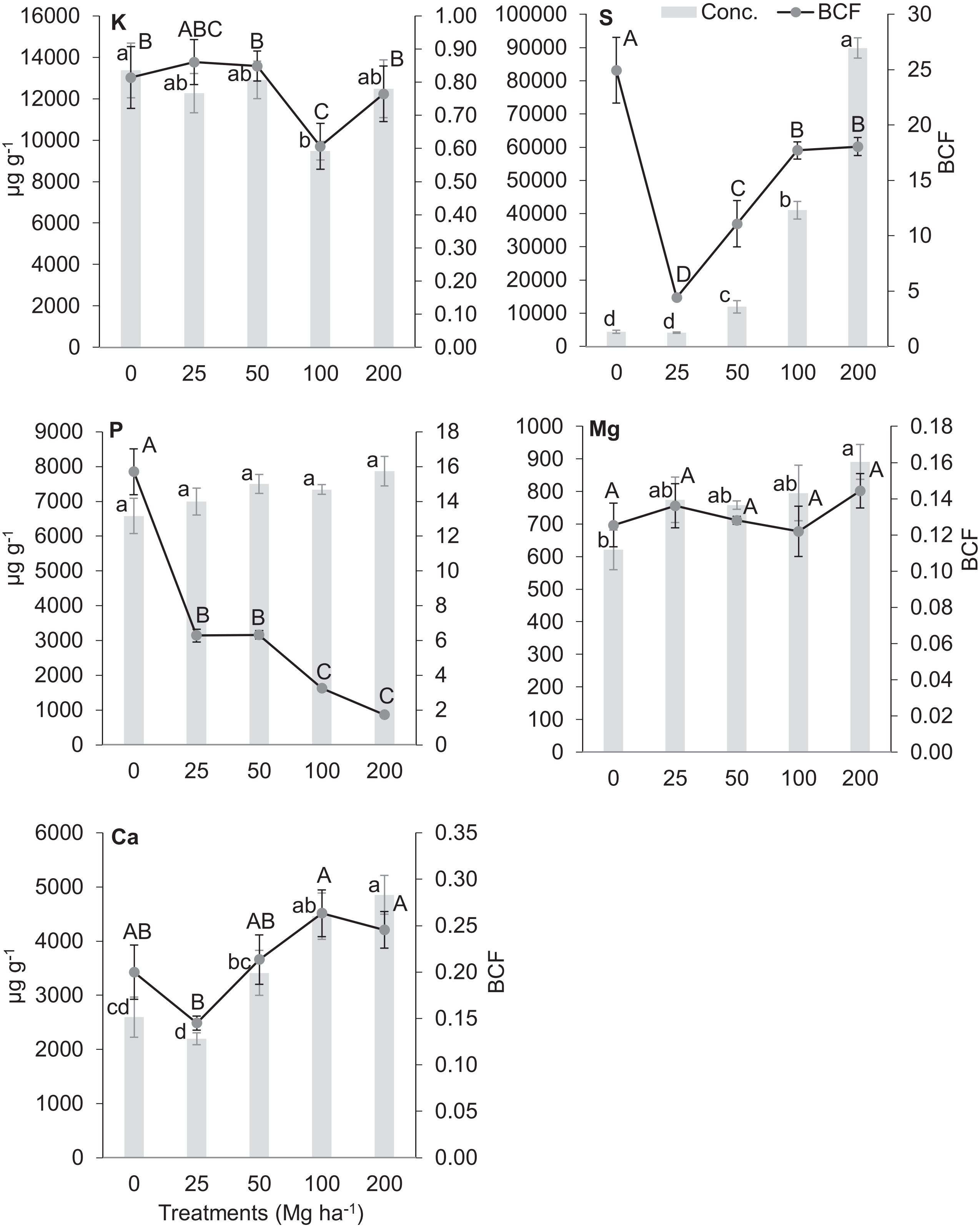

Fig. 6. Ca, K, Mg, P and S earthworm concentrations and bioconcentration factors (BCF) in different SS treatments. Error bars represent standard errors (SE). Different letters indicate significant difference ($p < 0.05$) among the treatments: uppercase for BCF and lowercase for earthworm concentrations (LSD test).

inversely correlated with soil Ca content ($r = -0.90$, $p < 0.01$).

As heavy metals removal efficiency varied depending on sewage sludge concentration and specific element (Fig. 4), we tried to elucidate whether heavy metals concentrations in the earthworms were related to earthworms' growth and survival. Ni, Mn, Cu and Cr bioconcentrations were closely related with earthworm mortality ($r > 0.59$, $p < 0.05$), correlation for Zn was close to significant ($r = 0.61$, $p = 0.06$). Earthworms which accumulated higher amounts of heavy metals have shown a tendency to gain lower weight during the vermiremediation period, however the relationship was insignificant.

4. Discussion

For ensuring vermiremediation feasibility and efficiency, a balance between proper environmental conditions (climatic conditions, food availability, substrate compatibility, etc.) and pollution level must be maintained (Zeb et al., 2020). Therefore, during the vermiremediation process normal earthworm physiological activity and survival should be kept. SS application leads to significantly changed soil properties (pH, SOM) and nutrients, metals content. Changed soil pH and SOM might influence physico-chemical processes in the soil and affect physiology, behaviour of soil dwelling organisms. Our data are in line with other studies reporting pH reduction and SOM increase in SS amended soil (Bourioug et al., 2015; Méndez et al., 2012; Singh and Agrawal, 2010). As soil pH showed a significant decrease with increasing SS dose (Table 1), it might induce heavy metals activation and increase bioavailability as acidic condition usually facilitate their solubility and uptake (Giska et al., 2014). At the same time, decreasing pH might alter earthworm fitness. Neutral pH has been shown to be optimal for many earthworm species, while at $pH < 5.2$ inhibited *E. fetida* survival, growth, and reproduction was recorded (Wu et al., 2020a). Increasing SOM content with SS dose has been shown to decrease (Bradham et al., 2006) and increase (Bai et al., 2017) chemicals bioavailability and this impact was chemical dependent. In our study, vermiremediation has stabilized soil pH and decreased SOM. Most studies have recorded that vermicomposting of different wastes leads to pH increase (Paul et al., 2020) and SOM reduction and net organic matter stabilization is explained by organic matter mineralization and consumption by earthworms converting it to earthworm biomass (Lin et al., 2019; Mondal et al., 2020; Tripathi and Bhardwaj, 2004).

Although SS used in the present study did not exceed heavy metals permissible levels for agricultural application, different intensity fertilization has led to the significant changes in heavy metals, alkaline earth, alkali metals and nutrients concentrations in soil. Cr concentrations in SS amended soil in our study were around Northern Europe regional background level, Ni concentrations were below median Ni concentration in Europe (Albanese et al., 2015), Co concentrations were below mean Co topsoil Europe concentration (Tóth et al., 2016). Zn soil concentrations in SS amended soil exceeded EU guidelines when SS application rate was more than 50 Mg ha^{-1} and soil Zn concentration increased along with SS dose ($R^2 = 0.84$, $p < 0.05$). For Cu soil level, only the treatments with 200 Mg ha^{-1} of SS showed Cu soil concentrations above the Northern Europe regional average Cu soil concentrations (20.1–20.2 mg kg) (Albanese et al., 2015), though did not exceed EU limit values (Council of European Communities, 1986). Mn concentration in SS amended soil did not exceed EU limit values (Reimann et al., 2018), though at the highest SS dose the average background (300–600 mg kg^{-1}) Mn level was slightly exceeded (Morgan et al., 2007). Although Cu and Mn soil content after SS amendment were below the limit values established by EU regulations, significant increase in their amount with SS dose (Cu $R^2 = 0.86$, Mn $R^2 = 0.95$, $p < 0.05$) should be considered, especially in long-term applications. Significant increase in Cu and Zn soil concentration after 15 years SS amendment (in the range of 13–69 t FW ha^{-1} y^{-1}) was recorded in experimental site in Spain (Iglesias et al., 2018). Considerable accumulation of Cu and Zn in the topsoil and plants was found after 3 years SS application in China (Wei and Liu, 2005).

Plant macro- and micronutrient deficiency in soils is one of the key factors limiting crop productivity, therefore fertilization with SS rich in nutrients might increase soil nutrient pool and improve crop productivity. As north-central Europe region soils are characterized by having low Mg content (Négrel et al., 2021), fertilization with SS could help to increase the content of this essential plant nutrient. After SS application Mg soil level was in the range of 0.90–1.19 of median value in European agricultural soils (5488 mg kg^{-1}). However, this increase in Mg content might be insufficient to meet plant requirements as decreasing soil pH with SS dose may interfere Mg uptake due to competition with H^+ at the site of rhizosphere (Senbayram et al., 2015). S concentrations in SS amended soil were far above the background values in Europe (Matschullat et al., 2018). High S content in SS amended soil was shown to increase heavy metal solubility due to lowered pH (Dede and Ozdemir, 2016) and may lead to higher metal bioavailability to soil biota. This is in line with our data showing inverse relationship between soil pH and S content ($r = -0.82$, $p < 0.01$) presupposing subsequent higher heavy metals bioavailability. Amount of K, Ca and P in SS amended soil were in the range of Q50-Q98 of concentrations in agricultural and grazing land soils of Europe (Reimann et al., 2012). Remarkable increase in P after soil amendment with SS might pose a risk of P runoff and leaching (Wang et al., 2020b).

Present study has shown that SS fertilization doses did not induce risk of soil pollution with heavy metals, except for Zn, and enriched soil with macro- and micronutrients. Potential hazard assessment of SS application currently is based on the physicochemical parameters of SS and receiving soils, though chemical data alone do not allow evaluation of possible toxic effects to soil biota. Soil dwelling organisms' response, in contrast to physico-chemical analysis, integrate the biological effects of all compounds present and other factors such as bioavailability, toxicants interaction and others. Therefore, earthworm survival and growth could be used as the indicators of vermiremediation suitability for soil cleaning and its efficiency. In all treatments metal concentrations were far below the previously determined lethal concentrations (LC_{50}) for earthworms (56-days LC_{50} for Cu 555 mg kg^{-1}, for Zn 745 mg kg^{-1} (Spurgeon et al., 1994), 14-days LC_{50} for Cr 241.13 mg kg^{-1} (Yang et al., 2018), for Ni 1069.32 mg kg^{-1} (Wang et al., 2020a), 28-days for Mn 1970 mg kg^{-1} (Kuperman et al., 2004)). However, these LC_{50} were mostly determined when earthworms were exposed to single heavy metal, and in SS amended soils earthworms are subjected to the mixture of different substances. Furthermore, it should be considered that generally metal toxicity in field or aged soil is less pronounced than in freshly spiked soil even at the same metal concentrations (Lock et al., 2006; Smolders et al., 2009). Previously we have shown that fresh SS was extremely toxic to *E. fetida* compared to aged SS (Žaltauskaitė et al., 2017). Based on earthworm survival and growth data (Fig. 1), we presume that vermiremediation could be efficiently applied to remediate aged SS amended soil in case of low and moderate doses, up to 100 Mg ha^{-1}. Higher doses may temporarily inhibit earthworm growth or even cause a mortality, and this was proved by fitted survival model showing the significant increase in mortality with SS dose and time. Hence, even the single heavy metals concentrations in soil were far below reported LC_{50}, significant earthworm mortality and retarded growth could be explained by the fact that earthworms were exposed to the mixture of chemicals and soil pH was slightly lowered. Retarded growth and mortality could be partially also linked to high soil S content. Highly significant effect of local S pollution gradient within 200 m from sulphur block in Canada on the number of earthworms was found and no earthworms were found in the site where S content in the soil was around 6673 mg kg^{-1} (Cárcamo et al., 1998).

The main mechanisms of soil vermiremediation leading to chemicals content changes in the soil are (Rodriguez-Campos et al., 2014; Sanchez-Hernandez et al., 2019; Zeb et al., 2020): chemicals bioaccumulation by earthworms, physical and physiological activity of earthworms, and leaching and runoff of chemicals. Vermiremediation had different impact to alkaline earth, alkali metals and non-metals concentration in soil than to heavy metals (Figs. 2–4). The concentrations of heavy metals sharply decreased, K and Mg decreased to a moderate extent, whereas an increase was observed in Ca content. Final soil concentrations of all studied heavy metals were below the threshold posing an ecological risk, indicating high efficiency of vermiremediation. Similar high removal efficiency for heavy metals was recorded in case of different types of wastes, SSs, ashes as well as spiked soils with single heavy metal (Cheng et al., 2021; Lacalle et al., 2020; Wu et al., 2020b) remediated with different earthworm species (*E. fetida, Eudrilus eugeniae, Lumbricus rubellus*) (Azizi et al., 2013; Paul et al., 2020, 2018; Suthar et al., 2014; Usmani et al., 2017). The data on K changes during the vermitreatment are controversial: both an increase (Gupta and Garg, 2009; Usmani et al., 2017) and decrease (Mondal et al., 2020; Paul et al., 2018) were reported. An increase in Ca soil content after 50 and 100 days of SS remediation with *E. fetida* was reported by Ahadi et al. (2020). To our knowledge where are no data on Mg changes during the vermiremediation, therefore we cannot compare our results. The substantial reduction in metal soil content upon vermiremediation could be attributed to metal bioaccumulation in earthworms (Figs. 5–6), showing close relationship both with SS dose and soil heavy metal content. The earthworms modify the physical and chemical structure of ingested substrate leading to accumulation and changed bioavailability of different substances in processed substrate. Although heavy metal earthworm concentrations increased with soil concentrations, the increase in earthworm concentration was proportionally less than that in soil, thus illustrating earthworms' ability to regulate uptake and producing a negative relationship between BCFs and soil concentrations. Detected low values of BCFs (mostly < 1) and inverse relationship between BCFs and soil metal content are consistent with other field or laboratory studies (Coelho et al., 2018; Nahmani et al., 2009; Rorat et al., 2017; Suleiman et al., 2017; Suthar, 2008). Meta-analysis of 56 studies has shown lower bioaccumulation factors at higher soil concentrations and this was likely driven by reduced uptake due to saturation, more efficient detoxification and elimination (Ardestani et al., 2014; Richardson et al., 2020). Inverse relationship between metal removal efficiency and BCFs suggests that metals adsorption, precipitation or leaching might be also important during the vermiremediation (Paul et al., 2020; Rorat et al., 2016; Suthar et al., 2014).

Earthworms metal uptake and bioaccumulation differ among earthworm genera, ecophysiological groups, metals and their speciation, exposure duration, soil properties and environmental conditions (Richardson et al., 2020). In most cases endogeic and epigeic earthworms because of different food preference, skin exposure and behaviour have shown distinct metal tissue concentrations and BCFs (Dai et al., 2004; Richardson et al., 2020). Hobbelen et al. (2006) examined 15 field sites in Netherlands and found higher Cd, Cu and Zn concentrations in endogeic *Aporrectodea caliginosa* compared to those in epigeic L. *rubellus*. Similar pattern of Cd, Cu, Zn and Pb earthworm concentrations was observed in Wales (Morgan and Morgan, 1999). On the contrary higher BCFs for Cd, Hg and Pb were found in endogeic *A. rosea, O. cyaneum* than in epigeic *L. terrestris* and *E. fetida* (Ernst et al., 2008).

Distinct uptake and excretion kinetics are attributed to essential and non-essential metals, with rapid uptake and equilibrium for essential metals and slow excretion for non-essential ones (Spurgeon and Hopkin, 1999). Continuous uptake of non-essential metals with time of exposure was observed in *E.fetida* and L. *rubellus*, whereas body concentrations of essential metals were regulated very efficiently (Giska et al., 2014; Spurgeon and Hopkin, 1999). This is in line with our results, showing that earthworms have regulated very efficiently Cu and Zn concentrations as body concentrations has increased only up to 1.5-fold, while soil concentrations have increased 3.41- and 11.76-fold, respectively. Whereas SS addition had no effect on soil Ni and Cr concentrations, though earthworm concentrations have increased up to 1.64- and 3.5-fold, respectively. Notwithstanding, Nahmani et al. (2007) observed a broad range of essential metals (Cu and Zn) in earthworms, suggesting that at high soil concentrations the regulation efficiency of essential metals weakens resulting in a higher influx and toxic effects subsequently. Different accumulation of metals in earthworms could also be explained by metal ions competition with other cations (Ca^{2+}, Mg^{2+}, etc.) for uptake or toxic site, i.e., biotic ligand model. Ca^{2+}, Mg^{2+} and Na^{+} inhibited the uptake of Ni by *Enchytraeus crypticus* and mitigated its toxicity, while K^{+} and pH had no effect (He et al., 2014). Whereas no effects of Ca on Zn uptake by *E. fetida* suggesting that Zn uptake was not exerted at a Ca channel (Li et al., 2010). However, our results did not support this theory as only earthworm accumulation of Co and partially of K could be explained by the competition with Ca or Mg.

Metal removal from SS amended soil due to bioaccumulation by earthworms is highly dependent on soil characteristics. Among soil properties pH was the most often explaining variable of the metal uptake and bioaccumulation (Spurgeon et al., 2006), followed by organic matter content, CEC and others. In our study decreasing soil pH and increasing SOM with increasing SS dose have facilitated the uptake and bioaccumulation of heavy metals and macronutrients (Mg, P and S). Soil OM increases forming of soluble organo-metal complexes (Rieuwerts et al., 2006) and P ad S labile forms in the soils (Breda et al., 2020) leading to changed chemical bioavailability and bioaccumulation in earthworms. However, the impact on the whole soil remediation efficiency was significant only for Cu and Mn implying that soil pH and SOM only partially could be used for the prediction of remediation efficiency.

High efficiency of heavy metals removal in the treatments with 100–200 Mg ha^{-1}, despite the slow growth and mortality from the 4th week could be explained by the rapid HM uptake and accumulation during the early phase of exposure (Bernard et al., 2010; Nahmani et al., 2009; Spurgeon and Hopkin, 1999). Moreover, heavy metal earthworm concentrations were positively correlated with subsequent earthworm lower fresh weight and mortality. This is in agreement with Suleiman et al. (2017) who reported the most substantial decrease in heavy metal soil content in SS amended soil during the first 10 days of vermiremediation, afterwards the rate of heavy metal removal was dropped or remained constant. The negative SS impact on earthworms' performance could be partially counterbalanced by the addition of supplemented materials (organic waste, plant material, biochar, cow dung, etc.) to the SS amended soil (Kończak and Oleszczuk, 2018; Rorat et al., 2016; Sanchez-Hernandez et al., 2019; Suleiman et al., 2017; Wang et al., 2013).

The substantial increase in soil concentrations of Ca and nutrients (P, S) (Fig. 3) could be attributed to earthworms' activity in the soil leading to enhanced microbial and enzymatic activities in the soil. Earthworm activity (borrowing, feeding, casting, mucus excretion) strongly increase microbial abundance and activities leading to the enzyme activation, nutrient elevation and changes in their bioavailability (Hoang et al., 2017; Huang and Xia, 2018; Lavelle et al., 1997). An increase in Ca soil concentration after vermiremediation period could be explained by a secretion of intestinal Ca and NH_4-N (Suleiman et al., 2017) allowing to maintain neutral pH and through neutralization of carboxylic and phenolic groups of humin acids. pH shift towards neutral range during vermiremediation was also observed in our study (Table 1). Moreover, during the vermiremediation process organically bound nutrients (such as P, K) are transformed into bioavailable forms (Sahariah et al., 2015). Therefore, even the K concentrations have change only moderately during the vermiremediation, though vermitreatment could be beneficial as K was transformed to more bioavailable forms. Very sharp increase in P and S soil concentration compared to initial values was recorded (Fig. 3), despite very prominent their accumulation in earthworms (Fig. 6). An increase in P and S level after vermiremediation was explained by the enhancement of enzyme activity in soil mediated by earthworms (Breda et al., 2020; Gupta and Garg, 2009; Suthar and Singh, 2008) The earthworm gut produces considerable amount of alkaline phosphatases, an essential enzyme of biogeochemical cycle of P, which facilitate the P mineralization process when soil passes through the worm gut (Le Bayon and Binet, 2006; Pramanik et al., 2007). Furthermore, long-term (5–9 years) field experiments with SS application has shown that high rate SS application and high metal accumulation reduced urease and phosphatase activities in soil (García-Gil et al., 2000). This was supported by our data showing that the higher increment in P were found at low SS doses (Fig. 3) and no relationship were found between P earthworm concentration and soil P level ($r = 0.5$, $p > 0.05$). Therefore, we may conclude that the presence of earthworms might be crucial in maintaining sufficient soil enzymes activity in SS amended soils.

5. Concluding remarks

The present study has shown that earthworms significantly improved the SS amended soil quality and resulted in a highly efficient remediation effect. Earthworms accelerated organic matter mineralization, stabilized soil pH, significantly reduced heavy metals concentrations in the soil or their bioavailability and increased major nutrient soil content. However, the use of vermiremediation is limited under high load of sewage sludge. The obtained results confirmed that the most efficient vermiremediation and soil quality improvement at the same time could be achieved under the doses of 25–50 Mg ha^{-1}. Higher ($\geq$ 100 Mg ha^{-1}) application doses have led to higher heavy metal content in earthworms tissue, lower weight increment and induced mortality. These adverse effect on earthworm life cycle parameters might further interrupt remediation process and its efficiency. Though we have not detected significant relationship between earthworms reduced survival and growth and remediation efficiency. This could be explained by the fact that SS had not imposed acute lethal toxicity and lethal consequences were recorded only after four weeks of exposure. Same tendence was found in the growth rate: growth rate decreased at the end of remediation process. However, questions of possible further heavy metal transfer to higher trophic level remain and future research in the mechanisms of earthworms' abilities to cope with accumulated toxicants are needed.

CRediT authorship contribution statement

Jūratė Žaltauskaitė: Conceptualization, Methodology, writing and Supervision. **Inesa Kniuipytė**: Investigation, Formal analysis, Visualization. **Marius Praspaliauskas**: Investigation, Methodology, Resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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