

HEAVY METAL ACCUMULATION IN DIFFERENT PARTS OF TREES GROWN ON SEWAGE SLUDGE

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Sewage sludge is a rest product deriving from the wastewater treatment plants. It is rich in nutrients and essential elements. Therefore sludge on-land utilisation can create an added value by recovering energy from biomass produced on marginal lands. However, widespread on-land recycling of sewage sludge might be limited due to high heavy metal content. Health organisations show an increasing concern about the risks posed to the environment and human health as many countries worldwide already are facing the heavy metal contamination problem. In the scientific literature it can be found, that high total heavy metal content is not directly related to an intensive metal uptake by plants. In this study samples were collected from three woody plant species (black locust, silver birch and aspen) growing directly on the sewage sludge in a storage site near Kaunas, Lithuania. Heavy metal content was detected separately in the leaves, stems and roots. It was determined that nearly all analysed heavy metals (lead, chromium, nickel, copper and zinc) were accumulated within normal range despite high total concentrations in the growing media, and only cadmium was accumulated at elevated concentration. Based on bioaccumulation factor, aspen could be considered as a Cd-accumulator and used for phytoremediation purposes.

Keywords: heavy metals, cadmium, bioaccumulation, woody plants, aspen, black locust, silver birch

INTRODUCTION

Geological processes, heavy industry, agriculture and most often poor waste disposal practises have led to significant accumulation of heavy metals (HM) in soils. Concentration and speciation of HM, soil properties and rate of uptake by plants determines the entry of HM into the food chain. Health organisations show an increasing concern about the risks posed to the environment and human health as many countries worldwide face the HM contamination problem. Heavy metals cannot be degraded during biological and microbial processes, so the contamination with HM in soil persists long time after the contaminants have entered the soil (Bolan et al., 2014).

Due to intensive farming soil quality in arable lands has decreased drastically in recent decades. Main threat for the agriculture is a lack of nutrients, phosphorus (P) in particular. With regards to bioavailable P, sewage sludge (SS) is considered as a valuable source (Ciešlik and Konieczka, 2017). It is also rich in other essential nutrients as well as organic matter. Sewage sludge derives from wastewater treatment plants. Wastewater collection network has been constantly expanding and the treatment facilities became more efficient due to technological advances. For this reason, loads of the generated SS are higher than its utilisation rates. However, widespread on-land utilization of SS might be limited due to a high HM content and presence of pathogens (Pecia and Westerhoff, 2015). Even if SS is used as fertilizers for energy crops, it is commonly believed that plants will accumulate HM at high levels, which later on can hinder energy recovery process or create management problems for the rest products (e.g. ashes, digestate). On the other hand, uptake of HM by plants is very complex because it depends on so many biotic and abiotic factors that it cannot be measured on a linear scale. Therefore, studies involving HM accumulation deriving from SS-fertilisation are very important as they can help to enhance the utilisation of SS and create added value by recovering energy from biomass produced on marginal lands.

Aim of this study was to assess potential of HM to be transferred from the SS to the tissue of woody plants as well as capability of the selected plant species to accumulate HM in different plant parts: roots, stems and leaves.

RESEARCH METHODS

Woody plant samples were collected in October, 2015 from SS storage site in Ežerėlis, Kaunas district, Lithuania. Stem, leaf and root samples were taken from three woody plant species: black locust (*Robinia pseudoacacia*), silver birch (*Betula pendula*) and aspen (*Populus tremula*). Roots were dug out using stainless-steel spade and cut with the gardening scissors. Stem samples were taken using a fine cut saw. Leaves were plucked from the branches. All plant samples (sealed in plastic bags) were taken back to the laboratory where they were thoroughly washed with distilled water, dried at 60 °C for 24 hours and finely chopped or pulverized. Elemental analysis was performed at an accredited laboratory “Centre of Agrochemical Research” (Lithuania). Lead (Pb) and cadmium (Cd) were detected with graphite furnace atomic absorption

spectrometer following the standard LST EN 15550:2008. Chromium (Cr), copper (Cu), nickel (Ni) and zinc (Zn) were determined with inductively coupled plasma – atomic emission spectrometer following the standard LST EN 15621:2012.

Samples of the growing media (SS), were also collected. They were taken at a distance of not more than 1 m from the plant centre and from 0.4-0.6 m depth. Joint soil samples of approximately 4-5 kg were pooled from 3-5 different places around every woody plant; bigger roots and other debris were removed. After SS homogenization in the lab, subsamples of 0.5 kg were dried until air-dry status. Elemental analysis was also performed at “Centre of Agrochemical Research”. Cadmium, Cu and Zn were detected with electrothermal or flame atomic absorption spectrometer following the standard ISO 11047:1998. For chromium, Ni and Pb – the ISO 22036:2008 standard was used. pH was detected with potentiometer in 1 mol/L potassium chloride (KCl) suspension following the LST ISO 10390:2005 standard. Mineral nitrogen (N) in SS was determined using a spectrometric flow method; the concentration of water soluble P (as P₂O₅) and potassium (K) (K₂O) was determined by the Egner-Reihem Domingo method.

To determine age of the trees, increment borer was used. A section of wood tissue from all three-investigated species was taken. Core samples were dyed with potassium permanganate. Due to different density between early wood and late wood, annual rings exhibit varying intensity of the colour. Thus, it is possible to count them and determine tree age. Annual growing rings were counted using LINTAB instrument and TSAP (Time Series Analysis and Presentation) software.

All instrumental analyses were performed in triplicates. Significance level between the means was calculated using analysis of variance (ANOVA) tool pack for a *t*-test (two sample analysis assuming unequal variances) when $p < 0.05$. Bioaccumulation factor (BCF) was calculated as a ratio of the total concentration of an element in the roots to its concentration in the growing media. Translocation factor (TF) was calculated as a ratio of the total concentration of an element in the aboveground parts of the plant to the concentration in the roots. Mobility ratio (MR) was calculated as a ratio of the total concentration of an element in the aboveground parts of the plant to its concentration in the growing media.

RESEARCH RESULTS

Heavy metal content in the growing media is presented in Table 1 together with detected pH, concentrations of mobile P and K, and mineral N. The growing media of woody plants mainly consisted of SS. According to the LAND 20-2005, SS is classified into three categories based on HM content. Only the first category's SS can be used for agricultural or re-cultivation purposes. Maximum permissible concentrations (MPC) of HM for each category are also presented in Table 1. Numbers in bold indicate that these elements were exceeding MPC and such SS could not be utilized as soil amendment. Due to the elevated HM content, SS from Kaunas wastewater treatment plant after anaerobic digestion and dewatering usually is transported and stored in a specially constructed sludge storage facilities. Nevertheless, high concentrations of essential plant macronutrients (N, P, K) showed potential for SS utilisation in agriculture or forestry as a valuable resource, if only it would meet requirements for HM content.

Heavy metal content was uneven between three different composite samples of the growing media (Table 1), but in all cases exhibited high concentrations of Cd, Cu and Zn. Elevated concentrations of Cd, Cu, Zn as well as other HM is a common problem with SS, originated in the areas with high population density and industrial activity, and thus limiting its further utilisation. Sewage sludge, deriving from wastewater treatment plans in smaller and non-industrial towns, usually contain lesser HM content (Somasundaram et al., 2012).

Table 1. Analytical characteristics in the growing media of each plant and heavy metal maximum permissible concentrations for the different sewage sludge categories according to the LAND 20-2005 (average ± standard deviation, n = 3)

Analytes	Concentration, mg/kg dry weight			MPC for different SS categories, mg/kg dry weight		
	Black locust	Silver birch	Aspen	1 st	2 nd	3 rd
Pb	57 ± 6	105 ± 12	64 ± 7	≤ 140	141–500	> 500
Cd	16 ± 3	52 ± 8	4.1 ± 0.7	≤ 1.5	1.6–6.0	> 6.0
Cr	82 ± 14	143 ± 25	63 ± 11	≤ 140	141–400	> 400
Ni	22 ± 2	32 ± 3.3	33 ± 6	≤ 50	51–300	> 300
Cu	298 ± 35	418 ± 49	266 ± 31	≤ 75	76–600	> 600
Zn	913 ± 119	1270 ± 166	1560 ± 204	≤ 300	301–2000	> 2000
Total N	6073	8261	1876	-	-	-
P ₂ O ₅	8320	3434	2435	-	-	-
K ₂ O	815	615	519	-	-	-
pH _(KCl)	6.0	7.1	6.6	-	-	-

Heavy metal accumulation in the biomass of woody plants growing on SS in the storage site is presented in Figure 1. It was difficult to establish a clear pattern how HM accumulated in different plant parts. A very generic trend was observed that leaves accumulated the highest HM amount (28–75%), lower concentrations were found in roots (15–57%), and the lowest – in stems (9–17%). Gradeckas (1998) found similar HM distribution trend in willows (*Salix*) fertilized with SS, where leaves contained 50-80%, roots – 15–50%, and stems – 1–25%. Heavy metal accumulation in the biomass is highly dependent on plant species as well as adaptation of the specific genotype to tolerate elevated HM concentrations (Chibuike and Obiora, 2014). According to Gradeckas (1998), live organisms have biological accumulation limits for all elements.

It was determined that the black locust was five-, silver birch – fifteen- and aspen – eleven years old. The black locust has accumulated similar total concentrations of Pb as compared to silver birch and aspen though they were growing for longer time. Furthermore, growing media at black locust contained almost twice less of Pb. Therefore, it could be that

black locust have reached its biological accumulation limit for Pb and despite being exposed to higher Pb content in the growing media, the concentration in plant was increasing but only at slow pace.

Total content of Cd in aspen and silver birch was similar, although Cd content in the growing media of aspen was nearly 12 times lower than in silver birch location.

No significant differences were detected between Ni, Cu and Zn concentrations either in the growing media or in plants.

In general, all analysed metals, but Cd, were within normal accumulation ranges summarized by Kabata-Pendias (2011). This supports findings from other authors, that high total HM concentrations in the growing media does not determined intensive uptake by plants a priori (Zeng et al., 2011; Tchounwou et al., 2012).

Heavy metal transfer from soil to roots and to aboveground plant parts is expressed through the factor of bioaccumulation and translocation and mobility ratio. Calculated values are presented in Table 2.

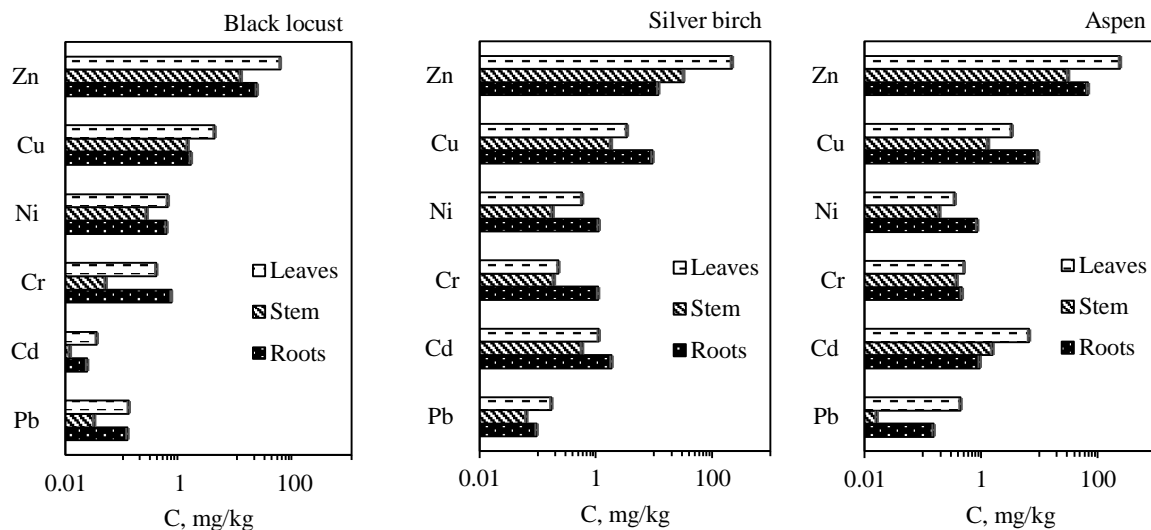


Figure 1. Heavy metal concentrations in the parts of black locust, silver birch and aspen plotted on the logarithmic scale

Bioaccumulation factor is a ratio between HM concentration in plant roots and HM content in the sewage sludge. As presented in Table 2, BCF in almost all cases was barely reaching 0.045, indicating that these woody plant species don't have a tendency to accumulate the investigated HM. Only aspen exhibited BCF = 0.23 for Cd. Marmioli et al. (2013) reported that genus of *Populus spp.* which included aspen, can have morphological adaptations favourable for Cd transfer from roots to aboveground parts and accumulation of Cd compounds in leaves and roots. Therefore, woody plants from this genus can be successfully used for phytoextraction purposes in the cases when Cd is the target element.

Table 2. Bioaccumulation and translocation factors, and mobility ratio of heavy metals in the analysed woody plant species

Species	Element	Bioaccumulation	Translocation factor		Mobility ratio	
		factor	Stem	Leaves	Stem	Leaves
Black locust	Pb	0.002	0.26	1.1	0.001	0.002
	Cd	0.001	0.50	1.5	0.001	0.002
	Cr	0.009	0.07	0.55	0.001	0.005
	Ni	0.027	0.44	1.0	0.012	0.028
	Cu	0.005	0.88	2.6	0.005	0.014
	Zn	0.024	0.53	2.6	0.013	0.062
Silver birch	Pb	0.001	0.67	1.8	0.001	0.002
	Cd	0.036	0.31	0.60	0.011	0.022
	Cr	0.008	0.17	0.21	0.001	0.002
	Ni	0.037	0.16	0.51	0.006	0.019
	Cu	0.023	0.19	0.36	0.004	0.008
	Zn	0.009	2.7	18	0.026	0.17
Aspen	Pb	0.002	0.10	2.9	0.000	0.007
	Cd	0.23	1.7	7.1	0.38	1.6
	Cr	0.007	0.81	1.1	0.006	0.008
	Ni	0.026	0.22	0.41	0.006	0.011
	Cu	0.036	0.14	0.35	0.005	0.013
	Zn	0.045	0.45	3.5	0.020	0.16

Translocation factor allows to evaluate HM transfer from roots to aboveground parts. In case, TF is greater than unity, the translocation is high and such plant species can be considered for phytoextraction purposes. From the values, given in Table 2, it is clear, that HM translocation rate from roots to stem was significantly lower/weaker than from roots to leaves. Results indicate that all three woody plant species were very active to transfer Zn to the leaf area. This

microelement is required while producing chlorophyll, thus rapid Zn transfer is essential for plant development. Usually plants are adapted to tolerate and accumulate only certain elements. Heavy metal transfer to aboveground parts depends on plant tissues which are responsible for water and nutrient transport – xylem and phloem. Transpiration speed is the most important parameter deciding how quickly nutrients can be utilized. The faster the transpiration, the quicker nutrients, water and HM are being “consumed” or accumulated in plant tissues (Orsini et al., 2012). Transpiration effect explains why HM were actively transported to the leaf area. However, HM accumulation in the leaves can also have negative consequences, because with the dead leaves HM are returned back to the growing media. Contrary to that, because water and nutrient transport towards tissues in the stem is slow, HM are accumulated weaker in the stem. However, since stem is a long-lasting part, HM are accumulated/“locked” in there permanently. In other words, HM accumulation in leaves is intensive but renewed every season, while accumulation in stems is slow but always increasing.

Mobility ratio, expressed as a ratio between HM concentration in the aboveground parts and HM concentration in the growing media, evaluates the potential of a plant to accumulate HM in certain parts. When MR is less than unity, then accumulation is weak. Mobility ratio close to unity shows medium accumulation potential. Finally, MR higher than unity shows a strong potential to accumulate HM. Analysed woody plant species exhibited very weak potential to accumulate HM both in the stems and in the leaves. Only aspen showed MR higher than unity (1.6) for Cd and could be considered as a suitable plant species for Cd phytoextraction from contaminated media.

In general, when $TF > BCF$, then HM transfer from roots to aboveground parts is more intensive than the uptake from the growing media. This can occur due to intrinsic traits of the species and/or low HM mobility in the growing media.

CONCLUSIONS

Woody plants, growing on the sewage sludge, accumulated Pb, Cr, Ni, Cu and Zn within normal ranges although total metal content in the growing media was considered as unacceptable for on-land utilization. This proves the fact, that metal accumulation in plants is highly dependent on HM mobile fraction in the growing media and not only on its total concentration. Determination of the bioavailable HM fraction could be useful for further studies. Only Cd was being accumulated at elevated concentrations.

Analysed plants species accumulated HM most intensively in the leaves, to a lesser extent in the roots and with a least extent in the stems. Heavy metals accumulated in tree biomass can be listed in the following order from the highest to the lowest: $Zn > Cu > Cr > Pb > Ni > Cd$. Results showed that HM transfer from the roots to the leaves is more intense than the transfer from the roots to the stems. This is due to a higher demand of water and nutrients in the leaf area than in stem. Based on bioaccumulation factor and mobility ratio, aspen could be considered for phytoremediation purposes to remove Cd from contaminated soil.

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