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PHYTOREMEDIATION POTENTIAL OF *MISCANTHUS* X *GIGANTEUS* IN SOIL CONTAMINATED WITH HEAVY METALS

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Abstract

There has been carried out a comparative research, which allow us to evaluate the efficacy of Miscanthus x giganteus for phytoremediation of contaminated soils. The effects of organic soil amendments (compost and vermicompost) on uptake of heavy metals in Miscanthus x giganteus were studied. Experiments have been implemented in controlled conditions. The soil used in this experiment was sampled from the vicinity of the area contaminated by the Non-Ferrous-Metal Works (MFMW) near Plovdiv, Bulgaria. The soils were amended or not with 5, 10, 15 or 30% of compost and 5, 10, 15 or 30% of vermicompost. Heavy metal contents in roots, rhizomes, stems, and leaves of Miscanthus x giganteus were analysed. The quantitative measurements were carried out with inductively-coupled plasma (ICP). The application of soil amendments favoured plant growth and development. Compost and vermicompost application led to effective immobilization of Pb, Zn, and Cd mobile forms in soil. A correlation was found between the quantity of the mobile forms and the uptake of Pb, Zn, and Cd by the Miscanthus x giganteus. Compost and vermicompost treatments were effective organic amendments and reduced heavy metals in leaves of Miscanthus x giganteus, but the effect differed among them. Also, there was a dose effect for amendments. The 30% compost and 15% vermicompost treatments led to the maximal reduction of heavy metals in Miscanthus x giganteus biomass. The depots for accumulation followed the order: roots > rhizomes > leaves > stems. The high concentration of heavy metals in the roots and rhizomes and the low translocation factor indicated the possibility of Miscanthus x giganteus to be used in phytostabilization. Miscanthus x giganteus harvested from heavy metals contaminated soil may be used for energy production.

Key words: *heavy metals, Miscanthus x giganteus, organic amendments, phytoremediation, polluted soils.*

INTRODUCTION

Phytoremediation is an emerging technology, which should be considered for remediation of contaminated sites because of its cost effectiveness, aesthetic advantages and long-term applicability (Chaney et al., 1993; Marques et al., 2008). This technology can be defined as the efficient use of plants to remove, detoxify or immobilize environmental contaminants in soils, waters or sediments through the natural, biological, chemical or physical activities and processes of the plants (Ciura et al., 2005; Lone et al., 2008). It is best applied at the sites with shallow contamination of organic, nutrient or metal pollutants (Yang et al., 2005).

The use of crop plants for phytoremediation of contaminated soils has the advantages of their high biomass production and adaptive capacity to variable environments (Fässler et al., 2010; Komarek et al., 2007). However, to succeed they must be tolerant to the contaminants and be capable of accumulating significant concentrations of heavy metals in their tissues. Additionally, crops could make the long time-periods for decontamination more acceptable, economically and environmentally. If the contaminated biomass may be further processed for added value products (not only concentrated on deposits of hazardous wastes), then such fact represents an improvement of economic efficiency of phytoremediation technology. Industrial plants, i.e. energy crops or crops for bio-diesel production, are therefore the prime candidates as plants for phytoremediation.

The use of biofuel crops for phytoremediation of heavy metal contaminated soils is of increasing interest (Pidlisnyuk et al., 2014). The main reason for this is the increase in demand for biomass as alternative energy sources, as well as the possibility of remediation of contaminated soils. Energy crops include fast-growing varieties of trees and annual and perennial grasses. Among perennial grasses, *Miscanthus* x *giganteus* is considered the most promising biofuel plant. *Miscanthus* x *giganteus* is a sterile hybrid between *M. sinensis* and *M. sacchariflorus*, propagated vegetatively through its rhizomes. It is also called "elephant grass". The species has been distributed in Europe since the early 1980s.

The cultivation of *Miscanthus* x *giganteus* in Bulgaria began 5-6 years ago with the development of technology for the reproduction and cultivation of a perennial grass energy crop similar to reeds. It has rapid growth, low mineral content and high biomass yield. It reaches a height of more than 3.5 meters in one season and the annual dry weight yield can reach 25 tons per hectare. Preliminary studies have shown that *Miscanthus* x *giganteus* can be used for phytoremediation of contaminated land after the Chernobyl disaster in Ukraine and contaminated soils from mining in Slovakia. The use of *Miscanthus* x *giganteus* biomass for energy production seems promising in terms of the costs for phytoremediation and can find real application in practice compared to the use of expensive conventional methods. Studies have shown that the utilization of biomass obtained as a source of energy is feasible and can make the phytoremediation process profitable (Dornburg et al., 2005).

On the other hand, growing *Miscanthus* x *giganteus* on contaminated soils creates a potential risk of heavy metal pollution in the environment. The studies of Pogrzeba et al. (2011) show that the cultivation of *Miscanthus* x *giganteus* on contaminated soils leads to increased levels of heavy metals in the above-ground part of the plant. Contaminated biomass has to be treated as a hazardous material and its incineration has to be done in facilities equipped with filters for capture of metal oxides. That is why soil preparation before planting is a crucial step in the cultivation of *Miscanthus* x *giganteus.* It is necessary to propose measures to reduce the bioavailability of metals in the soil and very slow removal of metals from the soil associated with this perennial crop.

Growth and development of plants on soils contaminated with heavy metals depends on many factors, and above all on the concentration of these metals in the soil, the physicochemical properties of the soil, such as pH, organic matter content, as well as plant tolerance (Kabata-Pendias, 2001). Decrease in the amount of bioavailable (accessible) forms of heavy metals in the soil after the use of soil ameliorants can lead to a decrease in heavy metal content in biomass. According to Placek et al. (2017) the addition of inorganic fertilizers and/or lime leads to an increase of the biomass produced from *Miscanthus* x *giganteus* in contaminated soils but does not affect the uptake of the metals from the plant, whereas organic amendments are able to modify heavy metals bioavailability (Angelova et al., 2017). Addition of organic matter amendments, such as compost, and vermicompost in soil leads to immobilization of heavy metals and soil amelioration of contaminated soils (Angelova et al., 2013; Clemente et al., 2005).

Organic amendments are able to improve soil physical, chemical and biological properties by:

i. raising the pH,
ii. increasing the organic matter content,
iii. adding essential nutrients for plant growth,
iv. increasing the water holding capacity,
v. modifying heavy metals bioavailability (Angelova et al., 2013; Walker et al., 2003; 2004).

The aim of this experiment was to compare the effect of the selected organic additives on accumulation of heavy metals by the *Miscanthus* x *giganteus*, as well as to evaluate the possibilities to use the plant for phytoremediation of heavy metal contaminated soils.

MATERIALS AND METHODS

The soil used in this experiment was sampled from the vicinity of the area contaminated by the Non-Ferrous-Metal Works (NFMW) near Plovdiv, Bulgaria. Characteristics of soils and organic amendments are shown in Table 1. The soil used in this experiment was slightly alkalic, with moderate content of organic matter and essential nutrients (N, P and K). The pseudo-total content of Zn, Pb and Cd is extremely high (2540.8 mg/kg Zn, 2429.3 mg/kg Pb and 51.5 mg/kg Cd, respectively) and exceed the maximum permissible concentrations (320 mg/kg Zn, 100 mg/kg Pb and 2.0 mg/kg Cd).

The pot experiment was conducted on soil with organic amendments (compost at 5, 10, 15 or 30% and vermicompost at 5.0%, 10.0%, 15% and 30% addition rates (calculated on soil dry weight basis). Soils were passed through a 2 cm sieve. Amendments were added and thoroughly mixed by hand. The pots were filled with 3 kg soil. All treatments were performed in triplicate. Three control pots were also set up without amendment. Pots were watered and stored in a greenhouse, where they were left to settle a minimum of 6 weeks at room temperature before planting the *Miscanthus* x *giganteus*.

Table 1. Characterization of the soil and the organic amendments used in the experiment

Parameter	Soil	Compost	Vermicompost
pH	7.6	6.9	7.5
EC, dS/m	0.2	0.2	2.2
Organic matter, %	3.99	72.10	38.58
N Kjeldal, %	0.24	2.22	1.57
C/N	9.41	32.43	24.59
Pseudo-total P, mg/kg	731	12654	10211
Pseudo-total K, mg/kg	4674.7	6082	10495
Pseudo-total Pb, mg/kg	2429.3	12.0	32.3
DTPA – extractable Pb, mg/kg	849.1		
Pseudo-total Zn, mg/kg	2540.8	170.8	270.3
DTPA – extractable Zn, mg/kg	236.8		
Pseudo-total Cd, mg/kg	51.5	0.19	0.69
DTPA - extractable Cd, mg/kg	36.8		

The *Miscanthus* x *giganteus* plants were grown in a climate chamber with regular watering and random rotation of the position of the pots. Rhizomes were sown in each pot. After 180 days, all plants were harvested.

The plants were gathered and the contents of Pb, Zn and Cd in their different parts – roots, rhizomes, stems, and leaves, were analysed separately. The contents of heavy metals in the plant material were determined.

To determine the effect of the organic amendments, soil samples were collected 1 month after addition of organic amendments. A soil subsample was air-dried, passed through a 2 mm sieve and characterized for soil pH (H_2O) in deionised water suspension of 1: 5 (w/v); total nitrogen by the Kjeldahl method (N Kjeldahl); total oxidizable organic carbon according to Tube digestion method (with titration) (Sparks, 1996).

The pseudo-total and DTPA-extractable concentration of heavy metals in the soils, after four weeks' equilibration were determined. Pseudo-total content of metals in soils was determined in accordance with ISO 11466.

The available (mobile) heavy metals contents were extracted by a solution of DTPA (ISO 14870).

The plant samples were treated by the method of microwave mineralization. To determine the heavy metal content in the plant and soil samples, inductively coupled emission spectrometer (Jobin Yvon Horiba "ULTIMA 2", France) was used.

RESULTS AND DISCUSSIONS

Accumulation of heavy metals in vegetative organs of Miscanthus x giganteus without amendments (control)

To clarify the issues of absorption, accumulation and distribution of heavy metals in vegetative organs of *Miscanthus* x *giganteus* samples of rhizomes, roots, stems, and leaves were analysed.

Table 2 presents the results obtained for the content of heavy metals in the vegetative organs of the study crop.

Considerably higher amounts were established in the roots of *Miscanthus* x *giganteus* compared to the above- ground parts of *Miscanthus* x *giganteus*. This is consistent with the results obtained by Fernando and Oliveira (2004), who found that metals accumulate primarily in the underground parts of *Miscanthus* x *giganteus* rather than in the above-ground parts. The content of Pb in the roots of *Miscanthus* x *giganteus* without amendments reached to 529.8 mg/kg, Zn -

365.8 mg/kg and Cd - 30.8 mg/kg, whereas in the rhizomes of *Miscanthus* x *giganteus* without amendments Pb reached to 119.5 mg/kg, Zn - 106.5 mg/kg and Cd - 5.94 mg/kg. Our results indicate that a considerable part of the heavy metals are accumulated in the roots and rhizomes, which is consistent with the results of other authors (Fernando and Oliveira, 2004). This is explained by the fact that during the penetration of heavy metals in the plasma there is inactivation and disposal of significant quantities of them, as a result of the formation of slightly mobile compounds with the organic substance.

Table 2. Content of heavy metals (mg/kg) in vegetative organs of *Miscanthus* x *giganteus* (without amendments, control)

Element	Roots x±sd	Rhizomes x±sd	Stems x±sd	Leaves x±sd
Pb	529.8±2.5	119.5±1.5	44.3±0.8	261.3±1.5
Cd	30.7±2.1	5.9±0.3	13.0±0.9	27.3±1,2
Zn	365.8±5.3	106.5±2.1	132.0±2.0	281.4±2.8

x - average value (mg/kg) from 5 repetitions; sd - mean standard deviation

The heavy metals contents in the stems and leaves of the *Miscanthus* x *giganteus* were considerably lower compared to those in the root system, which showed that their movement through the conductive system was strongly restricted. The content of Pb in the stems of *Miscanthus* x *giganteus* without amendments reached to 44.3 mg/kg, Zn - 132.0 mg/kg, and Cd - 13.0 mg/kg. The content of Pb in the leaves of *Miscanthus* x *giganteus* without amendments reached to 261.3 mg/kg, Zn - 281.4 mg/kg, and Cd - 27.3 mg/kg.

The results we obtained shown that the content of Pb in the above-ground parts of *Miscanthus* x *giganteus* is high despite the low level of bioavailable forms of Pb in the soil.

The studies of Pogrzeba et al. (2011; 2013) shown that the absorption of metals strongly depends on the level of accessible forms. In the cultivation of *Miscanthus* x *giganteus*, 2 mg/kg Pb, 0.3 mg/kg Cd and 25 mg/kg Zn are accumulated on clean soils, while at cultivation on contaminated soils - up to 200 mg/kg Pb, 5 mg/kg Cd and 700 mg/kg Zn. Barbu et al. (2009; 2010) found that there is a correlation between the Pb content of the above-ground parts of *Miscanthus* x *giganteus* and the Pb content in the soil.

Barbu et al. (2009; 2010) found that the amount of accumulated metals in leaves and stems of *Miscanthus* x *giganteus* when grown on contaminated soil (680 mg/kg Pb and 13 mg/kg Cd) is very low. Similar results were obtained by Nsanganwimana et al. (2015) who found that, despite the high content of Pb in the soil, plants accumulate small amounts of this element in their above-ground mass, which may be due to the accumulation of Pb in the roots of the plant.

The Translocation Factor (TF) provides information on the ability of plants to digest heavy metals through the roots and to move them to the above-ground mass (leaves).

The results obtained shown that, with respect to Pb, the translocation factor for plants without the importation of additives reaches up to 0.47, for Cd up to 1.1 and for Zn up to 0.88.

The effectiveness of phytoremediation is also determined by the bioconcentration factor (BCF), (McGrath and Zhao, 2003). BCF root is a ratio of the content of heavy metals in plant roots to soil content (BCFroots = [Metal] roots/[Available metal] soils).

The results obtained shown that this ratio reaches up to 0.72 for Pb, up to 0.94 for Cd and up to 1.37 for Zn.

BCF shoot is defined as the ratio of the metal concentration in the above-ground mass of the plant and in the soil (BCFshoots = [Metal] shoots/[Available metal] soils) and is a measure of the plant's ability to digest and move the metals to the above-ground mass, which can be easily harvested. In hyperaccumulators case, the enrichment factor is higher than 1 and in some cases it may reach 50-100 (McGrath and Zhao, 2003).

The results obtained shown that, with respect to Pb, the bioconcentration factor for plants without the importation of additives reaches up to 0.34, for Cd up to 1.03, and for Zn up to 1.20. The results obtained shown that BCF is higher for Zn and Cd than for Pb. Higher values for Zn and Cd are probably a consequence of the greater ability of these elements to accumulate in the above-ground mass than in the roots, which is consistent with the results of Korzeniowska et al. (2011) and Yoon et al. (2006).

According to McGrath and Zhao (2003), the BCF value for the above-ground parts of the accumulator plants should be higher than that of the plant roots and should exceed 1. The results of the study show that *Miscanthus* x *giganteus* cannot be classified as an accumulator type, although the BCF for Cd reaches up 1.03, and up to 1.2 for Zn. Our results are in accordance with Pogrzeba et al. (2011) and Krzyzak et al. (2017) according to whom the *Miscanthus* x *giganteus* accumulates moderate amount of heavy metals in the leaves and has a relatively lower potential for phytoextraction.

Figures 1, 2 and 3 presents the results obtained for impact on organic amendments on the content of heavy metals in the vegetative organs of the *Miscanthus* x *giganteus*.

The results obtained by us shows that the absorption of the elements by the *Miscanthus* x *giganteus* is influenced by the organic amendments imported to the soil (type and quantity).

The addition of compost and vermicompost significantly affect the absorption of heavy metals (Pb, Zn and Cd) of the tested plants, and depends to a considerable extent by their quantity.

The addition of compost results in a decrease in Pb, Zn and Cd content in the roots of *Miscanthus* x *giganteus*, as this decrease is more pronounced when 30% of compost is imported. The content of Pb in the roots decreases to 396.2 mg/kg, Cd to 13.4 mg/kg and Zn to 289.9 (Figures 1, 2 and 3). The addition of compost leads to a decrease in Pb content in the stems and leaves of the *Miscanthus* x *giganteus*, as this decrease is more pronounced when 30% of compost is imported. Similar is the trend of decreasing the amount of Zn and Cd in the leaves of *Miscanthus* x *giganteus* where the Zn content decreases from 281.8 mg/kg to 201.8 mg/kg and Cd from 27.4 mg/kg to 3.5 mg/kg in the leaves.

On the other hand, the addition of compost leads to an increase in the content of Pb, Cd and Zn in the rhizomes of the *Miscanthus* x *giganteus*. Pb content in rhizomes increases from 119.5 mg/kg in the control to 382 mg/kg, Cd from 5.9 mg/kg to 11.4 mg/kg, and Zn from 106.5 mg/kg to 379.9 mg/kg.

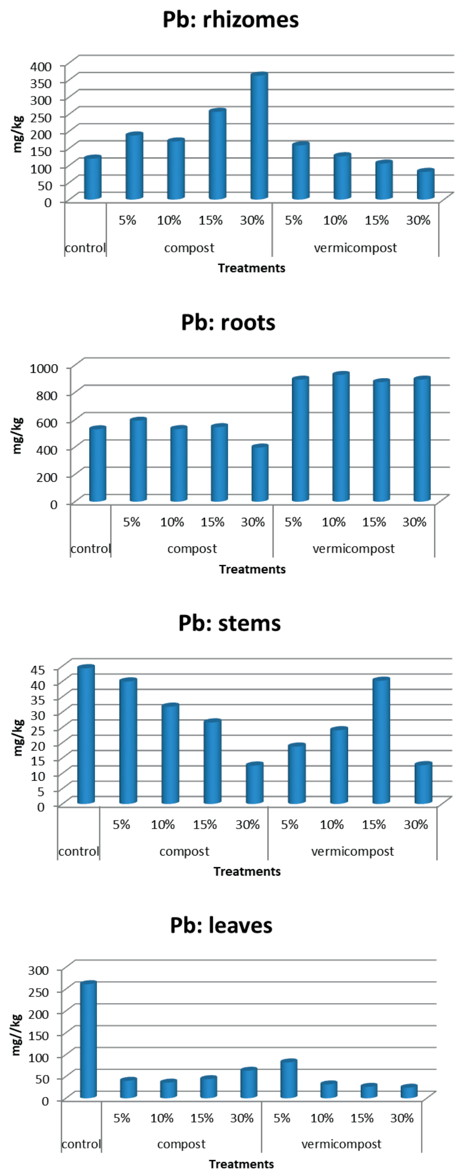

Figure 1. Effect of different organic amendments (compost and vermicompost) applications to accumulation of Pb in the vegetative parts of *Miscanthus* x *giganteus*

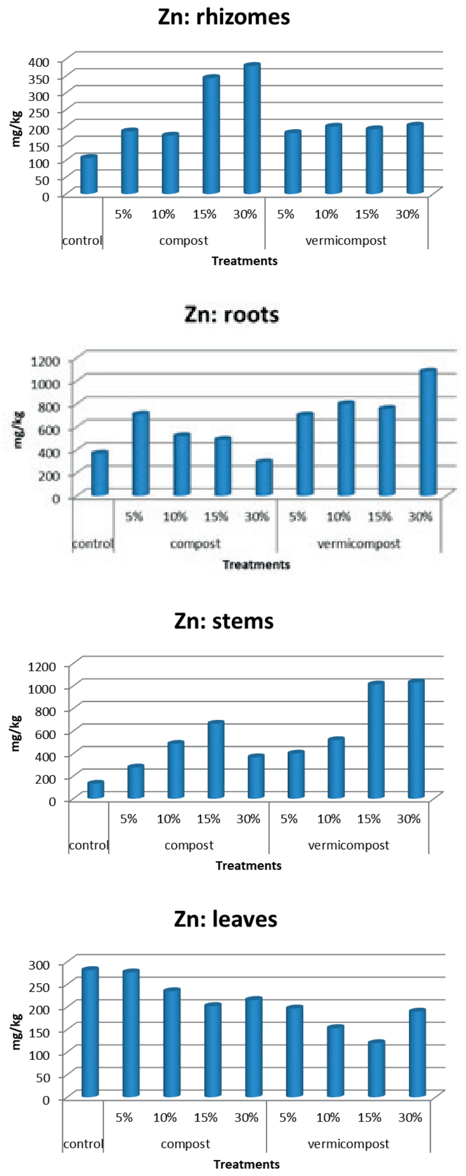

Figure 2. Effect of different organic amendments (compost and vermicompost) applications to accumulation of Zn in the vegetative parts of *Miscanthus* x *giganteus*

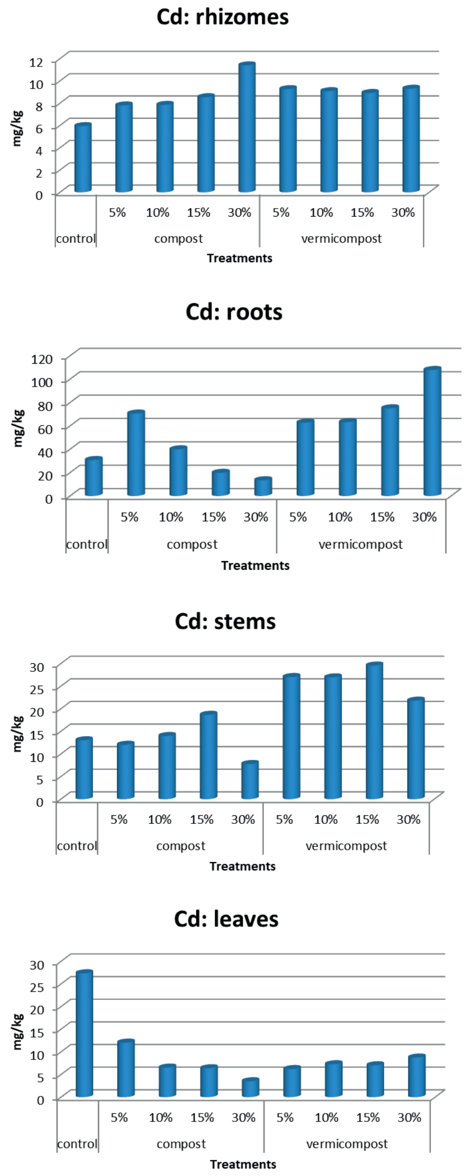

Figure 3. Effect of different organic amendments (compost and vermicompost) applications to accumulation of Cd in the vegetative parts of *Miscanthus* x *giganteus*

The addition of a vermicompost leads to a decrease in the Pb content and an increase in Cd and Zn content in the rhizomes of the *Miscanthus* x *giganteus*. Pb content in rhizomes decreases from 119.5 mg/kg in the control to 80.8 mg/kg, while Cd content increases from 5.9 mg/kg to 11.4 mg/kg and Zn from 106.5 mg/kg to 202.3 mg/kg. The addition of a vermicompost leads to a decrease in the Pb, Cd and Zn content in the leaves of the *Miscanthus* x *giganteus*. Pb content decreased from 261.3 mg/kg to 24.2 mg/kg, Zn from 281.4 mg/kg to 119.5 mg/kg, and Cd from 27.3 mg/kg to 7.0 mg/kg (Figures 1, 2 and 3). On the other hand, the addition of a vermicompost leads to an increase in Pb, Cd and Zn content in the roots and stems of the *Miscanthus* x *giganteus*, this increase being more pronounced with the importation of 30% vermicompost in the roots and 15% vermicompost in the stems.

The results obtained can be explained by the amount of mobile forms of heavy metals. The addition of compost and vermicompost to the soil leads to a decrease in the mobile forms of Pb, Zn and Cd (data are not shown), resulting in a significantly lower content of these elements in the leaves of the plants.

The addition of compost and vermicompost results in a decrease in the translocation factor for Pb and Cd, which indicates the lower ability of the plants to bioaccumulate these elements compared to the control (Figure 4). In the addition of 15% and 30% of compost, TF values for Zn increased from 0.87 to 1.04, while in the use of 15% vermicompost - to 1.2. In the variants mentioned, the TF values indicate that a significantly greater amount of Zn moves to the leaves compared to other variants.

The distribution of heavy metals in the *Miscanthus* x *giganteus* organs has a selective character, it is specific for the individual elements. The main part of Pb accumulates in the underground parts of *the Miscanthus* x *giganteus* (roots and rhizomes) - 68%. In terms of Cd, the content in the above-ground part of the Miscanthus x giganteus is slightly higher than in the underground part (52% in the above-ground part and 48% in the underground part), while in the Zn the opposite trend is observed (47% in the above-ground part and 53% in the underground part).

The distribution of Pb in plants organs for all options with the addition of compost follows the same correlation observed in the control. The highest content of Pb was found in the roots, followed by the rhizomes, leaves and stems (Figure 4).

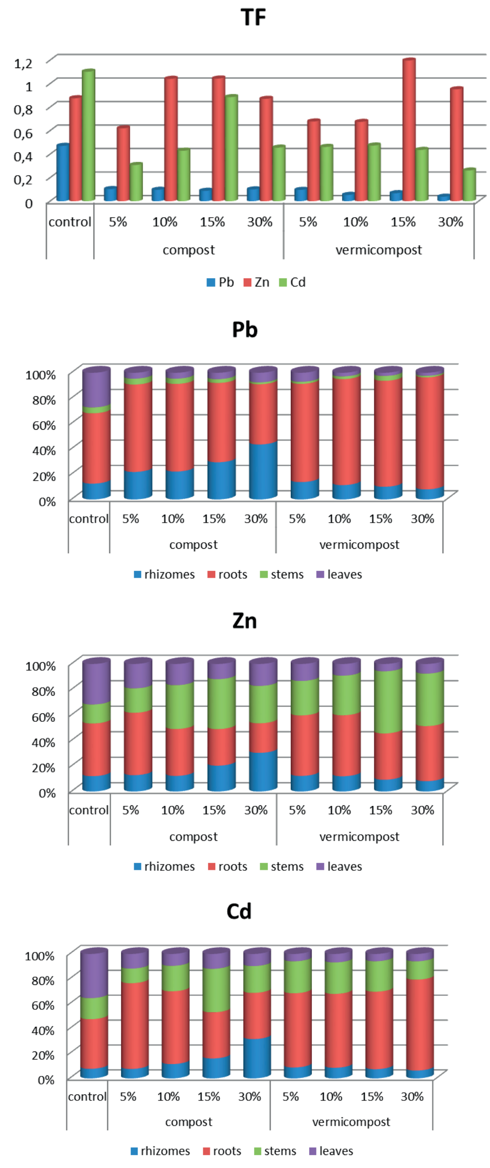

Figure 4. Effect of organic amendments on Translocation factor and distribution of heavy metals in vegetative organs of *Miscanthus* x *giganteus*

These results correspond to the results of Fernando and Oliveira (2004) who found that in the aboveground parts of the plants the accumulation of heavy metals is less than that in the roots.

Similar results were obtained for the influence of vermicompost on the accumulation of heavy metals from *Miscanthus* x *giganteus*. There is a correlation between the amount of amendments and their content in the leaves, as with the increase of the amount of compost and vermicompost the content of heavy metals in the leaves is decreasing.

According to Pidlisnyuk et al. (2014) and Kocon et al. (2012) *Miscanthus* x *giganteus* can be grown on contaminated soils which are not contaminated with a high concentration of metals. However, the results we obtained clearly shows that *Miscanthus* x *giganteus* is a crop tolerant to heavy metals and can be grown on highly contaminated soils (2544.8 mg/kg of Zn, 2429.3 mg/kg of Pb and 51.5 mg/kg of Cd). The high concentration of heavy metals in the roots and the low translocation factor indicates the possibility of *Miscanthus* x *giganteus* to be used in phyto stabilization. Our results confirm that *Miscanthus* x *giganteus* has phytostabilization potential. The addition of compost and vermicompost further reduces the ability of Miscanthus x giganteus to transfer the heavy metals in the leaves, allowing its use as a safe energy crop.

CONCLUSIONS

Based on the results obtained the following conclusions can be made:

1. The *Miscanthus* x *giganteus* is a plant tolerant to heavy metals and which can be grown in heavy metal polluted soils (2540.8 mg/kg Zn, 2429.3 mg/kg Pb and 51.5 mg/kg Cd) and can be successfully used in the phytoremediation of heavy metal polluted soils.
2. The distribution of the heavy metals in the organs of the *Miscanthus* x *giganteus* has a selective character. *Miscanthus* x *giganteus* decreases in the following order: roots > leaves > rhizomes > stems.
3. The high concentration of heavy metals in the roots and the low translocation factor indicates the possibility of *Miscanthus* x *giganteus* to be used in phytostabilization.
4. Compost and vermicompost treatments were effective organic amendments and reduced heavy metals in leaves of *Miscanthus* x *giganteus*, but the effect differed among them.
5. The 30% compost and 15% vermicompost treatments led to the maximal reduction of heavy metals in the *Miscanthus* x *giganteus* leaves, allowing its use as a safe energy crop.

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