Environment Protection Engineering

Vol. 44 2018 No. 3

DOI: 10.37190/epe180310

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EVALUATION OF THE CONTENT OF TRACE ELEMENTS IN THE AERIAL AND UNDERGROUND BIOMASS OF PERENNIAL GRASSES OF THE GENUS *MISCANTHUS*

The content of lead, zinc, copper, nickel and chromium in the aerial and underground parts of *M. sinensis* from eleven years old plantation and *M. sacchariflorus* and *M. giganteus* from nine years old plantations were analysed in order to recognize what organs of the plant play the most important function as a metal accumulator. It was found that in the aboveground parts, lead, zinc and copper were accumulated mostly in leaves and nickel and chromium in stems of the studied species. In underground plant parts, especially in roots, zinc, copper and nickel were most abundantly accumulated, while rhizomes accumulated higher amounts of lead and chromium. The content of lead, zinc and copper was definitely lower in those plant organs than their content in soil. The content of nickel and chromium, on the other hand, showed the opposite dependence. A similar capacity for uptaking trace elements from soil was observed for *M. sacchariflorus* and *M. giganteus*, while *M. sinensis* it was much lower, which is confirmed by the values of the bioaccumulation factors. The translocation factor for trace metals in the studied grass species indicated great translocation of lead and nickel from the roots to rhizomes, and that of zinc to aboveground parts.

1. INTRODUCTION

Trace elements which occur in plants in inconsiderable quantities are essential for their adequate growth and development. Some of them, e.g., lead, zinc or chromium, occurring at elevated concentrations, are toxic to living organisms. They also deteriorate the soil quality and physiological activity of plants [1–3]. A natural source of those

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elements in soil is parent material; they are released and made available to plants and, usually in such quantities (form), they pose no threat to living organisms [3–5]. A total amount of trace elements in soil exceeding the norms is most often due to pollution of the environment with exhaust gases, industrial dusts or crop protection chemicals [5– 7].

The availability of trace elements to plants mainly depends on soil reaction, texture, sorption properties as well as the content of organic matter [8, 9], while the amount of heavy metals uptaken by plants is considerably affected by their form and total content in soil. The interaction of metal ions stimulates or inhibits the process of their uptake by plants [2, 10]. In the areas polluted with heavy metals, it is recommended to grow non-consumption crops, e.g., crops used for energy purposes with a high phytoremediation potential [11, 12]. Due to their well-developed root system, even up to 2.5–3.0 m deep down the soil profile, they are considered to be the so-called bioaccumulators.

A group of such plants covers the plantation of grasses of the genus *Miscanthus* [13–15]. Due to considerably low ecological and fertilization requirements as compared with other crops, the plants are quite environment-friendly [11]. They tolerate a wide variation in soil and in climate conditions [16]. As grasses of C-4 type, they are capable of an effective use of elements, which leads to a reduction of greenhouse gasses emissions [14]. They are considered extensive crops, limiting the process of steppe encroachment and contamination of ground waters [11,16]. Establishing long term energy crop plantations is a method of reclamation of soil polluted with chemicals, which complies with the provisions of *Law on Preventing Environmental Damage and its Remediation* [5].

The aim of the research was to track down the process of an uptake and accumulation of selected trace elements in the system of soil – root, rhizomes – aerial parts of grasses of the genus *Miscanthus*, for the recognition of plant organs most effective as accumulators of metals from contaminated soils.

2. MATERIALS AND METHODS

The research has been performed in the Botanic Garden of the Plant Breeding and Acclimatization Institute (IHAR – PIB) in Bydgoszcz. Multi- annual collections of three grass species representing the genus *Miscanthus* were evaluated. The experiment did not consider the genotypic features of grasses. The seedlings for the *M. sinensis* (Thunb.) Anderss plantation set up in 2001 were derived from a collection of the Institute of Plant Genetics of the Polish Academy of Sciences (PAN) in Poznań. The material for plantings of *M. sacchariflorus* (Maxim) Benth was taken from a collection held by IHAR in Bydgoszcz and *M. giganteus* – from a laboratory of VITROPLANT in Klein Wanzleben, Germany. The research areas of the latter ones were established in 2003. Each year the plant species (research objects) were fertilized with mineral fertilizers:

N (ammonium nitrate) – 88 kg·ha^{-1}, P (superphosphate) – 64 kg·ha^{-1}, K (potassium salt) – 96 kg ha^{-1}.

On the grass plantations under study, in autumn 2012, namely 9 and 11 years after their establishment, aboveground parts were randomly sampled from ten sampling points, in total from the area of 1 m^2. The samples were separated into stems and leaves to determine their dry weight expressed in kg·m^{-2}, whereas the samples of underground parts were separated from soil in a form of soil monolith, each from the area of 500 cm^2 down to 30 cm deep. Plant material was separated from the soil and soil debris by hand, using tweezers. Plants were separated into leaves and stems (aerial part) and roots from two depths (0–15 and 15–30 cm) and rhizomes – underground parts. Plant material was washed gently in tap water and then in distilled water. The biomass was dried at 60 °C to reach air dry matter. Plant material was ground in a laboratory grinder type WZ-IS. The soil samples were sieved through a 2 mm diameter sieve. The total contents of selected trace metals (Pb, Zn, Cu, Ni and Cr), separately in plant leaves, stems, rhizomes and roots from two depths as well as in soil, were assayed by the atomic absorption spectrometry (AAS) with a spectrometer PU 9100X, following a preliminary mineralization applying the microwave technique in concentrated HNO_3. Microwave mineralization was performed at five stages with varing mineralization time and microwave power. Granulometric composition of soil was determined by the Cassagrande method modified by Prószyński. pH was measured by the potentiometric method in H_2O (1:2.5 ratio) and in KCl solution at the concentration of 1 mol·dm^{-3} (1:2:5 ratio). Total organic carbon content (TOC) was determined by the sulfochromic wet oxidation method in the potassium dichromate solution [17–19].

The research objects were located on sandy loam soils [19] with a low humus content (0.8–1.2%). The soils reaction for surface horizons (0–15 cm) was as follows: pH_{H_2O} 7.24 and pH_{KCl} 7.20 as well as sub-surface horizons (15–30 cm) – pH_{H_2O} 6.35 and pH_{KCl} 5.88. Base saturation in the soils accounted for 96%.

To illustrate the effect of the content of the microelements in soil on their content in the grasses analyzed, the bioaccumulation factor (*BF*) was determined to define the ratio of the element content per plant to its content in soil and to assay the mobility of microelements in the plants, the translocation factor (*TF*) was determined to define the content of microelements in aboveground parts to their content in roots [20, 21]. Due to the formation of rhizomes in those grasses, the translocation factor was separately determined in aboveground parts and rhizomes.

The data analysis was performed with the STATISTICA 10 programme. To determine the significance of differences between the content of microelements in the biomass of grass species and in soil, the Tukey test was used at the significance level of $p \leq 0.05$. To demonstrate the standard deviation of grass and soil richness in microelements, diagrams were plotted following a standardization of their values.

3. RESULTS AND DISCUSSION

The study has shown that the content of trace elements accumulated in the organs of grasses of the genus *Miscanthus* was different and depended on the species and place of their accumulation (Table 1). The content of heavy metals was evaluated in the aboveground grass biomass (leaves and stems) by comparing it to the threshold value defined for the plant material allocated to animal feed; for Pb < 10 mg·kg^{-1}, Zn < 100 mg·kg^{-1}, Cu < 30 mg·kg^{-1}, Ni < 50 mg·kg^{-1}, Cr < 20 mg·kg^{-1} [5]. Of all the plants tested, the highest mean content of lead, zinc and nickel in aboveground parts was recorded for *M. gigantheus*, zinc – for *M. sacchariflorus*, and chromium – for *M. sinensis*.

Table 1

Content of trace elements [mg·kg^{-1}] in the organs of grass species of the genus *Miscanthus*

Element	Leaves	Stems	Rhizomes	Roots		Soil		$LSD_{p \le 0.05}$ for plant organs
				0–15 cm	15–30 cm	0–15 cm	15–30 cm	
Miscanthus sacchariflorus								
Pb	6.6	4.3	10.4	3.8	5.9	30.4	24.2	1.72
Zn	16.6	3.2	2.1	39.8	45.6	74.8	76.4	0.89
Cu	6.7	3.0	4.3	19.6	14.7	21.8	20.2	1.02
Ni	2.2	28.1	34.9	78.9	54.3	17.8	14.3	0.54
Cr	2.9	9.1	3.0	2.8	2.5	11.5	20.0	0.62
Miscanthus sinensis								
Pb	6.4	2.4	8.0	5.7	7.2	28.3	25.2	0.65
Zn	14.1	4.4	2.4	39.8	34.0	68.7	65.8	2.01
Cu	6.5	2.4	6.3	24.9	22.1	26.8	24.3	0.43
Ni	2.2	26.5	31.8	22.1	62.0	20.2	20.4	0.67
Cr	1.3	10.9	3.1	1.4	1.5	13.0	20.0	0.31
Miscanthus giganteus								
Pb	6.7	4.8	9.0	3.6	4.6	29.1	26.9	0.47
Zn	17.1	7.2	1.4	60.7	58.7	65.1	77.6	0.62
Cu	6.4	4.5	3.6	15.8	16.4	17.5	19.3	0.73
Ni	1.9	29.0	40.4	79.2	74.6	16.3	18.8	0.55
Cr	1.3	10.4	7.7	1.5	1.3	18.6	22.5	0.67
$LSD_{p \le 0.05}$ for microelements								
Pb	n.i.	0.40	0.53	0.25	0.66	2.12	0.10	
Zn	0.47	0.43	0.49	0.68	0.72	0.8	2.75	
Cu	n.i.	0.65	0.75	0.34	0.6	0.74	1.10	
Ni	n.i.	0.66	0.39	0.49	0.48	0.53	0.54	
Cr	0.46	8.3	6.0	5.5	3.9	0.92	0.75	

The amount of chromium, accumulated mostly in stems, was the only one which exceeded the critical values. The presence of microelements at high concentrations in the aboveground parts of plants could have been due to a specific content of heavy metals in soil and atmospheric dust fall onto the plant and soil [22]. In the aboveground biomass, irrespective of the grass species, the leaves are the organs more abundant with lead, zinc and copper, whereas stems – in nickel and chromium. Non-negligible in leaves, the lowest content of zinc was reported for *M. sinensis,* while the highest content of chromium in those organs was found in *M. sacchariflorus.* The amount of the other trace elements in all the species studied was similar.

The underground biomass was also identified with a high variation in the content of trace metals, both uptaken by roots and accumulated in rhizomes. In rhizomes, irrespective of the plant species, lead was accumulated at significantly higher amounts than in the other plant organs. Those organs contained also high amounts of nickel and chromium, especially in *M. gigantheus*. This phenomenon and the tendency to accumulate metals are very promising from the remediation point of view of contaminated soils by phytoextraction and the removal of rhizomes rich in metals from the soil.

In the roots, the content of trace elements was different than that in rhizomes. They accumulate mostly zinc, copper and nickel in the quantities significantly higher than in the other plant organs, irrespective of their soil penetration depth. Zinc and nickel get mostly accumulated in *M. gigantheus* roots (60.7–58.7 $mg \cdot kg^{-1}$, and 79.2–74.6 $mg \cdot kg^{-1}$, repectively), and copper – in *M. sinensis* roots (24.9–22.1 $mg \cdot kg^{-1}$). Chromium content in roots varied, being species-specific. A high content of trace elements in underground parts of plants is due to the fact that heavy metal uptake occurs mostly through roots where their considerable amounts are also immobilized and retained [3, 23, 24].

The study has shown that, irrespective of the grass species of the genus *Miscanthus*, the content of lead, zinc and copper was higher in soil than in the plant organs evaluated, while the content of nickel and chromium – just opposite.

The content of trace elements in the entire soil profile (0 to 30 cm) under all the studied plantations was similar and it was below the norms established for agricultural soils (Regulation 2016). In the soil samples it was, on average: Pb 27.3 $mg \cdot kg^{-1}$, Zn 71.4 $mg \cdot kg^{-1}$, Cu 21.6 $mg \cdot kg^{-1}$, Ni 17.9 $mg \cdot kg^{-1}$, Cr 11.5 $mg \cdot kg^{-1}$ (Table 1). The content of metals in the soil horizons varied with the soil sampling depth. A significantly higher lead accumulation was found in all the objects in the soil surface layers from 0 cm to 15 cm, whereas chromium was more abundant in the samples from 15–30 cm horizon. The amount of the other elements at specific soil profile depths varied and it was species--specific. The elements content and mobility in soil is considerably related to the soil texture and its mineral composition. Trace elements in soil, in general, are associated with soil management, organic matter content and their availability to plants depends on soil reaction and soil sorption capacity [5, 25].

For a better comparison of the content of respective microelements in the grass species studied and in soil under plantations, the experimental data was standardized and presented in figures (Figs. 1, 2). The broken line stands for the confidence interval of standard deviation (–1, 1), thus determining the limits of average results in the set [Statistica 10].

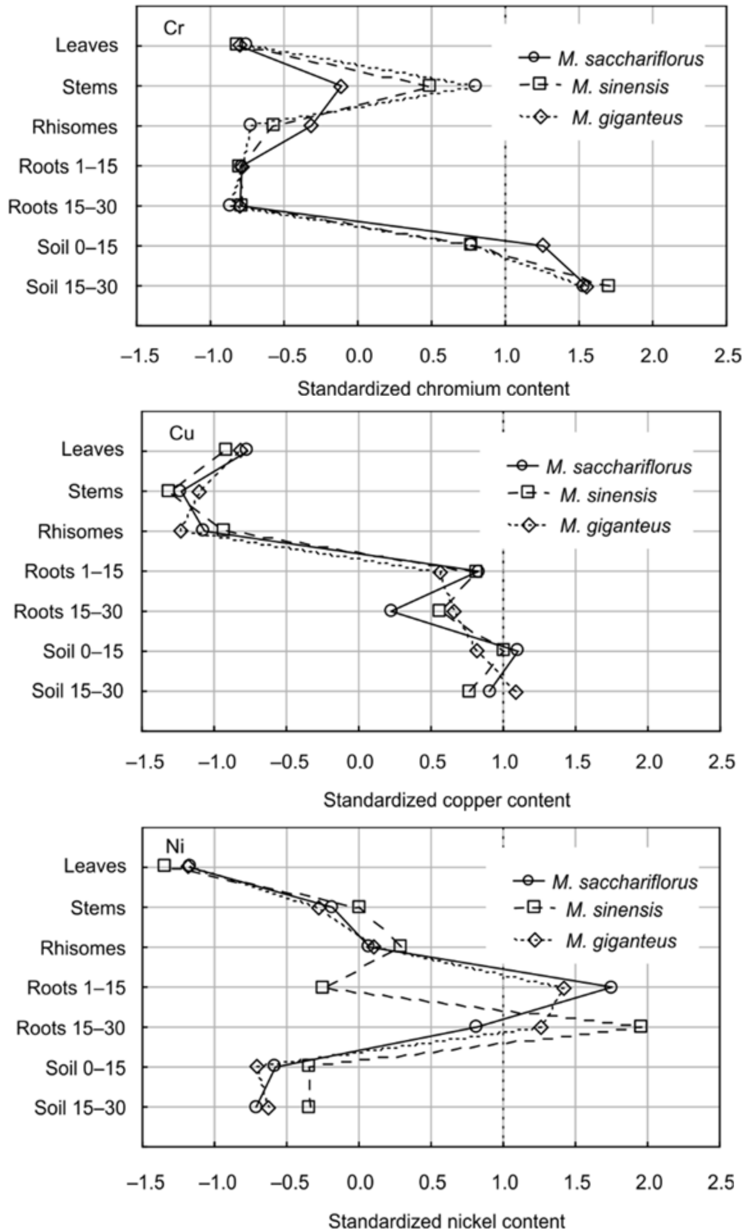

Fig. 1. Content of Cr, Cu and Ni in the organs of *Miscanthus* genus grasses

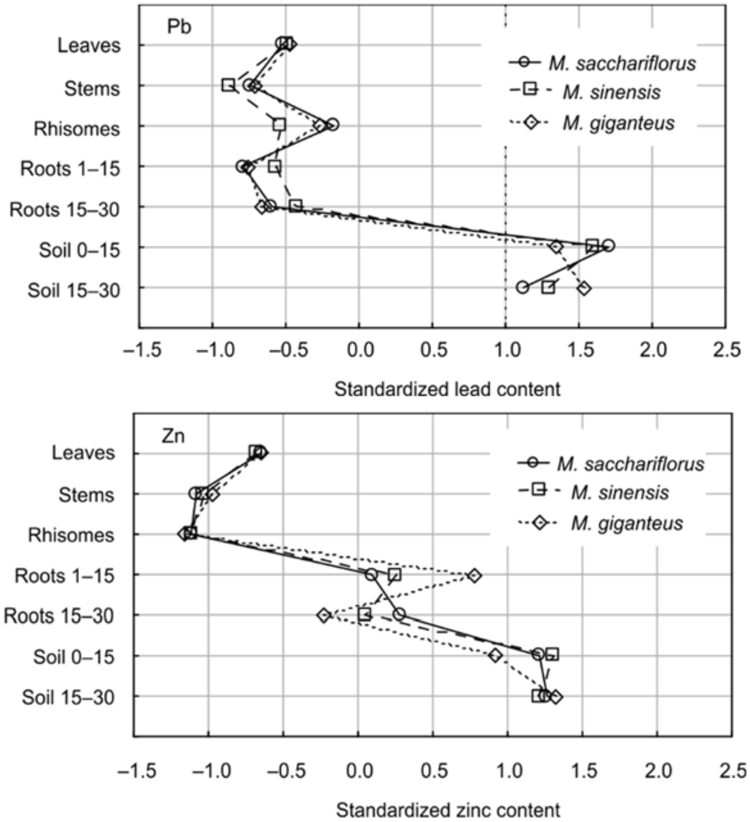

Fig. 2. Content of Pb and Zn in the organs of *Miscanthus* genus grasses

The greatest dispersion of the content of trace elements was observed for zinc, copper and nickel, exceeding the average results limits (–1, 1). The statistical analysis confirmed that the content of lead, zinc and, less considerably, copper, was much higher in soil than in plant organs. Nickel was mostly accumulated in roots, while chromium, exceeding the average results limit, in *M. sacchariflorus* and *M. sinensis* stems and in *M. giganteus* stems and rhizomes. Moreover, the study showed that the biomass weight of the aerial parts of the grass species was for *M. sacchariflorus* 15.8 Mg·ha^{-1}, for *M. giganteus* 36.0 Mg·ha^{-1} and for *M. sinensis* 32.6 Mg·ha^{-1}. The underground biomass was higher for all studied miscantus species and was as follows: 34.2 Mg·ha^{-1}, 41.4 Mg·ha^{-1} and 49.1 Mg·ha^{-1}, respectively.

A good indicator of the relationship between the element concentration in the plant and in soil is the bioaccumulation factor – *BF*. The capacity for an uptake of metals by *Miscanthus,* expressed as *BF* value, varies (Fig. 3a). The highest average capacity for the uptake of trace elements from soil in the grass species under study was found for chromium and nickel.

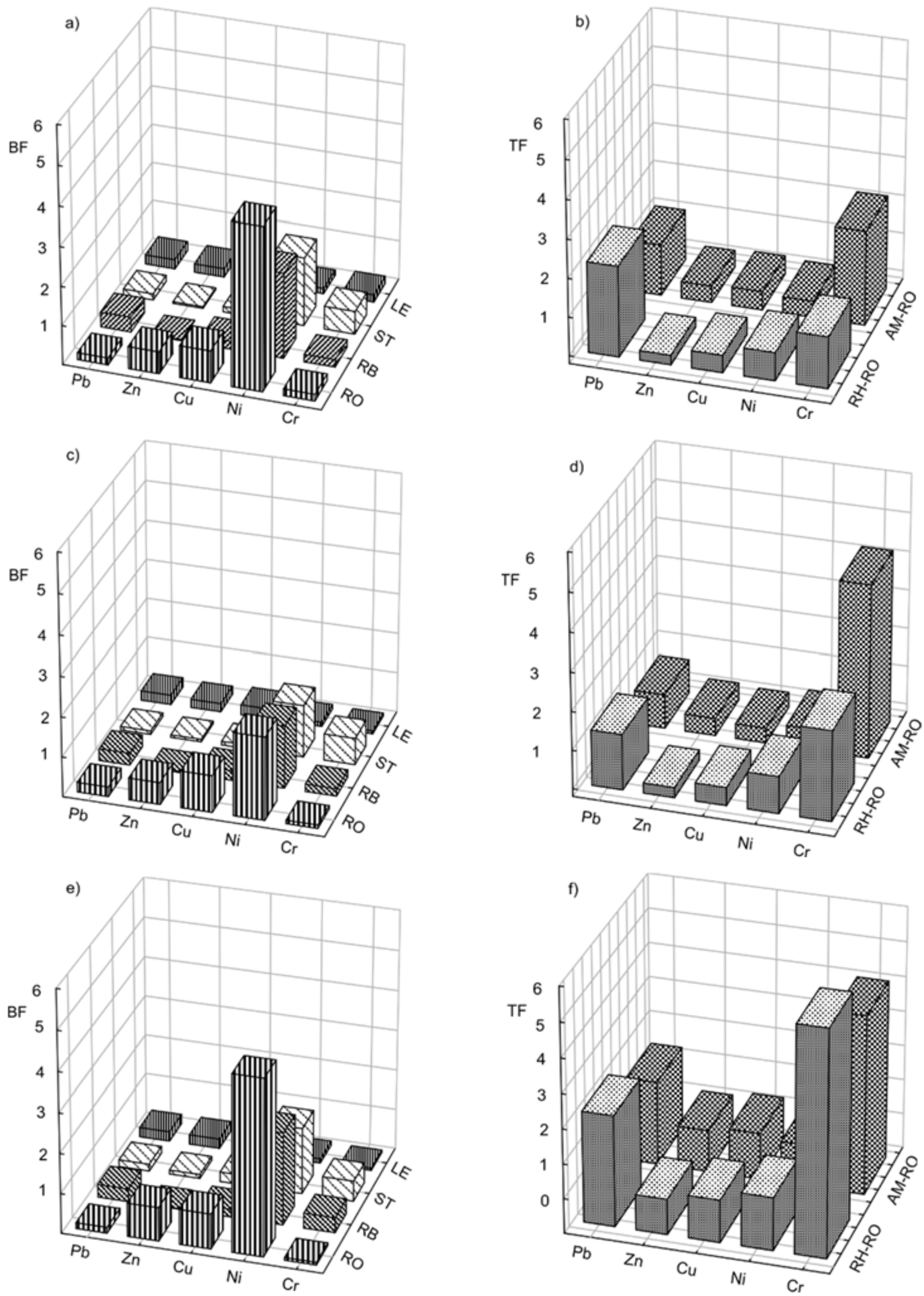

Fig. 3. Values recorded in under- and aboveground organs in *Miscanthus* genus grasses: a, c, e) bioaccumulation factor *BF*, b, d, f) translocation factor *TF*; a, b) *M. sacchariflorus*, c, d) *M. sinensis*, e, f) *M. giganteus*; LE – leaves, ST – stems, RH – rhizomes, RO – roots; AM-RO – the aboveground mass:roots ratio, RH:RO – rhizomes:roots ratio

The highest *BF* values for nickel were found in all the species in an order of decreasing *BF*: roots (from 2.07 in *M. sinensis* to 4.38 in *M. giganteus*) > rhizomes (from 1.57 in *M. sinensis* to 2.30 in *M. giganteus*) > stems (from 1.31 in *M. sinensis* to 1.75 in *M. sacchariflorus*) > leaves (from 0.11 in *M. sinensis* and *M. giganteus* to 0.14 in *M. sacchariflorus.*

The *BF* values for the other trace elements were much lower and, depending on the species and the place of accumulation, for zinc from 0.02 to 0.84, copper – from 0.09 to 0.92 and lead – from 0.09 to 0.38. Once the mean *BF* values were determined for respective grass species, it was found that the capacity for the uptake of microelements from soil in *M. sacchariflorus* (*BF*– 0.63) and *M. giganteus* (*BF*– 0.65) were similar. The *BF* value in *M. sinensis* was much lower and it was 0.49. Such discrepancies in terms of the capacity of elements uptake from soil and their accumulation in plant organs can be due to a rhizome (*M. sacchariflorus and M. giganteus*) or tuft (*M. sinensis*) type of grass growth. To evaluate the distribution of trace elements among plant organs, the translocation factor (TF) was calculated (Fig. 3b). A series for the accumulation of elements for aboveground parts and roots (AM:RO) in decreasing values order was as follows: for *M. sacchariflorus* 2.25 (Cr) > 1.12 (Pb) > 0.28 (Cu) > 0.23 (Zn and Ni), for *M. sinensis* 4.27 (Cr) > 0.68 (Pb) > 0.34 (Ni) > 0.25 (Zn) > 0.19 (Cu), for *M. giganteus* 4.16 (Cr) > 1.40 (Pb) > 0.34 (Cu) > 0.20 (Zn i Ni).

What is important, the TF values calculated, determining a capacity for translocation of the trace elements from roots to rhizomes (RH:RO), show dependences which are similar to the ones earlier calculated for the ratio of aboveground parts and roots (AM:RO) in grasses (Fig. 3b). Higher TF values in rhizomes, as compared with the aboveground parts, were found for lead and nickel, and in the case of *M. giganteus* – also for chromium. Zinc was accumulated at inconsiderable amounts in rhizomes.

The above study and the results reported by other authors [14, 16, 21] confirm that grasses of the genus *Miscanthus* can accumulate considerable amounts of trace elements in their organs. A capacity for elements penetration from soil to plants is, to much extent, species-specific. They are mostly accumulated in stems and rhizomes, which makes it possible for long term plantations of those grasses to be used for the remediation of soils polluted with heavy metals like lead, chromium, zinc and copper.

4. CONCLUSIONS

- The content of respective trace elements in the biomass of grasses of the genus *Miscanthus* varied depending on the species. The content of lead, zinc, copper and chromium was definitely lower in the plant organs under study than their content in soil, while the content of nickel was just opposite.

• In aboveground parts, lead, zinc and copper were mostly accumulated in leaves and nickel and chromium – mostly in stems. The underground plant parts, especially roots, accumulated mostly zinc, copper and nickel and rhizomes – higher amounts of lead and chromium.

• Due to a rhizome-like soil penetration, a greater capacity for bioaccumulation of the microelements under study was recorded for *M. sacchariflorus* and *M. giganteus* than for *M. sinensis*.

• During phytoextraction, grasses of the genus *Miscanthus*, accumulating considerable amounts of trace elements, can be used for remediation of chemically polluted soils, thus preventing damage to the environment and enhancing soil quality.

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