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Municipal sewage sludge as a source of microelements in sustainable plant production: a laboratory lysimeter study

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Article info

Abstract

The aim of the work was to characterize the samples of sewage sludge (SSL) originated from the Wastewater Treatment Plant Piešťany (TAVOS, a.s., Trnava, Slovakia) in terms of their potential application into the soils. Within the physico-chemical characterization of SSL, the samples were analysed in terms of the values of pH, cation exchange capacity (*CEC*), total organic carbon (*TOC*), water holding capacity (*WHC*), as well as the presence of heavy metals. It was found that SSL contained significant amounts of microelements Zn (1,269 mg.kg^{-1}, d.w.) and Cu (224 mg.kg^{-1}, d.w.). Laboratory lysimeter experiments involving the application of SSL into the top layer of agriculturally used soil (0 – 10 cm) forming a soil column, in which seedlings of tobacco (*Nicotiana tabacum* L.) were cultivated, showed that in the case of SSL application in the highest permitted amounts (15 t.ha^{-1}) only 0.11 % Zn and 0.07 % Cu were released into the soil eluate during a 28 day of exposure, while in tobacco plants 0.13 % Zn and 0.05 % Cu were accumulated from the total amount of Zn and Cu originated from the application of SSL (Zn – 133 mg and Cu – 23.5 mg). When applying SSL in amount 30 t.ha^{-1} i.e., in the dose exceeding the permitted limits, only 0.02 % Zn and 0.04 % Cu were released into the soil eluate and 0.16 % Zn and 0.09 % Cu were accumulated in tobacco plants from the applied amount of SSL (Zn – 267 mg and Cu – 47.0 mg).

Introduction

Sewage sludge (SSL) represent large-volume wastes annually produced in the whole world. The current global SSL rate is about 45 million dry tons of sludge per year. Approx. 6 million tonnes of dry SSL were produced in China in 2013, while approx. 14 million tons of dry SSL per year were released during wastewater treatment in the United States (Seiple *et al.* 2017; Teoh and Li 2020). Currently, it is estimated that at least 10 million tons of dry SSL will be produced within the European Union annually (Wang *et al.* 2019). The composition of SSL is dependent on its origin in

terms of the type of wastewater treatment, and therefore is variable and unpredictable as well. In this way the public concern and definition of regulations or directives are primarily associated with toxic metals, organic xenobiotics or pathogens occurrence in this type of wastes (Zhang *et al.* 2017). All these factors play an important role in decision with regard to the ultimate SSL disposal, which can involve: composting, landfilling, agricultural application, as well as sea or surface waters disposal and incineration (Donatello and Cheeseman 2013). In the Slovak Republic, SSL production represents more than 50,000 tons/year and SSL is predominantly used for compost production (62.7 %), reclamation (16.4 %), landfilling (13.5 %), incineration (5.5 %) and direct application into the soil (1.9 %) (according to data of MŽP SR, 2012).

SSL have increasingly been used in the last decades on farmland, mostly for their potentially appropriate fertilizing properties, due to its high content of organic matter as well as of macro- and micronutrients of plant nutrition (Usman *et al.* 2012). The application of SSL into the agriculturally used soils partially solves the problem of SSL disposal but on the other hand, may pose risks related to the contamination of soil and groundwater by inorganic contaminants (especially metals), organic xenobiotics (especially pharmaceuticals or chlorinated compounds) or microbial pathogens (coliform and faecal coliform bacteria) (Santoro *et al.* 2017). In particular, toxic metals (Cd, As, Pb, Hg) entering into agriculturally used soils through the application of SSL may contaminate the food chain through plants and the surface or ground water. However, high amounts of heavy metals belonging to microelements (Zn, Cu, Mn) in SSL may also represent risks associated with the contamination of soils and ground water. In the EU, the application of SSL in agriculture is regulated by the EU Council Directive 86/278/EEC. Also, this directive determines the limit values for the content of heavy metals in SSL in this way. In Slovak Republic, the handling of SSL as a large-volume waste produced is regulated by the Act No. 79/2015. In terms of the application of SSL into the soils, these activities are regulated by the Act No. 188/2003. This Act also determines the characteristics of the soil which must be evaluated, especially in terms of soil pH. The mentioned Act No. 188/2003 indicates the value of the amount of SSL that can be applied into the soil, whereby the permitted amount of SSL applied shall not exceed 15 tons of dry matter per 1 ha over a period of five years.

In many studies, the application of SSL in agriculture is studied and evaluated mainly from the point of view of SSL utilization as a source of important macro- and microelements for plant nutrition, plant production, risks of pathogens transfer present in the SSL, and translocation of toxic elements or xenobiotics to the soils and plants (Bettiol and Ghini 2011; Marguí *et al.* 2016; Mossa *et al.* 2017; Borba *et al.* 2018). The availability and mobility of macro- and microelements or toxic metals in the systems SSL/soil have been examined in short-term laboratory studies using both leaching columns and incubation (Toribio and Romanyà 2006), lysimeters (Borba *et al.* 2018), and under conditions of experimental fields (Wolejko *et al.* 2013).

Lysimeter devices currently represent important tools for studying and predicting the behaviour of a wide range of substances in terms of their movement and overall balance within the soil biosystems defined by soil – soil solution – plant, as well as in the solution of various issues in agriculture, forestry, pedology or hydrology. Lysimeter systems also offer the possibility to assess and analyse the impacts of climate change, the hydrodynamic changes associated with the construction of water structures or other large structures, the transport of a wide range of pesticides, xenobiotics, toxic metals, and radionuclides into the food chain or subsurface water, as well as in the analysis of microbial activity, whether from the point of view of agricultural production or microbial degradation of organic substances present in the soil. Lysimeters also allow to analyse the impacts of the use of fertilizers and various chemicals on soil, groundwater and plant physiological status. In this way, it is possible to optimize the doses not only of the substances mentioned in such a way that they bring maximum benefit to the soil, but also the irrigation can be optimized for the given location. The latest lysimeter systems allow evaluate movement of water in large monolithic soil

columns with a diameter of 1 m and a height of 1.5 m with high accuracy at a level of ± 20 g (Meissner *et al.* 2014).

In previous papers, samples of municipal SSL were characterized as a potential soil additive (Šuňovská *et al.* 2013) or metals sorbent (Frišták *et al.* 2013) and evaluated from the point of view of responses of rhizosphere bacterial (Ondreičková *et al.* 2019a) or fungal (Ondreičková *et al.* 2019b; 2021) communities to the sewage sludge application into soil as well. The aim of this work was to characterize samples of SSL originated from the Wastewater Treatment Plant of company TAVOS, a.s. (municipal wastewater treatment plant, Piešťany, Slovak Republic) in terms of their potential application into the soils using a laboratory lysimeter system. In the first part of the work, a physico-chemical characterization of obtained SSL samples as well as samples of reference soil – an agriculturally used soil (AUS) was carried out. Subsequent laboratory lysimeter experiments were focused on the total balance of the movement of metals – microelements Zn and Cu occurring in significant amounts in the obtained samples of SSL within the soil biosystem defined by SSL/AUS – soil solution – root system and aboveground parts of tobacco (*Nicotiana tabacum* L.) plants as a model of fast-growing crops.

Experimental

Sewage sludge and soil samples

The sewage sludge (SSL) was sampled from the wastewater treatment plant of company TAVOS, a.s. (municipal wastewater treatment plant Piešťany, Slovakia). Obtained samples of SSL represented activated sludge treated into the form of solid particles using a mechanical drainage system. In the experiments, SSL dried under laboratory conditions, mechanically homogenized and sieved to achieve a particle size < 0.150 mm was used. A reference soil – an agriculturally used soil (AUS) was obtained from experimental fields of Plant Production Research Institute (location Borovce, Slovakia, 48°34′N, 17°44′E, altitude 160 m). The individual soil samples were taken gradually in layers with the aim to keep the natural soil stratification: 1. layer 0 – 10 cm; 2. layer 10 – 20 cm; 3. layer 20 – 30 cm, and 4. layer 30 – 40 cm.

Plant seedlings

As a model for fast-growing plants, tobacco (*Nicotiana tabacum* L.) seedlings were chosen. Tobacco plants were obtained by germination of seeds and cultivation under hydroponic conditions in the inorganic carrier Perlite watered by a nutrient solution according to Hoagland (1920) diluted with deionized water in the volume ratio of 1 : 3 (25 % concentration strength). Plant cultivation was carried out in a plant growth chamber KBWF 720 (Binder, Germany) under the following conditions: photoperiod 16 h light/8 h dark (max. photosynthetic photon flux density PPFD = 200 $\mu mol.m^2.s^{-1}$ with gradual reaching of maximum and minimum in the steps of 0 %, 40 %, 60 %, and 100 % light intensity), temperature 28/18 ºC and relative humidity 60 – 80 %. For experimental purposes, uniform plants with slight variations in the growth phase, height (10 – 12 cm), mass (wet material, 2 – 4 g), number of leaves (5 – 6 leaves) after four weeks of cultivation were obtained.

Characterization of studied matrices

The determination of pH or cation-exchange capacity (CEC) of SSL and AUS were realized according to ISO standard method No. 10390 or No. 11260, respectively. The value of pH was measured in the suspension of solutions 0.01 $mol.dm^{-3}$ $CaCl_2$, 1 $mol.dm^{-3}$ KCl, or deionized water in the volume ratio 1 : 5 (sample : solution) after 1 h of sedimentation. CEC determination was carried out using $BaCl_2$ solutions. Water holding capacity (WHC) of individual samples of SSL and AUS or mixture SSL : AUS in different weight ratio: 1 : 76, 1 : 38, 1 : 19, and 1 : 9 were evaluated using glass columns with an inner diameter of 2.5 cm and length of 30 cm. The elemental analysis of mechanically homogenized SSL (particle size of < 0.063 mm) was performed by X-ray fluorescence spectrometry using the high-performance X-ray fluorescence spectrometer X-LAB 2000 (Spectro, Germany). Total organic carbon (TOC) was determined using TOC analyzer (TOC-VCPN, Shimadzu, Japan).

The determination of bioavailable amount of Zn and Cu in studied samples of SSL and individual layers of AUS was realized by one-step extractions using solutions according to Mehlich (1984) – Mehlich II (in mol.dm^{-3}: NH$_4$Cl – 0.2; CH$_3$COOH – 0.2; NH$_4$F – 0.015; HCl – 0.12) and Mehlich III (in mol.dm^{-3}: EDTA – 0.001; HNO$_3$ – 0.001; NH$_4$F – 0.015; NH$_4$NO$_3$ – 0.25; CH$_3$COOH – 0.2). The suspension of SSL or AUS in Mehlich II or Mehlich III solution (10 g in 100 cm^3 of solution) was incubated for 10 min under agitation (250 rpm) at 25 °C. After exposure, the mixture was filtered through a filter paper and then through a syringe filter (13 mm diameter; 0.45 µm permeability; CHS FilterPure PTFE hydrophobic) and the content of Zn and Cu obtained in the filtrate was determined by atomic absorption spectrometry.

Laboratory lysimeter experiments

The laboratory lysimeter system (EcoTech, Germany) was self-designed and used to perform the lysimeter experiments. This system consisted of two parts (Fig. 1A), a vessel to create a soil column (diameter 30 cm and height 40 cm) and a cultivation part for growing plants in a soil column supplemented by a watering head. In this system, it is possible to install the soil monolith or create a soil column with a dry weight of 30 to 40 kg. The lysimeter contains on each side of the lysimetric vessel 3 inputs for sensors recording soil moisture tension, humidity, or temperature. Bottom probe input is located at the interface of the lowest fourth and third layer of the soil, the middle probe input at the interface of the third and second layer of the soil, and the upper probe input at the interface of the second and top layer of the soil. Data from individual tensiometers and temperature sensors are analysed and collected by a datalogger (Fig. 1B), from which they can be wirelessly transferred to a computer. Before, during and at the end of the experiment, the whole lysimetric system can be weighed using a weighing device with the aim to evaluate the total water balance (Fig. 1C). The lysimeter system also includes apparatus for watering the soil column via a peristaltic pump and watering head, as well as apparatus for suction of soil eluate by a vacuum pump and through the drainage system at the bottom of the lysimeter vessel (Fig. 1D).

Within the preparation of the lysimeter experiment, 8 kg of the soil was transferred from each obtained layer of AUS (1. layer 0 – 10 cm; 2. layer 10 – 20 cm; 3. layer 20 – 30 cm, and 4. layer 30 – 40 cm) into the lysimeter vessel (dry weight, particle size < 2 mm, humidity < 5 %). The individual soil layers were inserted into the lysimeter sequentially in the order in which they occurred naturally at the sampling site, thus respecting their natural vertical distribution. In the first lysimeter experiment A, a homogeneous mixture of SSL : AUS in the weight ratio 1 : 76 (105 g of the studied SSL) was added to the top (first) layer of the prepared soil column. In the second experiment B, a mixture of SSL : AUS with double amount of SSL (210 g of SSL; SSL : AUS = 1 : 38) was applied. The first addition of SSL represents the amount of 15 tons of SSL per hectare, which corresponds with the limits permitted by Act No. 188/2003. In the case of the second lysimeter experiment, this amount was increased to 30 t.ha^{-1} thus exceeding the permitted limit value of 15 t.ha^{-1}.

Fig. 1. The design of the laboratory lysimeter system used in experiments.

Due to the fact that dry soil samples were placed into the lysimeter vessel, prior to planting fast-

growing tobacco (*N. tabacum* L.) plants, it was necessary to water the soil column using 3.5 dm^3 of artificial rainwater prepared according to the authors Do Lago *et al.* (2013). After equilibration (24 h) in terms of soil moisture tension distribution analysed by installed tensiometers, the soil eluate was drained from the drainage bottom of the lysimeter vessel before planting the plants of tobacco. Subsequently, 3 seedlings of tobacco plants were planted into the topsoil layer of the soil column and the whole lysimeter system was placed in the growth chamber, where plants were cultivated under the following conditions: photoperiod 16 h light/8 h dark (max. photosynthetic photon flux density PPFD = 200 µmol.m^{-2}.s with gradual reaching of maximum and minimum in the steps of 0 %, 40 %, 60 %, and 100 % light intensity), temperature 28/18 ℃ and relative humidity 60 – 80 %. During the experiments, the soil column was subjected to watering by a watering head with the application of 1.5 – 2.0 dm^3 of artificial rainwater at precisely defined intervals. After each watering and equilibrium reaching (after 24 h), the soil eluate was drained from the drainage bottom of the lysimeter vessel by a vacuum pump. The total water balance in the lysimeter system was also evaluated during the experiment by weighing the whole system. A continuous analysis (every 5 min) of soil moisture tension and temperature, as well as air humidity and temperature was carried out using installed probes. Data was collected and stored by a datalogger.

Atomic absorption spectrometry

The presence of Zn and Cu as microelements in the samples of soil layers, soil eluates, or individual parts of tobacco plants at the end of the lysimeter experiments and the bioavailability of Zn and Cu in AUS and/or SSL samples were determined by atomic absorption spectrometry (AAS) using atomic absorption spectrometer iCE 3000 (Thermo Scientific, USA). In the case of soil and plant biomass, before the analysis the samples were mineralized in concentrated HNO$_3$ at 210 °C and 20 bars using a microwave mineralization system Multiwave 3000 (Anton Paar, Austria). Based on the estimated amount of studied metals in the given

samples, the determination was carried out either by flame atomization (acetylene/air; ppm amounts) or by electrothermal atomization (argon as an inert gas; ppb amounts). In the measurement of absorbance of samples, hollow cathode lamps (Heraeus, Germany) as radiation sources were used. Deuterium background compensation was applied for correction of background effect.

Results and Discussion

Characterization of studied matrices

From the comparison of the values of physico-chemical parameters pH, cation-exchange capacity (*CEC*) and total organic carbon (*TOC*) (Table 1) it is clear that the samples of sewage sludge (SSL) and samples of agriculturally used soil (AUS) differed significantly in these evaluated parameters. In the case of samples of individual soil layers, such significant differences in the mentioned values were not found. Soil samples showed pH values within the range from 7.29 to 7.87, while for SSL pH values from 6.04 to 6.11 were determined. The slightly acidic character of the studied SSL samples after their application to soils similar to the soil studied in terms of their pH reaction may have a positive effect on reducing the pH values of typical alkaline soils to values pH of neutral soils. A similar observation was confirmed by Žaltauskaitė *et al.* (2022). However, the reduction in pH is variable depending on the type of soil, sludge dosage, and characteristics, incubation time etc. (Dhanker *et al.* 2021). It is generally known that soil pH value plays an important role in the mobility and bioavailability of some elements, e.g. significant microelements in the soil (see e.g. Hechmi *et al.* 2021). At alkaline and neutral pH, metals tend to form insoluble minerals, whereas at acidic pH, metals are in more soluble and bioavailable forms (Alloway 2012). For typical microelements (Zn, Mn, Cu), their mobility and bioavailability for plant uptake in the soil increased with decreasing of pH value (below pH 7.0) of the soil. From the point of view of agricultural production, it can have a positive effect. On the other hand, from an ecotoxicological point of view, it can be evaluated rather negatively due to: (i) risk of heavy metals entering the groundwaters and

food chain, and (ii) risk of increased heavy (toxic) metals uptake by plants and their transport into the food chain.

In the case of *CEC* values, the studied SSL showed a 1.5-fold higher *CEC* value and in the case of *TOC* values up to 40-fold higher value in comparison with AUS samples (*CEC* = 15.1 – 16.8 meq/100 g and *TOC* = 0.21 – 0.76 %) (Table 1). For individual samples of vertical soil layers, the *CEC* and *TOC* values decreased slightly from the topsoil layer (0 – 10 cm) to the lowest soil layer (30 – 40 cm). From the literature it is evident that soils showing *CEC*

values in the range of 11 – 50 meq/100 g can be characterized as soils with low sand content, high clay content, medium or high organic matter content, and high *WHC*. Soils showing low *CEC* values in the range of 0 – 10 meq/100 g are negative in this respect (Daniels and Haering 2006). Based on this, it can be assumed that the addition of the studied SSL to soils can have a positive effect in terms of increasing the *CEC* and *TOC* values of soils, as well as improving the characteristics of the soils important, especially from the point of view of agricultural production.

Table 1. The values of pH (pH$_{H2O}$, pH$_{CaCl2}$, and pH$_{KCl}$), cation exchange capacity (*CEC*) and total organic carbon (*TOC*) determined for SSL and individual layers of AUS.

Sample	pH$_{H2O}$	pH$_{CaCl2}$	pH$_{KCl}$	*CEC* [meq/100 g]	*TOC* [%]
SSL	6.11	6.04	6.04	24.6	27.7
AUS1 0 – 10 cm	7.87	7.29	7.42	16.6	0.76
AUS2 10 – 20 cm	7.84	7.34	7.53	16.8	0.53
AUS3 20 – 30 cm	7.80	7.41	7.47	15.7	0.32
AUS4 30 – 40 cm	7.87	7.40	7.51	15.1	0.21

According to performed analyses determining the values of water holding capacity (*WHC*), it was found that SSL samples had more than 4-times higher *WHC* compared to AUS samples (*WHC* in the range of 44 to 48 g H$_2$O/100 g, d.w.) (Fig. 2).
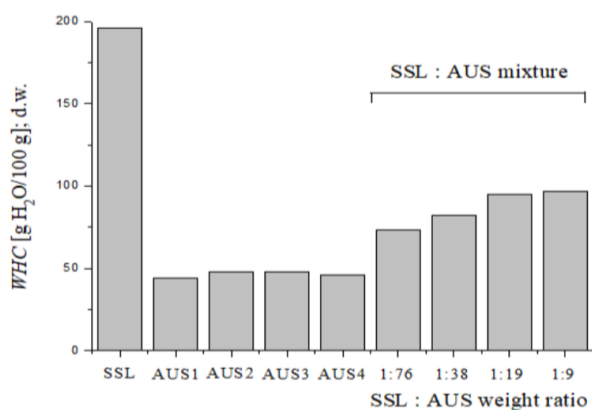

Fig. 2. Water holding capacity (*WHC*) obtained for studied matrices – sewage sludge (SSL) and individual layers of agriculturally used soil (AUS) (AUS1: topsoil layer 0 – 10 cm; AUS2: 10 – 20 cm; AUS3: 20 – 30 cm; AUS4: 30 – 40 cm) or for mixtures SSL : AUS prepared in different weight ratio.

Individual experiments also showed that the addition of SSL to soil had a positive effect in terms of gradual increase in *WHC* values, when with increasing SSL : AUS weight ratio *WHC* values increased from 73 g H$_2$O/100 g (d.w.) found for SSL : AUS = 1 : 76 to the value of 97 g H$_2$O/100 g (d.w.) found for SSL : AUS = 1: 9. Boudjabi and Chenchouni (2021) found that the application of SSL at the soil surface significantly increased soil moisture (25.95 %) compared to SSL mixed treatment, which was 24.46 % and the controls.

Elemental analysis of SSL samples carried out by X-ray fluorescence spectrometry confirmed that these samples did not exceed the limits for heavy metals concentrations permitted by Act No. 188/2003 in terms of their direct application to soil (Table 2). However, an interesting finding was that the samples of SSL contained significant amounts of heavy metals − microelements Zn (1,269 mg.kg^{-1}, d.w.) and Cu (224 mg.kg^{-1}, d.w.).

Table 2. Metal concentrations in the studied sewage sludge.

				Total concentration of metal [mg.kg^{-1}; d.w.]; (LOQ)					
As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Se	Zn
3 (1)	< 5 (1)	36 (5)	224 (5)	2.69 (0.05)	0.21* (0.05)	22 (4)	46 (5)	6 (1)	1,269 (5)

* determined in %.

It is generally known that higher total amounts of important elements in a given substrate (soil) may not automatically mean a more favourable environment for plant growth. In addition to the total amounts of elements, their chemical form in which they occur in the given environment (free ionic forms, inorganic or organic complexes, and others) and the portion of their bioavailable amounts also play an important role. This results in the fact that the plant is not able to absorb the total amount of the element present in the given environment, but only its bioavailable part. The literature shows that the bioavailability of many heavy metals is strongly dependent on several factors, such as pH, organic matter content, clay content and other components (Kabata-Pendias 2011).

Following the above mentioned results regarding the total Zn and Cu content in the studied matrices, analyses with the aim to evaluate the bioavailability of these metals were carried out. For this purpose, one-step extraction procedures using Mehlich II and Mehlich III solutions were used. The choice of both extraction procedures was made in order to compare the obtained results, despite the fact that the Mehlich II solution is recommended for determination of the extractability of macroelements, e.g. Mg, K, Ca, and P. One-step extractions using Mehlich III solution showed that the percentage of the extractable Zn to the total amount of Zn occurring before extraction in the SSL sample was slightly higher compared to the AUS samples and reached the value 8.7 % (Fig. 3).
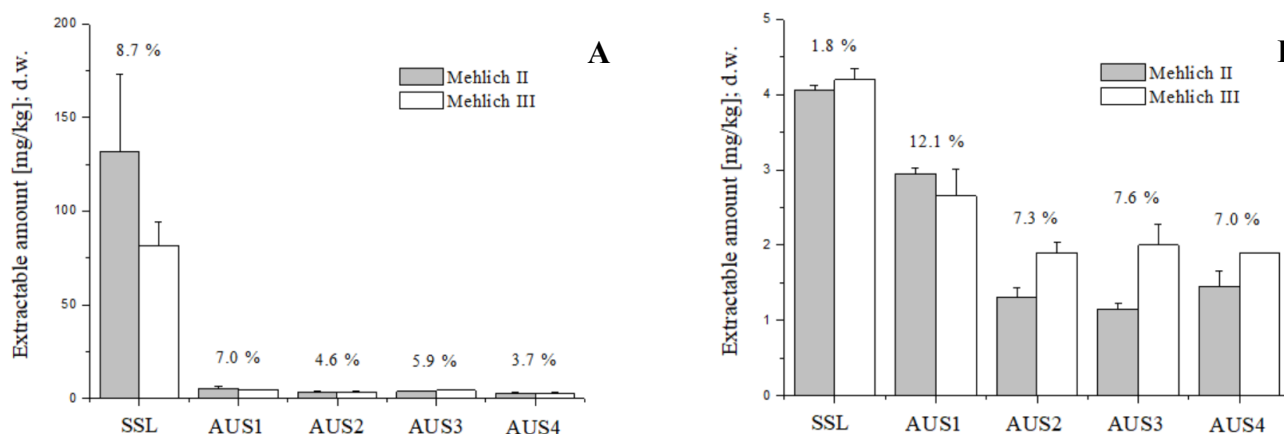

Fig. 3. Extractability of Zn (**A**) and Cu (**B**) from the samples of SSL and individual layers of AUS determined using Mehlich II and Mehlich III extractions. Extractability of metals expressed as the amount of metal released form 1 kg of the given matrix (in mg/kg), the percentages represent the ratio of the extractable amount of metal to the total amount of the metal present before extraction in the matrix (see Table 3). Error bars represent the standard deviations of the mean ($\pm$ SD; $n = 3$).

Table 3. Total concentration of Zn and Cu in the studied matrices.

Total concentration of metal [mg.kg^{-1}; d.w.]	Sample				
	SSL	AUS1	AUS2	AUS3	AUS4
Zn	1,269	71	70	70	73
Cu	224	24	22	21	23

Markowicz *et al.* (2021) found that the bioavailable fraction of Zn in the control soil was only 0.14 % of its total content and was 2.8 $\pm$ 0.7 mg.kg^{-1}. For SSL, the bioavailable fraction of Zn was only 7.7 $\pm$ 0.5 mg.kg^{-1}. In contrast, the percentage of extractable Cu in SSL (only 1.8 %), was approx. 6-

times lower compared to AUS samples. Thus, it can be stated that despite the significant presence of Zn and Cu in the studied sample of SSL, their bioavailability is relatively low. This result can be evaluated positively because high values of the bioavailability of Zn and Cu would mean the immediate availability of these metals for plants, and it could be the case that the root system would not be able to absorb this amount effectively at a given moment. It follows that the studied SSL can represent an interesting source of Zn and Cu as important microelements for plants, but relatively tightly bound, which can be released from this matrix in smaller proportions controlled by the gradual concentration equilibria $[M]_{solution} : [M]_{SSL}$

reaching or decomposition of this matrix in the soil environment.

Laboratory lysimeter experiments

In this part of the work, the laboratory lysimetric experiments involving the application of SSL (15 t.ha^{-1} – experiment A or 30 t.ha^{-1} – experiment B) into the topsoil layer of AUS (0 – 10 cm) forming part of a 40 cm soil column in which seedlings of tobacco (*N. tabacum* L.) plants were cultivated, allowed us to evaluate the overall balance of Zn and Cu in terms of their amount applied into the soil in the form of SSL. These experiments were focused on the overall balance of metal movement – Zn and Cu microelements occurring in significant amounts in the obtained samples of SSL within the designed soil biosystem: SSL/AUS – soil solution – root system and aboveground parts of tobacco as a model fast-growing plant. The first addition of SSL represents the amount of 15 tons of SSL per hectare, which corresponds with the limits permitted by Act No. 188/2003. In the case of the second lysimeter experiment, this amount was increased to 30 t.ha^{-1}, thus exceeding the permitted limit value of 15 t.ha^{-1}. It follows that the first experiment involving the application of SSL within the permitted limits focuses primarily on the evaluation of the positive effects of SSL as potential effective carriers of important microelements for plant growth. On the other hand, the second experiment with a double dose of SSL application (30 t.ha^{-1}) makes it possible to assess their negative effects, especially in terms of the risks of heavy metals release from SSL into groundwater or the food chain through the plant uptake.

Fig. 4 graphically shows the changes in the values of water pressure potential (soil moisture tension) measured by tensiometers placed in individual layers of the prepared soil column caused by watering, suction of the soil eluate or evapotranspiration of water under given conditions. The value of pressure potential near 0 hPa represents saturated soil with water and values up to -600 to -700 hPa represent soil with minimal water content.
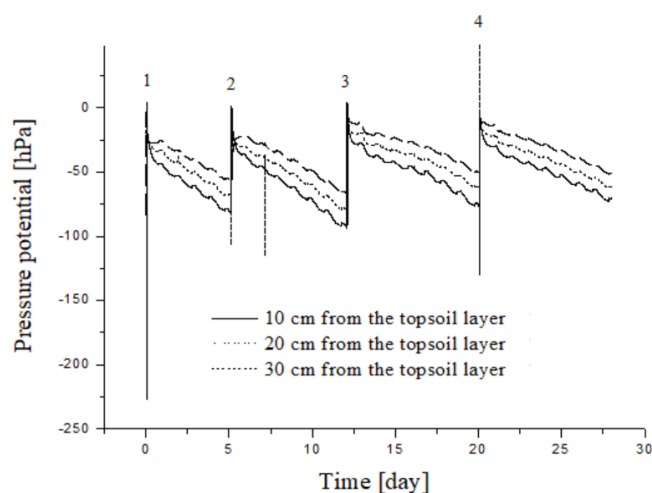
During a 28 day experiment A, the analysis of Zn

Fig. 4. Soil moisture tension changes analysed by tensiometers placed in individual layers of the prepared soil column caused by watering, suction of the soil eluate or evapotranspiration of water under conditions given by the growth chamber during 28 days of the lysimeter experiment A and B.

and Cu amount in the samples of soil eluates drained from bottom of the lysimeter vessel confirmed a gradual increase in the concentration of Zn and Cu (0.21 mg.dm^{-3} for Zn and 0.03 mg.dm^{-3} for Cu to 0.96 mg.dm^{-3} and 0.19 mg.dm^{-3}, respectively) in the soil eluates collected. A similar phenomenon was also observed in the case of the experiment B. The gradual increase of concentrations of Zn and Cu in the soil eluates is probably related to the gradual release of these metals from the SSL applied into the topsoil layer (0 – 10 cm).

At the end of the experiments, the grown tobacco plants and the individual soil layers were removed from the lysimeter vessel and subjected to quantitative analysis of the content of Zn and Cu. In the first step, the extractability of these microelements was evaluated by a one-step extraction procedure using a Mehlich III solution in order to determine the bioavailability of Zn and Cu within the individual layers of the soil column. The highest extractability of Zn and Cu was determined in the topsoil layer (0 – 10 cm) involving the application of the studied SSL (Fig. 5A), when 13.8 % of Zn of the total amount of Zn in the analysed topsoil occurred in the extractable – bioavailable form. In the case of Cu and the topsoil layer, the bioavailable form of this metal represented only 8.4 % of the total amount of Cu under the same

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conditions. From a quantitative point of view, it can also be said that the bioavailability of Zn and Cu in the topsoil layer was up to 3-times and about 30 % higher than in the case of lower soil layers without the direct application of SSL, respectively. Also in the second experiment B (Fig. 5B), it was found that the highest extractability of Zn and Cu was in the topsoil layer involving the application of the

studied SSL, when up to 14.6 % of Zn and 9.2 % of Cu of the total amount of Zn and Cu in the analysed topsoil were occurred in the bioavailable forms. Similarly, the bioavailability of Zn and Cu in the topsoil layer was up to 2-times and about 40 % higher than in the lower soil layers without the direct application of SSL, respectively.
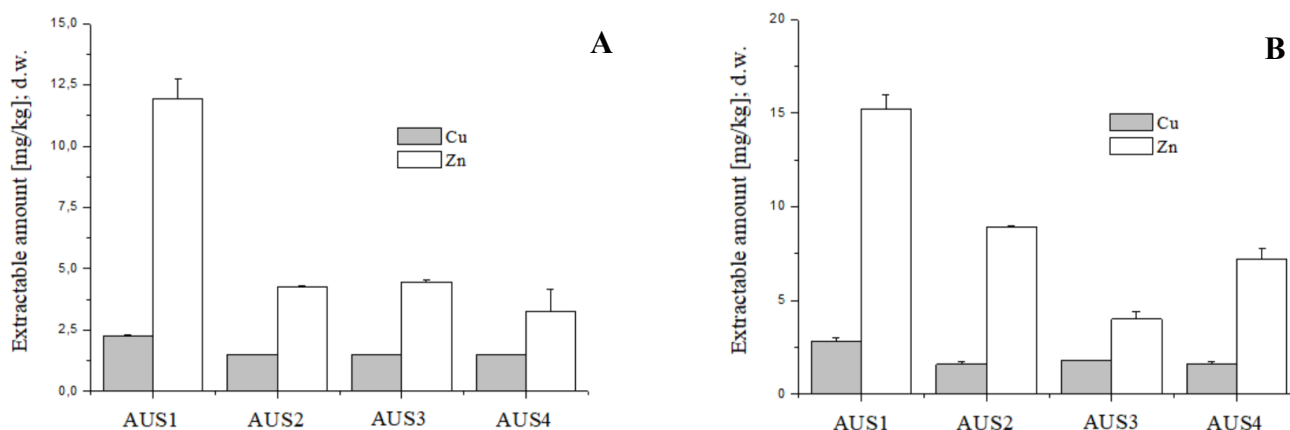

Fig. 5. Extractability of Zn and Cu from individual AUS layers (AUS1: topsoil layer 0 – 10 cm; AUS2: 10 – 20 cm; AUS3: 20 – 30 cm; AUS4: 30 – 40 cm) at the end of laboratory lysimeter **experiments A** and **B** expressed as the amount of metal released form 1 kg of the given soil layer. Error bars represent the standard deviations of the mean ($\pm$ SD; n = 3)

From the analysis of the samples of tobacco plants, it was observed that during experiment A, the plants increased their quantitative parameters, such as height of plants, length of above-ground parts or weight of plants, at least 3-times. From the point of view of the presence of Zn and Cu in the tissues of tobacco plants, it can be said that Zn was accumulated in the concentration of 84.4 mg.kg^{-1} (d.w.) and Cu in the amount 21.9 mg.kg^{-1} (d.w.). The ratio between accumulated amounts of Zn and Cu corresponds to the initial concentration ratio of these metals in the topsoil layer involving the application of SSL (86.8 mg.kg^{-1} for Zn and 26.6 mg.kg^{-1} for Cu). Within this analysis, it was also evaluated the transfer factor (TF) defined as $[M]_{plant} : [M]_{soil}$ and the portion of these metals in the tissues of individual parts of plants – root, stem and leaves in order to determine the concentration factor (CF) defined as $[M]_{aboveground\ parts} : [M]_{root}$. The calculated TF values were 0.97 for Zn and 0.82 for Cu and CF reached the same value of 0.74 for both metals.

From the results of experiment B, it can also be concluded that during the experiment the plants increased their quantitative parameters at least 3-times. Due to the double dose of SSL (30 t.ha^{-1}), in terms of the amounts permitted by Act No. 188/2003 (limit 15 t.ha^{-1}), the toxic effects of this increased addition of SSL on the growth of tobacco plants were not observed. Based on the portion of Zn and Cu in the tissues of tobacco plants, it was found that in this case Zn was present in a concentration of 96.2 mg.kg^{-1} (d.w.) and Cu in the amount 15.7 mg.kg^{-1} (d.w.). Thus, these amounts represent similar concentrations as in the case of an experiment involving the application of half amount of SSL. When calculating the values of concentration factors (CF) and transfer factors (TF), we found slightly different data than in previous experiment A. In both parameters, higher values were obtained for Zn (CF = 0.74; TF = 0.94) in the comparison with the data for Cu (CF = 0.36; TF = 0.54).
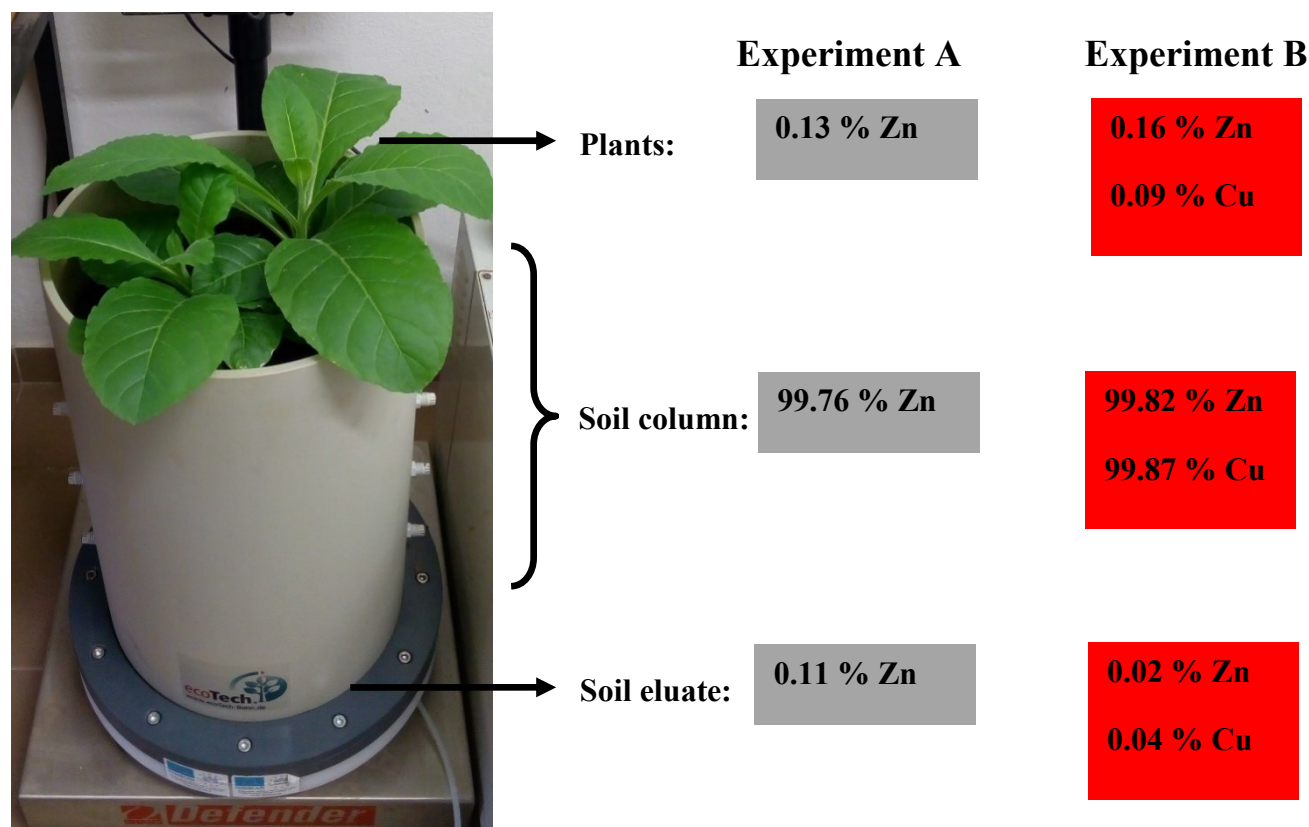
Fig. 6. Percentage distribution of the total amount of Zn and Cu occurring in the applied SSL (**Experiment A:** Zn – 133 mg and Cu – 23.5 mg; **Experiment B:** Zn – 267 mg and Cu – 47.0 mg) within the soil biosystem SSL/AUS – soil solution – 3 tobacco plants (*N. tabacum* L.) at the end of laboratory lysimeter experiments. Details are described in Fig. 4.

The use of a laboratory lysimeter system allowed to assess the total balance of Zn and Cu, based on the dose of SSL applied into the soil at the beginning of the experiment (Experiment A: Zn – 133 mg and Cu – 23.5 mg; Experiment B: Zn – 267 mg and Cu – 47.0 mg), the amount of Zn and Cu occurring in the collected soil eluates during the experiment and based on the amount of these metals accumulated in tobacco plants. In the case of the application of SSL in the highest dose permitted (15 t.ha^{-1}), the total balance of Zn and Cu originating from the applied amount of SSL into the soil at the beginning of the experiment showed that only 0.11 % Zn and 0.07 % Cu were released in the form of soil eluate during a 28 day of exposure, while in tobacco plants 0.13 % Zn and 0.05 % Cu were accumulated (Fig. 6). When applying SSL in the amount of 30 t/ha, i.e. in the amount exceeding the permitted limits, it was found that from the applied amount of SSL into the soil at the beginning of the experiment only 0.02 % Zn and 0.04 % Cu were released in the form of soil eluate during a 28 day exposure, while in tobacco plants 0.16 %

Zn and 0.09 % Cu were accumulated. The remaining percentages of Cu and Zn stayed bound in the prepared soil column. Similar minimal amounts of Zn and Cu released in the soil eluate or accumulated in giant reed (*Arundo donax* L.) plants were also observed in the work of Šuňovská *et al.* (2015), despite the increased bioavailability of these metals in the topsoil layer.

From the above mentioned results, it is clear that double dose of SSL applied into the topsoil layer had the effect of increasing the accumulated amounts of Zn and Co in plant tissues rather than releasing them into the lower soil layers or soil eluate at the bottom of the lysimeter vessel.

Conclusion

From the point of view of evaluating the possibility of application of the studied sewage sludge (SSL) to agricultural soils, it can be stated that the applied SSL represents an interesting source of Zn and Cu as important microelements in plant nutrition. These metals occur in the studied SSL

in a bioavailable form, while their bioavailable portion is higher than in the case of agriculturally used soil (AUS), but not enough to represent the risk of significant transport of these heavy metals into the lower soil layers or groundwater. In this regard, it was also shown that even exceeding the permitted limits in terms of application of SSL into the soil (30 t.ha^{-1}) did not have negative effects in terms of significant vertical transport of Zn and Cu to lower layers of the soil column or in terms of the growth of tobacco (*N. tabacum* L.) as a model of a fast-growing plant. However, it must be taken into account that long-term and especially repeated application of higher amounts of SSL into the soils can lead to a negative accumulation of heavy metals originating from this application and to soil contamination. In this sense, relevant legislation of the Slovak Republic is also drafted (Act No. 188/2003). An interesting scientific challenge, as well as a challenge for practice, is the application of SSL as a large-volume waste in terms of their application to degraded soils or soils deficient in some important elements in plant nutrition and for growing fast-growing plants (including trees) – energy crops.

Acknowledgement

Conflict of interest

The authors declare that they have no conflict of interest.

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