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Accumulation of heavy metals in short-rotation willow

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The article draws on the possibilities of using short-rotation coppices for removing heavy metals (Zn, Cu, Pb, Mn, Ni and Cr) from fermented waste filtrate or from soil by phytoremediation.

The aim of the study was to assess heavy metal accumulation in different stem parts of willow (*Salix dasyclados*) in the context of their soilless growing in sludge and grain filtrate and to estimate the influence of the pH of the filtrate and nutrients on their HM accumulation ability and biomass increase. HM concentration in willow biomass was evaluated from the phytotoxic concentrations of these elements in plants as a result of long-term observation.

The study has shown that insignificant differences in the capacity to accumulate heavy metals, recorded in the top, middle and bottom parts of the cuttings, depend on whether it is a ligneous part or leafage. It was determined experimentally that by their absorption capacity HMs can be ranged as follows: Pb > Cu > Cr > Zn > Ni. The data show a linear decrease in the accumulation of heavy metals with increasing filtrate concentration in the solution for 87% of plants, i.e. high concentrations of chemical pollutants cause an inhibitory effect, which was experimentally confirmed by growing cuttings in filtrates.

Key words: accumulation, heavy metals, fermented waste, phytoremediation

INTRODUCTION

According to the requirements of the European Union Landfill Directive, organic waste should be handled as a separate waste flow because of its influence on climate change. The national strategic waste management plan has set state waste management tasks. One of the aims of the strategic waste management plan is to reduce the amount of biodegradable waste in landfills. This requirement should be implemented gradually following the task to reduce waste amounts in landfills (Baltrėnas, 2004; 2005). Other organic waste and products made of it can be applied in agriculture without significant limitations, but the utilisation of organic waste from water treatment is still an unsolved issue because of pollution with heavy metals or other organic and inorganic pollutants.

Phytoremediation is a method of environmental treatment that makes use of the ability of some plant species to accumulate certain elements, including heavy metals, in amounts exceeding the nutrition requirements of plants (Raskin et al., 1997; Ebbs et al., 1998). Considering the possibilities for phytoremediation of waste landfill sites and areas surrounding industrial plants, important are both the ability of species to accumulate high amounts of elements per biomass unit and the possibility of high biomass production over a given time and area (Baltrėnaitė, 2007). This is not always possible for certain plant species, even if we provide optimal growth conditions and assume that high accumulations of cadmium or lead, for example, would not reduce this growth. Therefore, despite numerous studies on plant bioaccumulators (Raskin et al., 1997; Brown et al., 1994; 1995; Tolra et al., 1996; Greger, 1997), it is difficult to state definitely which species could be particularly useful in environmental phytoremediation.

Plants spontaneously growing on natural and anthropogenic grounds reflect their adaptation to the given conditions of growth. Plants fixing the mobile forms of elements "clean up" the surface of the substrate while drying up and stabilizing it. They also play the role of a protective barrier for the surrounding areas (Siuta et al., 1997). Knowledge on the biomass element accumulation capacity, which would enable developing a treatment method for the obtained plant mass, seems to be essential, as is also the assessment of the possibility of ground sanitation by plants. Plants with a particular ability of uptaking elements from the air, water and soil and of their accumulation are considered to be indicators and therefore are called bioaccumulators.

Heavy metals (HM) are important elements in biochemical cycles of plant ecosystems. The majority of HMs are important trace elements in the food chains or plant and

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animal organisms (Bohn et al., 2001). Mn is considered to be an important micronutrient, Zn, Ni, Cu and Cr are toxic HMs; Pb is toxic, and its importance as a nutrient has not yet been determined (Breckle, 1991; Nies, 1999; Karczmarczyk, 2007; Jankauskaitė, 2008). Plants can suffer from HM deficiency in case of low HM concentrations or low biological assimilation (Van Ginneken et al., 2007).

HM toxicity and biological assimilation directly depend on free ions or their labile forms in water. This confirms the hypothesis of free metal ions, which states that the portion of biologically assimilated HMs depends mostly on the amount of free metal ions in soil (Kabata-Pendias, 1993).

Due to a high content of phosphorus, nitrogen and organic carbon in it, the filtrate of organic waste is beneficial for soil enrichment with the nutrients that are necessary for the growth and development of cultural, food and other plants (Weggler-Beaton et al., 2000). Since ancient times, this feature has encouraged people to collect organic waste and use its filtrate as a fertiliser on farming lands, and later on woodlands or lumbering places (Baltrėnas, 2005).

Environmental pollution monitoring performed using indicator plants may help objectively evaluate the ecological situation in the changing environment (Ozolinčius, 2004). Willows have been selected for the research as such bioindicators.

Baker (1981) has distinguished three types of plants, viz. accumulators, excluders and indicators. In accumulator plants, the ratio of the concentration of elements in the plant to that in the soil is $\gg 1$. In excluder plants, metal concentration in aerial parts is maintained low and constant over a wide range of metal concentration in soil, up to a critical value above which the exclusion mechanism breaks down, resulting in unrestricted HM transport and toxicity, the plant / soil concentration factor being <1. In indicator plants, the uptake and transport of metals are regulated in such a way that the ratio of the concentration of elements in a plant to that in the soil is >1. In the present study, we focused on the safe disposal of low metal-contaminated fermentable waste filtrate, as well as heavy metal uptake and accumulation in different parts of willow.

The aim of this work was to assess heavy metal accumulation in different stem parts of willow (*Salix dasyclados*) in the context of their soilless growing in sludge and grain filtrate, and to estimate the influence of the filtrate and nutrients pH on HM accumulation ability and biomass increase. HM concentration in willow biomass was evaluated after long-term observations of the phytotoxic concentrations of these elements in plants.

METHODS

A short-rotation willow coppice was grown in the filtrate of fermented organic waste, and the total concentration of heavy metals was measured at the Vilnius Gediminas Technical University Laboratory of Environment Protection and Working Conditions in July–September 2007. Heavy metal concentrations were measured in the filtrate of fermented waste as well as in willow cuttings after 21 days of growth in the filtrate solutions. Metal concentration was measured in a total of 21 specimens. Quantitative measurements of pH in the filtrates of fermented sewage sludge and grain were taken employing a MultiCal 538 WTW pH meter with a glass electrode. The total phosphorus content in the filtrate was determined by the colorimetric method. The quantitative determination of total nitrogen in the filtrate was performed by the Kjeldahl method (Girgždys, 2000; Kabata-Pendias, 1993; Paliulis, 2004).

Willow of the Swedish cultural species "Gudrun" (*Salix dasyclados*) was selected for this research. Willow seedlings were planted in Dargužiai, Varėna District, in May 2003. These willow plants were cut in December 2006 and kept in a fridge.

Filtrate specimens were taken for research from tanks of anaerobic fermentation, in which sewage sludge and grain waste were fermented. After the fermented mass settled in the tank, the filtrate was taken with a glass flask at the depth of 0–20 cm. The filtrate was poured into a glass vessel and kept there until the beginning of willow research by the method of hydroponics (Kaunelienė, 2000).

Sixty units of willow cuttings, 180–200 mm in length and 9–18 mm in diameter, were taken from different parts of stem for growing them in the filtrate solutions. Willow stems were divided into three parts: I – top , II – middle, III – bottom (Fig. 1). Before analysis, the cuttings had been weighed. 18–20 cuttings were grown in solutions of each type of filtrate (25%, 50% and 100% concentrations) and 20 units in the control solution (35 mg $Ca(NO_3)_2$ per one litre of distilled water) (Gradeckas, 2000; Kaunelienė, 2000).

The willow growing experiment lasted 21 days at an average ambient temperature of 18–20 °C. The cuttings were kept outdoors in a well-lighted place protected against rain. When sunk, the solution was replenished. Upon completing the experiment, the cuttings' mass was recorded (Kaunelienė, 2000). Afterwards, the specimens were dried up to a steady weight at 105 °C, crushed, placed in crucibles and burnt in a muffle furnace at 550 °C for about 3 hours until burnt to ashes. Willow ashes were mineralised in concentrated nitric and saline acid and mineraliser ETHOS Touch control. Pb, Ni, Zn, Cr, Mn and Cu concentrations in mineralised solutions were measured with a BUCK 210 VGP flame atomic absorption spectrophotometer (AAS) (Paliulis, 2004).

The data were analysed using Systistica software (Version 7.0). As the samples were gathered from the same trees on different dates, the differences of treatment from control were tested by the repeated measures analysis (Girden, 1992). The parameter ratios were analysed after log-transformation to unify the scale of variances. The correlation (r) was calculated with MS Excel 9.0. Significances (p) of differences were accepted at the 95% confidence level.

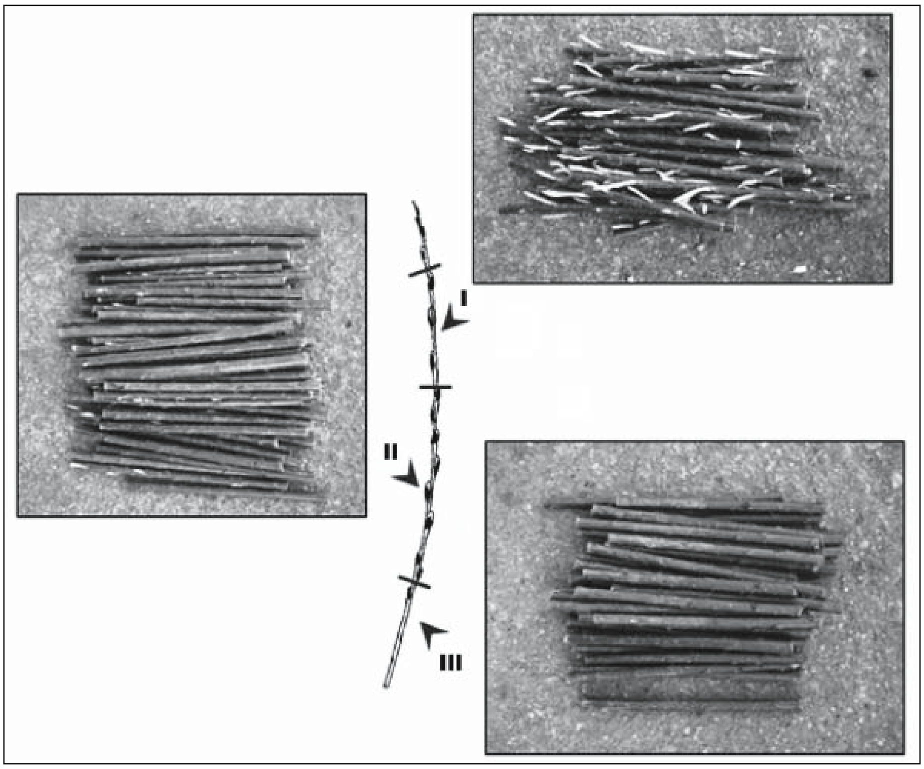

Fig. 1. Willow stem parts: I – top, II – middle, III – bottom

RESULTS AND DISCUSSION

The composition of a fermented organic waste filtrate depends on a number of factors such as the nature of waste, the parameters of its anaerobic treatment, waste keeping conditions and the filtrate's interaction with the environment. Table 1 shows the chemical analysis data on fermented sewage sludge and grain filtrates.

With the aim to evaluate the effect of phosphorus and nitrogen on HM assimilation in the ligneous part of willows, their content in solutions of different filtrates was measured (Table 1).

Figures 2–7 present data on heavy metal concentrations in the tissues of willows grown in different filtrates.

The negative effect of **nickel** is manifested as the chlorosis of plant leaf veins; the leaves acquire a greyish green colour and the roots become grey and weak. Very small amounts of Ni were detected in the tissues of all the cuttings, showing the smallest quantity of all the elements in question. The analysis of willow tissues affected by the filtrate of sewage sludge and grain substrates fermented under laboratory conditions showed that the willows accumulated Ni most efficiently in 25% filtrate solutions (Fig. 2). With increasing filtrate concentrations, heavy metal concentrations in all parts (top, medium and bottom) of willow cuttings that grew in solutions showed a linear decrease. The highest content of Ni was determined in the top part of willow cuttings; it was about twice as high as in the tissues of control willows.

The ratio of the analysed heavy metal concentrations in the tissues of willows that grew in 50% and 25% filtrate and control solutions was different. In many cases, this ratio for Ni did not exceed 2, showing that metals are accumulated only up to a certain limit of biological saturation. Also, HMs were not accumulated in willow tissues when the bottom parts of willow cuttings were grown in the sewage sludge filtrate solution and when all parts of cuttings were grown in the grain filtrate solution. The highest levels were twice as high as the lowest ones when the top parts of willows were grown in both types of filtrates; the difference was 1.5 and 1.4 times in the sewage sludge and grain filtrate, respectively, in case of the middle part, and 1.4 and 1.7 times in case of the bottom part. The capacity to accumulate smaller amounts of HMs or not to accumulate them at all could be predetermined by both the acid medium of the grain filtrate and too high concentrations of HMs and other organic pollutants (N_b = 147.3 mg/l and P_b = 7.02 mg/l). Ni levels did not exceed the established willow cutting phytotoxic concentrations which for this metal vary from 10 to 100 mg/kg.

Willow accumulated the biggest amounts of **zinc** (Fig. 3). This is natural as much more of this element is present in

Table 1. **Data of chemical analysis of the fermented waste filtrates in which willows were grown**

Filtrate sample	Mn, mg/l	Zn, mg/l	Cu, mg/l	Cr, mg/l	Pb, mg/l	Ni, mg/l	N_b, mg/l	P_b, mg/l	pH
Sewage sludge	0.138	0.485	0.122	1.224	0.541	0.079	246.2	4.66	6.96
Grain	0.266	0.755	0.075	0.185	0.09	0.079	147.3	7.02	3.66

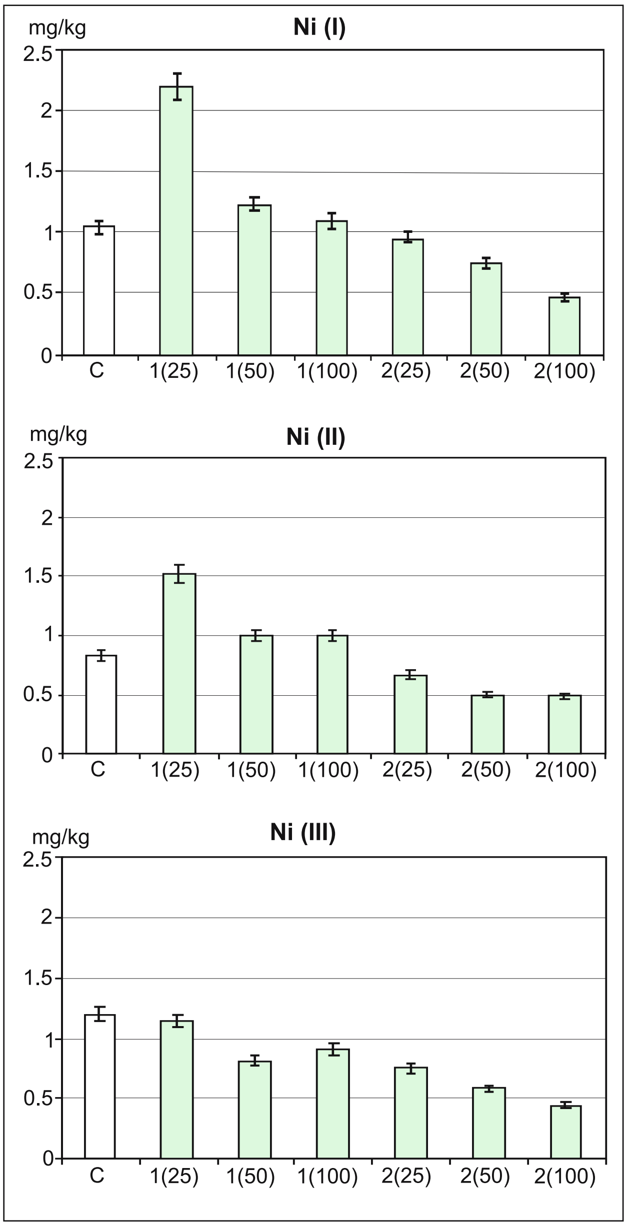

Fig. 2. Average Ni concentrations in the tissues of willow top (I), middle (II) and bottom (III) parts grown in control (C), sewage sludge (1) and grain (2) filtrates of different concentrations (25%, 50% and 100%)

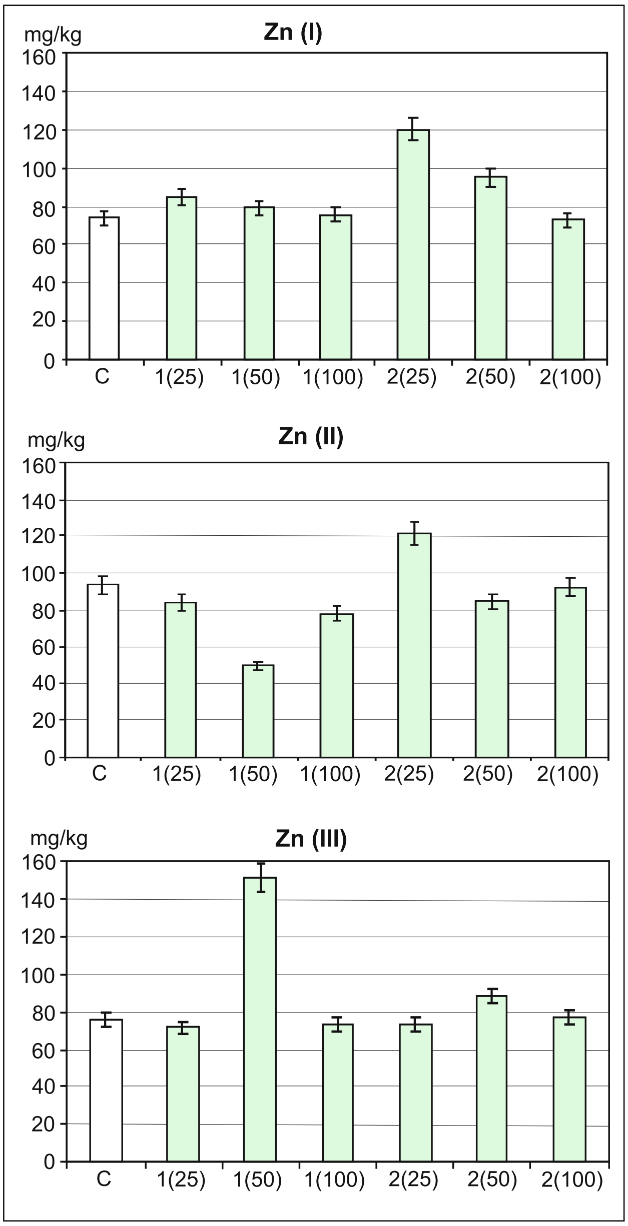

Fig. 3. Average Zn concentrations in the tissues of willow top (I), middle (II) and bottom (III) parts grown in control (C), sewage sludge (1) and grain (2) filtrates of different concentrations (25%, 50% and 100%)

the environment compared to other heavy metals that environment protection specialists show interest in. Zinc levels in the grain filtrate were also higher than in the sewage sludge filtrate (see Table 1).

The research has proved that in the middle and bottom parts of willow cuttings, Zn concentrations in the 25% solution decreased and in the bottom part increased. The same tendency was determined for both filtrate solutions. This allows establishing the best growth conditions for willow cuttings as well as fertilisation rates. In addition, zinc is characterized by a low phytotoxicity as well as weak carcinogenic and mutagenic properties (Mandre et al., 1999; Claus, 2007). Zn levels phytotoxic to plants, according to the literature (Kabata-Pendias et al., 1993), range from 100 to 4000 mg/kg.

Mobile **chromium** accounts for 10–20% of its total amount in soil. The maximum permissible concentration of total chromium in soil is 100 mg/kg. Especially hazardous are bivalent and trivalent chromium compounds (Kadūnas et al., 1999). Higher chromium concentrations cause chlorosis in young plant leaves and impede root growth. Chromium is ascribed to toxicity class II and is carcinogenic (Алексеев, 1987, Kadūnas et al., 1999). Its concentrations in control willow cuttings did not exceed 0.91 mg/kg (Fig. 4). Our findings show that with increasing filtrate concentrations in the solutions, Cr levels show a linear decrease, except the middle part willow cuttings in sewage sludge filtrate solutions. A deficient Cr concentration was determined in the same filtrate of 50% sewage sludge filtrate concentration, which was by

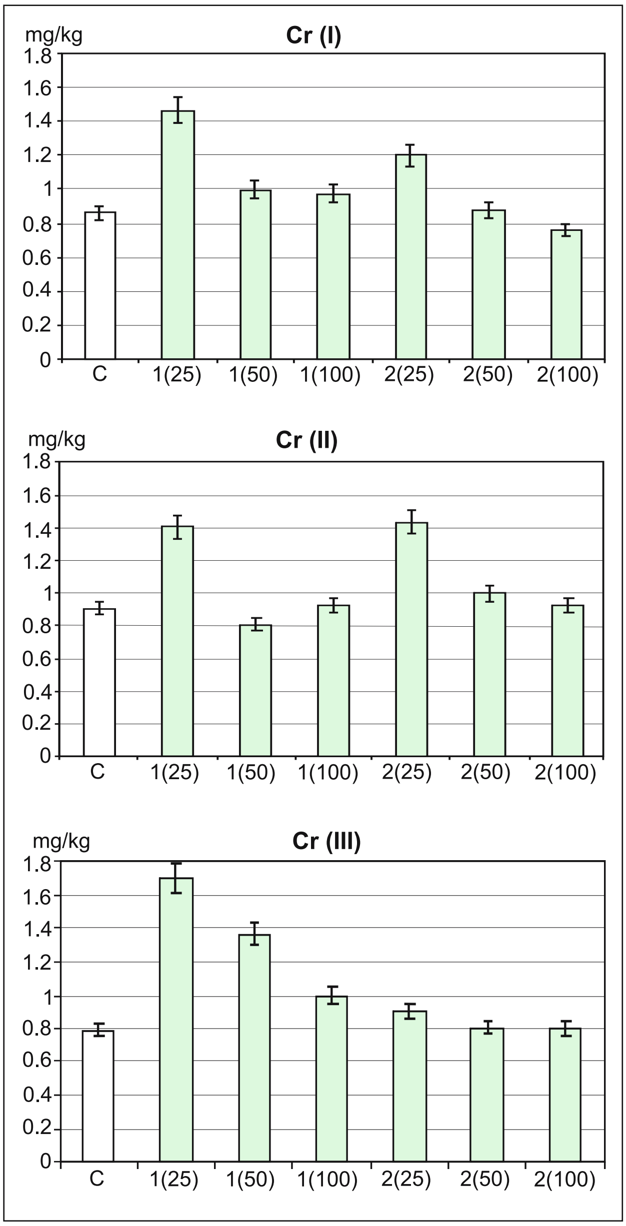

Fig. 4. Average Cr concentrations in the tissues of willow top (I), middle (II) and bottom (III) parts grown in control (C), sewage sludge (1) and grain (2) filtrates of different concentrations (25%, 50% and 100%)

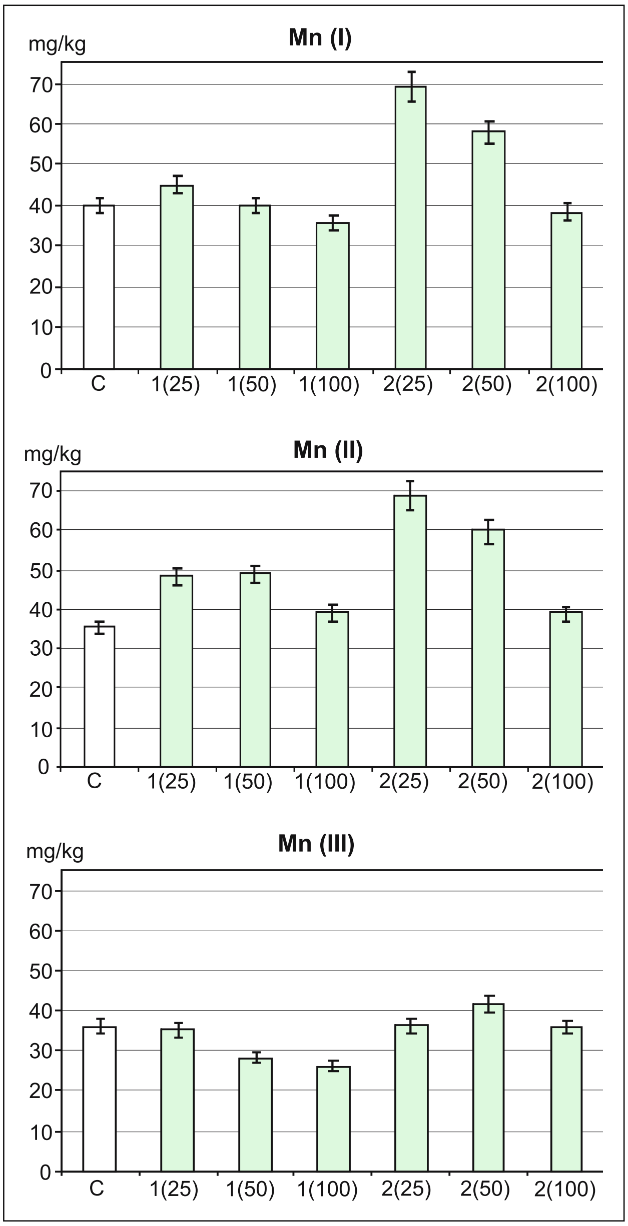

Fig. 5. Average Mn concentrations in the tissues of willow top (I), middle (II) and bottom (III) parts grown in control (C), sewage sludge (1) and grain (2) filtrates of different concentrations (25%, 50% and 100%)

0.11 mg/kg lower than the control one. The accumulation of this metal also decreases to a deficit level when the top parts of willow cuttings are grown in a 100% grain filtrate. A large content of chromium was accumulated in the cuttings of willow bottom part in which the highest concentration (1.7 mg/kg) was recorded in the 25% sewage sludge filtrate, i. e. 2.2 times more compared to the control specimen. Very low Cr levels were recorded in tissues of all the cuttings, showing the smallest content of all the elements in question.

The average **manganese** concentration determined in this research reached 37.7 mg/kg in control tissues (Fig. 5). The highest levels were determined in the top and middle parts of the cuttings in the grain solutions at the concentrations of 25 and 50% and were the same for both types of parts. The capacity of willows to accumulate Mn in solutions of the sewage sludge filtrates was not intensive, while the highest concentrations were no more than 1.3 times higher than the levels recorded for the cuttings in control solutions. Mn deficit was manifested in the top and bottom parts of willow cuttings in 100% sewage sludge solutions.

Manganese phytotoxic concentrations are often not determined because it is not considered as a toxic metal, but its participation in the fusion process may change the migration of other metals.

Copper (Cu) is a heavy metal of low chemical activity, which enters the environment with waste water, phosphoric fertilisers, limy materials, organic fertilisers and pesticides. It is one of the 20 elements mandatory for plant nutrition,

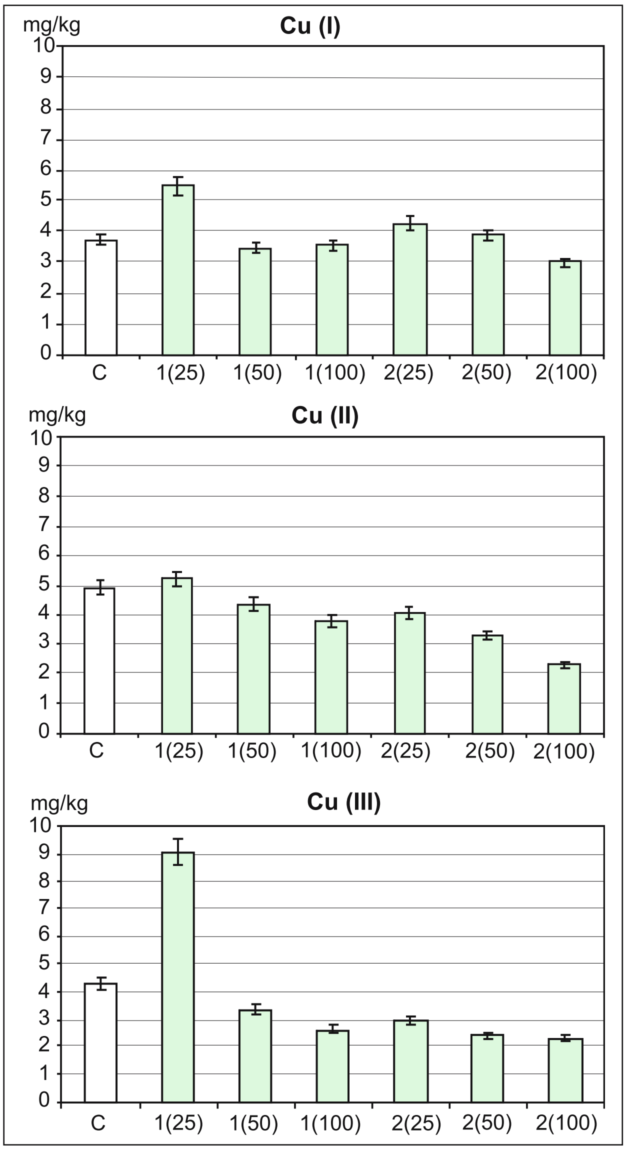

Fig. 6. Average Cu concentrations in the tissues of willow top (I), middle (II) and bottom (III) parts grown in control (C), sewage sludge (1) and grain (2) filtrates of different concentrations (25%, 50% and 100%)

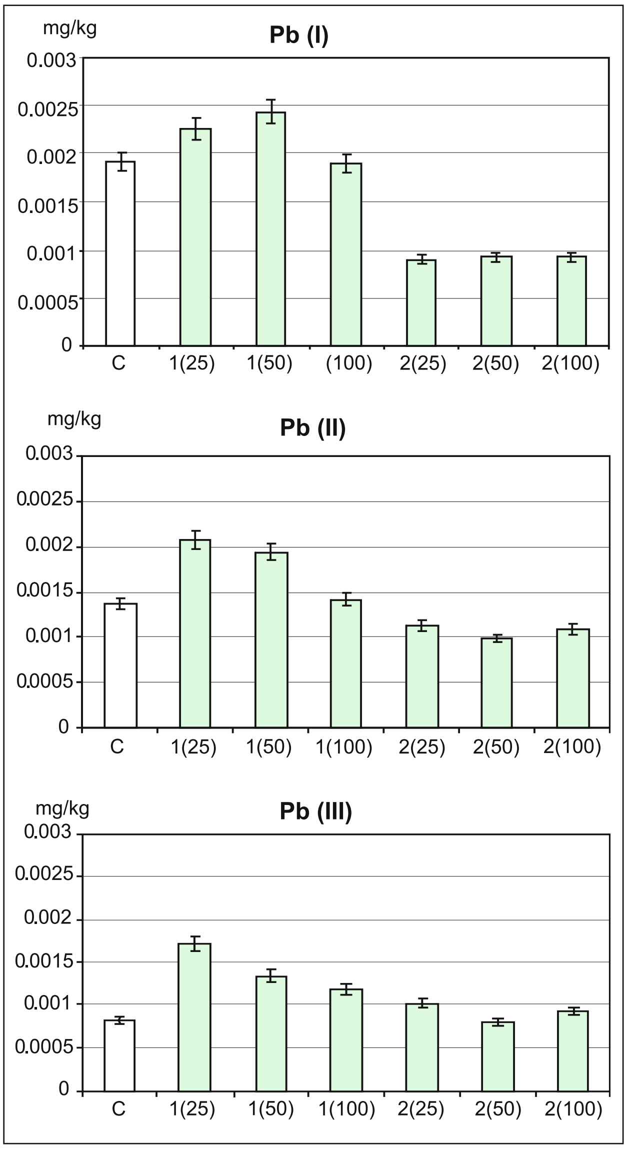

Fig. 7. Average Pb concentrations in the tissues of willow top (I), middle (II) and bottom (III) parts grown in control (C), sewage sludge (1) and grain (2) filtrates of different concentrations (25%, 50% and 100%)

therefore, if it is absent in soil, plants rot away and in case of its deficit become sick. High copper concentrations in soil cause plant vein chlorosis, and lateral sprouts become brown (Gradeckas, 2000; Mažvila, 2001).

Cu concentration in the sewage sludge filtrate is rather high (Table 1). Most probably, the filtrate contains Cu forms that are difficult for plants to assimilate (Fig. 6). Cu concentrations in the wood of willows fertilised with sewage sludge and of control willows was up to tree times higher compared with willows that grew in the grain filtrate.

The highest Cu concentration was recorded in the tissues of willow bottom part and reached even 9.05 mg/kg, which is 3.44 times higher than the control level. This can be explained by the optimum concentration of nutrients in the 25% filtrate solution compared to other solutions where willow cuttings were grown. A similar effect was recorded by other researchers (Kaunelienė, 2000; Gradeckas, 2000). An especially strong inhibitory effect was recorded in 100% filtrate solutions: willow cuttings looked poor, sprouts started withering, and roots were short and brown.

Lead (Pb), like copper, showed a low assimilation by willow tissues (Fig. 7). The phytotoxic concentrations of this metal range from 30 to 300 mg/kg (Kabata-Pendias, 1993). The highest intensities of accumulation were recorded for sewage sludge filtrate solutions, while the grain solutions produced deficit concentrations which were close to those of control cuttings, except for cuttings of the top (I) part. The difference between the highest and the lowest concentrations in each part of cuttings grown in different filtrates did not exceed 1.5 times. It can be naturally concluded that assimilation from filtrate is more intensive than from soil. Humus efficiently binds heavy metals in soil (Gradeckas, 2000).

It was determined experimentally that by their absorption capacity HMs can be ranged as follows: Pb > Cu > Cr > Zn > Ni. The absorption capacity of these elements is predetermined by the amount of biogenic substances, while cation sorption receptivity, the pH of the medium and the content of organic matter are of less importance (Gupta, 1997). Gradeckas (2000) and Kaunelienė (2002), who studied the capacity of several willow species fertilised with sewage sludge to accumulate heavy metals, presented a different sequence of concentrations in sprouts by the index of biological receptivity: Zn, Cu, Pb, Cr, Ni.

In the present study, the **concentration factor** (CF) of HM was expressed as the ratio of HM concentration in willow samples grown in fermented waste filtrate and in the control filtrate (Tables 2 and 3). The CF values were below unity, i.e. no accumulation of heavy metals from the filtrate was noted.

HM accumulation according to the CF in willow cuttings kept in sewage sludge filtrate may be shown in the following order: the top part cuttings (I) – Ni > Cr > Cu > Pb > Zn > Mn; middle part (II) – Mn > Cr > Zn > Cu, bottom part (III) – Cu > Cr, while in the filtrate from fermented grain: the top part cuttings (I) – Mn > Zn > Cr > Cu; middle part (II) – Mn > Cr > Zn > Cu; the bottom part cuttings accumulated Pb and Zn only.

It is known that according to the CF it is possible to select a plant species to clean contaminated soil (Zayed et al., 1998; Marchiol et. al., 2004). The higher mean CF values for Cu were found in bottom willow (III) parts growin in 25% sewage sludge filtrate in which Cu concentration was 2.4 times higher than in the control samples (Fig. 6). Also, the high CF value was determined for Ni, Pb and Cr (respectively 2.1 and 2.0) in the filtrate mentioned above. The absorption of trace amounts is believed to depend on iron oxides and clay content, which are less susceptible to the sorption of cations, to the pH value and organic matter content (Eitminavičiūtė, 2000; Ginneken, 2007). Other kinds of biological susceptibility of heavy metals were defined by Gradeckas (2000) and Kaunelienė (2002) in willow shoots. In their opinion, heavy metals can be removed from filtrates of landfill and sewage sludge in the following order: Zn, Cu, Pb, Cr, Ni. They studied some willow species, fertilized with landfill and sewage sludge filtrate, for the ability to accumulate heavy metals.

Table 2. **HM concentration factor (CF) in willow samples of control (C) and 25%, 50%, 100% filtrate solutions (1 – sewage sludge, 2 – grain)**

HM ratio in willow parts from different filtrate	Ni	Zn	Cr	Mn	Cu	Pb
I – top part						
C/1(25)	2.1	1.1	1.7	1.1	1.5	1.2
C/1(50)	1.2	1.1	1.2	1.0	0.9	1.3
C/1(100)	1.0	1.0	1.1	0.9	1.0	1.0
C/2(25)	0.9	1.6	1.4	1.7	1.1	0.5
C/2(50)	0.7	1.3	1.0	1.5	1.0	0.5
C/2(100)	0.4	1.0	0.9	1.0	0.8	0.5
II – middle part						
C/1(25)	1.8	0.9	1.6	1.2	1.4	1.1
C/1(50)	1.2	0.5	0.9	1.2	1.2	1.0
C/1(100)	1.2	0.8	1.1	1.0	1.0	0.7
C/2(25)	0.8	1.3	1.7	1.7	1.1	0.6
C/2(50)	0.6	0.9	1.2	1.5	0.9	0.5
C/2(100)	0.6	1.0	1.1	1.0	0.6	0.6
III – bottom part						
C/1(25)	1.1	1.0	2.0	0.9	2.4	2.1
C/1(50)	0.8	2.0	1.6	0.7	0.9	1.7
C/1(100)	0.9	1.0	1.1	0.7	0.7	1.4
C/2(25)	0.7	1.0	1.0	0.9	0.8	1.3
C/2(50)	0.6	1.2	0.9	1.0	0.6	1.0
C/2(100)	0.4	1.0	0.9	0.9	0.6	1.1

Table 3. **HM concentration factor (CF) in willow samples grown in 25% and 50% filtrate solutions (1 – sewage sludge, 2 – grain)**

HM ratio in willow parts from different filtrates	Ni	Zn	Cr	Mn	Cu	Pb
I – top part						
1(25)/1(50)	1.8	1.1	1.5	1.1	1.6	0.9
1(50)/1(25)	0.6	0.9	0.7	0.9	0.6	1.1
2(25)/2(50)	1.3	1.3	1.4	1.2	1.1	1.0
2(50)/2(25)	0.8	0.8	0.7	0.8	0.9	1.0
II – middle part						
1(25)/1(50)	1.5	1.7	1.8	1.0	1.2	1.1
1(50)/1(25)	0.7	0.6	0.6	1.0	0.8	0.9
2(25)/2(50)	1.3	1.4	1.4	1.2	1.2	1.1
2(50)/2(25)	0.7	0.7	0.7	0.9	0.8	0.9
III – bottom part						
1(25)/1(50)	1.4	0.5	1.2	1.2	2.7	1.3
1(50)/1(25)	0.7	2.1	0.8	0.8	0.4	0.8
2(25)/2(50)	1.3	0.8	1.1	0.9	1.2	1.3
2(50)/2(25)	0.8	1.2	0.9	1.2	0.8	0.8

The highest CF values were determined for Cu (2.4), Ni, Pb (2.1), Zn (2.0), Cr (2.0) in willow grown in sewage sludge filtrate and for Mn (1.7) grown in grain filtrate. Assessing the ability of HM accumulation in two filtrate types, we found that 61% of samples had accumulated Pb, 39% – Ni, 50% – Cu, 67% – Mn, 78% – Zn and Cr. The accumulation of all HMs was most intensive from sewage sludge filtrate and ranged from 56% to 89% for all collected samples, while accumulation from grain filtrate was less intensive: Zn, Cr and Mn were accumulated by over 50% of the samples, while no accumulation of Ni and Pb was found. As expected, there were some differences in the heavy metal CF in all three parts of willow stems grown in the 25% sewage sludge filtrate solution. Willow stems kept in other solutions accumulated HM in amounts that did not differ by more than 30% and therefore were not further evaluated.

Accumulation of Zn, Mn and Pb from the sewage sludge filtrate almost did not differ, while for other metals an unrelated accumulation was determined. The adsorption of Ni in willow top part (I) was 100%, in the middle part of stem (II) 87%, and in the bottom part (III) 52%, i. e. almost half as low as in part I.

Chromium was intensively accumulated in the bottom (III) part (100%), while 80% and 85% of the HM volume accumulated respectively in the middle and top parts of willow cuttings. The accumulated level of copper in willow part I comprised 63% compared with part III (100 %). The middle part of willow cuttings accumulated almost two times less Cu (58%). This can be explained by the mass gain of leaves, wood and root in each part of willow. Some authors (Kaunelienė et al., 2002) argue that under hydroponic cultivation of willow in laboratory conditions, metal concentration ratio in willow grown in the 50% filtrate and in control wood ranged from 1.2 to 7.8 and in roots from 4.6 to 31.3. Kaunelienė and others (2003) found the highest HM differences between willow grown in field conditions and control plants (1.3–2.1 times). These differences were not statistically significant in shoots, while the largest difference was found in roots (1.5 times). Gradeckas and others (2000) studied heavy metal levels in fertilization sewage sludge and willow tissue and found that in roots of fertilized willow grown in a peat bog the HM ratio ranged within 1.5 – 3.9 compared with control. The difference was probably due to two reasons: a relatively high concentration of heavy metals in the soil and the relatively small quantities of sewage sludge filtrate spill.

Biomass. After the first week of the experiment, all the cuttings in solutions differed slightly. At the end of the second week, differences among the cuttings were revealed. Cuttings showed a slower growth in 100% filtrate solutions. At the end of the third week, under the impact of HM, the growth inhibition of cuttings grown in the 100% and 50% filtrates was observed. At the end of the experiment (on day 21), the strapping mass of cuttings grown in the 100% sewage sludge solution was by 78% lower than of the control cuttings and by 70% lover than that of cuttings grown in grain solution. Meanwhile, the biomass of cuttings grown in the 50% sludge filtrate and grain filtrate solutions differed by 62% and 56%, respectively. The maximum increase of biomass was determined in the 25% sewage sludge filtrate and grain solutions and reached 55% and 60%, i.e. by 45% and 40% less compared to the control cuttings. Thus, best tolerated was the 25% concentration of the filtrate. Kaunelienė and others (Kaunelienė et al., 2003) recommend to repeat the experiment with deep-rooted willow to show its ability to tolerate higher concentrations of filtrate solutions. Gradeckas (1997) analysed the effects of different methods of sewage application on willow growth, including biomass gain. With sewage sludge inserted into the soil, the accumulated biomass of the first year was less than 36% compared with the control. However, the deeper root system developed in the first year led to the highest biomass during the second year (17% above the control version), in which sewage sludge was spread on the soil surface. All these data indicate that wastewater sediments favour willow rooting and growth. However, the best conditions, in Gradeckas' opinion (Gradeckas, 1997), for rooting and the growth of the growth of willow are ensured by rollers 105 cm wide and 30 cm high every 3 m. In this variant, the biomass increment was 50%, and within two years it also remained the highest (21% above the control variant). In the second year, the different methods of spreading sewage sludge had a less effect on willow growth. The effect of fertilization with sewage sludge on willow growth can be seen in not fertilized variants: the accumulated biomass was only 6% compared with the control (not fertilized) variant.

In order to assess the impact of biogenic substances (phosphorus and nitrogen) and pH value, we established a correlation between these parameters (Fig. 8).

Biogenic substances. Studies in Scandinavia and in other European countries show that the mean annual biomass increase during the first 10 years is reducible to an average of 5–6%. Thus, plant productivity is mainly caused by nitrogen losses and the negative balance of the base cations, which is associated with logging waste export from forest (Karczmarczyk, 2007; Shilev et al., 2007). It is believed that the liquidation of all forest waste-crossing triggers a negative impact on forest ecosystems by decreasing the quantity of litter fall, thinning the leaf litter, gradually decreasing organic matter and carbon content in the soil, decreasing soil fertility, weakening nitrate formation and elution, changing the moisture conditions, increasing soil acidity, changing vegetation species composition, increasing weediness, changing the microclimate, soil temperature and thus may increase the vegetation period and reduce the productivity of trees (Ginnecken, 2007; Nies, 1999; Greger, 1997). Many of us are aware of the need to fertilize our gardens and lawns, but we frequently forget that trees also benefit from fertilization. Like all plants, trees need sunlight, water, air, and certain mineral nutrients for normal growth. Under forest conditions, the annual fall of leaves and twigs and their eventual decomposition provide a fresh source of nutrient substances. However, trees in lawns or similar areas are usually denied this source of soil enrichment since most homeowners gather up these leaves. Over a prolonged period of time, this practice can lower the fertility of soil.

Organic waste filtrate, rich in phosphorus, nitrogen and organic carbon content, is useful for soil enrichment in nutrients and is necessary for cultural and other plants to grow and develop (Weggler-Beaton et al., 2000). A correlation was established between willow biomass increment and the content of biogenic substances in the filtrate (Fig. 8). The research showed a strong linear correlation between biomass growth and the total nitrogen concentration of the filtrate solutions. The correlation coefficient for these data was 0.8987 ($p = 0.05$). The poor growth of biomass was influenced by the level of phosphorus in the filtrate, and the correlation was not too close ($r^2 = 0.5907$, $p = 0.05$).

The assessment of biogenic substances in the grain filtrate depending on the total HM accumulated in the top part of willow showed an $r^2 > 0.5$ correlation with all HM except Ni and Pb. A strong correlation was determined only for two metals (Mn and Cr) in the middle part of willow cuttings.

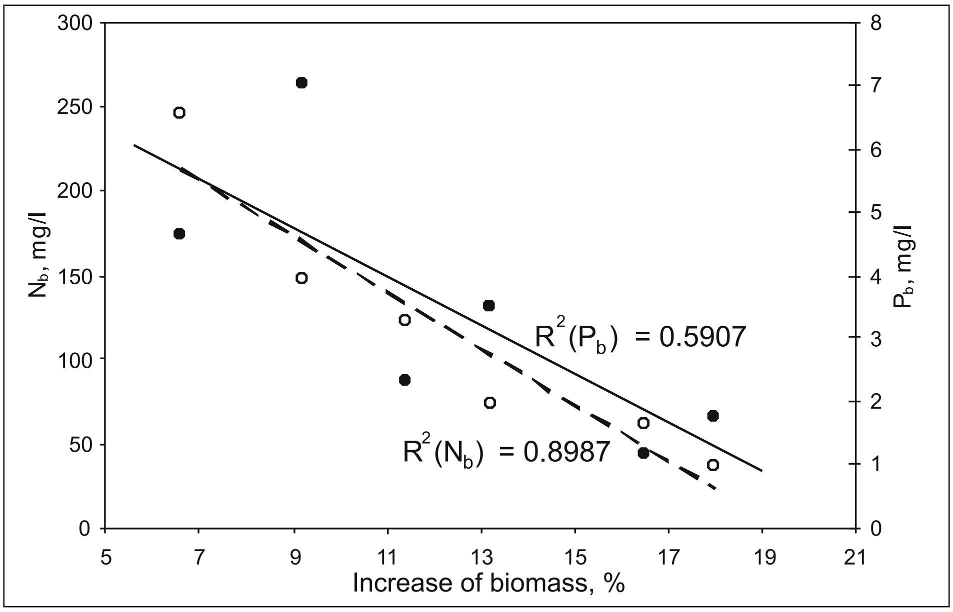

Fig. 8. Correlation between total nitrogen (N_b) and phosphorus (P_b) in fermented waste filtrate and biomass increase

The bottom part of willow cuttings was not evaluated for the correlation value because the HM concentration factor was below unity (Table 2).

pH value. One of the most important control factors of HM transport from soil is soil pH, in this particular case the filtrate pH (Table 1). According to Singh and Abrol (1985), most active HM adsorption processes occur when the soil medium pH is up to 6.0 and when it ranges within 6.0 – 7.9. The solubility of HMs is controlled not only by the processes of adsorption, but also by HM sedimentation. Soil pH value affects not only the content of HM in the soil, but also their movement. The value of Mn mobility is critical when the pH value is maintained at 5.6 to 6.0, of Zn – pH < 5.0–5.5, of Cu – pH < 4.5–5.0, and of Pb – pH < 4.0 (Baltakis, 1993). Therefore, the most favourable conditions would be to accumulate Mn, Zn and Pb from sewage sludge filtrate and Mn, Zn, Cu and Pb from grain filtate (Table 1). The results of our experiment showed that the previous rule for the sewage sludge filtrate was confirmed in full but for grain filtrate only in part, because zinc was found to show a weak correlation ($r^2 = 0.4447$, $p = 0.05$), and Cu was accumulated only in a very small quantity (CF = 1.1), and therefore the reliability of these data may be also doubtful.

The obtained findings should be evaluated with regard to the fact that metals could have entered wood not via roots but directly because of capillary phenomena as willow cuttings were directly dipped in landfill filtrate. The findings allow us, at least in part, reject the assumption that metals directly access wood. The concentrations of many of the metals in the tissues of willows grown in solutions of different concentrations did not differ much. In many cases, the ratio of metal concentrations in willow tissues grown in 50% and 25% filtrate solutions did not exceed 1.5. It is likely that the inhibitory effect of 50% and 100% filtrate solutions on the growth of cuttings predetermined the lower HM assimilation from these solutions. On the other hand, a certain limit of saturation could have been approached. Another fact worth mentioning is that the background content of heavy metals in ligneous parts of willow plants grown in control solutions did not differ much from that of cuttings grown in polluted solutions. The research findings show that the transport of heavy metals into willow tissues is a complicated process; it is individual for every metal. It can be assumed that metal access to willow tissues depends mostly on the compounds they are present in.

CONCLUSIONS

According to the values of the highest concentration factor, the heavy metals studied could be ranked as follows: Pb > Cu > Cr > Zn > Ni. Cuttings of willow stem top part best accumulated all heavy metals, except Ni and Pb from the grain filtrate and Mn from the sewage sludge filtrate.

Metal accumulation in willow tissues greatly depends on the compounds in which metals are present, and their transport to willow tissues is a complicated process individual for every metal.

The insignificant differences in the capacity to accumulate heavy metals recorded in the top, middle and bottom parts of the cuttings depend on whether it is a ligneous part or leafage.

Ni, Cr and Pb were intensively accumulating in the middle part of willow cuttings grown in the fermented sewage sludge filtrate, while zinc was the most difficult to assimilate. In willows grown in grain filtrate, mainly Cr and Mn were accumulated. The deficit concentrations were determined for Ni, Cu and Pb.

The most favourable conditions to accumulate Cr and Pb in the ligneous bottom part of willow stems were provided by the medium of fermented sewage sludge filtrate, while conditions for Ni and Cu accumulation were unfavourable in the solutions of both filtrates at different concentrations.

Heavy metals could be removed from sewage sludge filtrate in the following order: for the top part cuttings (I) – Ni > Cr > Cu > Pb > Zn > Mn, middle part (II) – Mn > Cr > Zn > Cu, bottom part (III) – Cu > Cr, while from the grain filtrate, for the top part cuttings (I) – Mn > Zn > Cr > Cu, middle part (II) – Mn > Cr > Zn > Cu. For the bottom part cuttings, no accumulation of heavy metals from filtrates was observed.

The highest CF values were determined for Cu (2.4), Ni (2.1), Zn (2.0), Cr (2.0) in willow grown in sewage sludge filtrate, and for Mn (1.7) in grain filtrate.

The data showed a linear decrease in the accumulation of heavy metals with increasing filtrate concentration in the solution for 87% of plants, i.e. high concentrations of chemical pollutants caused an inhibitory effect.

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SUNKIŲJŲ METALŲ KAUPIMASIS SPARČIAI AUGANČIUOSE GLUOSNIUOSE

Santrauka

Straipsnyje įvertintos galimybės naudoti sparčiai augančius miško želdinius sunkiesiems metalams (SM) – Zn, Cu, Pb, Mn, Ni ir Cr iš fermentuotų atliekų filtrato arba iš dirvožemio valyti fitoremediacijos būdu.

SM koncentracijos gluosnių (*Salix dasyclados*) biomasėje buvo vertinamos pagal eksperimento metu gautus SM kaupimosi gebos lygius bei ilgais stebėjimais paremtas fitotoksines SM koncentracijas augaluose. Tyrimų metu siekta įvertinti filtratų pH bei biogeninių medžiagų įtaką SM kaupimosi intensyvumui ir biomasės prieaugiui.

Paaiškėjo nedideli skirtumai tarp viršutinės, vidurinės bei žemutinės dalies auginių gebos kaupti sunkiuosius metalus dėl to, ar tai yra labiau sumedėjusi dalis, ar lapija. Nustatyta, kad SM pagal sugertį gali būti išdėstyti tokia tvarka: Pb > Cu > Cr > Zn > Ni. Duomenys rodo, kad 87 % augalų nustatyta tiesinis sunkiųjų metalų kaupimosi sumažėjimas didėjant filtrato koncentracijai, t. y. didelės cheminių teršalų koncentracijos, sukeliančios inhibicinį poveikį, kuris pasitvirtino pasibaigus auginių auginimo filtratuose eksperimentui.

Raktažodžiai: fermentuotos atliekos, fitoremediacija, kaupimas, sunkieji metalai