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# EFFECT OF VERMICOMPOST AND BIOCHAR AMENDMENTS ON UPTAKE OF HEAVY METALS, MICRO AND MACROELEMENTS BY *SEDUM PLUMBIZINCICOLA*

**Onyinye Anthonia Ezeifeka, Violina Angelova***

Agricultural University – Plovdiv, Bulgaria
***Corresponding e-mail: violina@au-plovdiv.bg**

## Abstract

Comparative research on the impact of organic amendments on the uptake of heavy metals and micro and macroelements of *Sedum Plumbizincicola* has been carried out. Experiments have been implemented in controlled conditions. The soil used in this experiment was sampled from the vicinity of the Non-Ferrous Metals Work near Plovdiv, Bulgaria. The pot experiment was a randomised complete block design containing five treatments and three replications (15 pots). The treatments consisted of a control (no organic amendments) and vermicompost and biochar amendments (added at 5% and 10%, respectively, recalculated based on dry soil weight). Applying organic additives to the soil influences the physicochemical properties. It leads to increased organic matter, electrical conductivity and content of macroelements (P, K, Ca and Mg) and microelements (Fe, Mn, Zn) in the soil. Applying organic additives to the soil affects the uptake of heavy metals and micro and macro elements by *Sedum Plumbizincicola*. Organic additives affect yield, influenced by the type of additive and dose. Cd and Zn yields were up to 3 times higher in the variant with 10% vermicompost application. Application of 10% biochar resulted in a negligible increase.

**Keywords:** phytoextraction, organic amendments, contaminated soils, *Sedum Plumbizincicola*

## INTRODUCTION

Heavy metal pollution is a global problem, and cleaning up the soil is a challenge. In recent years, more research has focused on phytoextraction as a technology for cleaning heavy metal-contaminated soils (Salt et al., 1995). This plant-based technology for removing heavy metals from contaminated soils has advantages as a green technology conducted *in situ* with low cost and not damaging soil quality. The success of phytoextraction mainly depends on the proper selection of plants. Hyperaccumulators are plants with an extremely high ability to accumulate heavy metals in the above-ground mass and have been the subject of numerous studies (Brooks, 1998; Baker et al., 2000). Plants accumulating over 100 mg Cd.kg$^{-1}$ in shoots are known as Cd hyperaccumulators (Baker et al., 2000). A few Cd hyperaccumulators, such as *Thalspi caerulescens* and *Arabidopsis halleri* are known due to the toxicity of the metal (Brown et al., 1995; Lombi et al., 2000; Kupper et al., 2000). *Sedum Plumbizincicola* is a plant of the *Crassulaceae* family that can hyperaccumulate Cd and Zn in very high concentrations (400 and 10,000 mg.kg$^{-1}$, respectively) (Liu et al., 2017). The species was discovered in 2005 near Qitong City (Zn-Pb mining area), northwest of Hangzhou City in western Zhejiang Province, eastern PR China and identified as a Cd-Zn hyperaccumulator in 2007 (Wu at al., 2013).

The main problem associated with using hyperaccumulators is their slow growth and small size and the need for agronomic practices related to their cultivation under field conditions. Greater biomass can be obtained by adding various amendments, e.g. inorganic fertilizers and organic amendments (such as compost, biochar and animal waste). According to Wu et al. (2012b) and Wu et al. (2018), organic additives can enhance the bioavailability of metals. They can be effective and environmentally friendly in improving the phytoremediation efficiency of Cd-contaminated soil.

The present study aimed to evaluate the effect of organic amendments (vermicompost, and biochar) on the uptake of heavy metals, micro and macroelements by *Sedum Plumbizincicola* grown on Bulgarian contaminated soils.

## MATERIALS AND METHODS

Vegetation experiments were conducted on contaminated soil from the area of Non-Ferrous Metals Work-Plovdiv (NFMW). The experiments were set in 0.25 l containers with dimensions 7x7x8 cm, in 5 variants with three repetitions according to the following scheme: 1. Control soil (no addition). 2. Soil + vermicompost (5%). 3. Soil + vermicompost (10.0%). 4. Soil + biochar (5%), and 5. Soil + biochar (10.0%).

The soils were sieved through a 2 $cm^2$ mesh sieve, and the tested organic soil amendments were added and carefully mixed with the soil by hand. After a 4-week incubation period, seeds of *S. Plumbizincicola* were sown in each pot. The containers bearing the plants were placed in a growth chamber called Growarm 100 propagators, which provided suitable constant temperature and humidity for optimal seed germination and plant growth. Harvesting took place 14 weeks (98 days excluding the day of harvest) after sowing. The plants were prepared by cutting approximately one inch above the soil, and they were also washed with distilled water and kept in appropriately labeled paper bags. Fresh and dry biomass yield was recorded. Soil samples from each treatment were air-dried and sieved through a sieve with a hole diameter < 2 mm, following storage in paper bags for routine analyses. Soil pH (ISO 10390), electrical conductivity (ISO 11265), total organic matter (BS EN 13039), CEC (ISO 11260), total (extracted with aqua regia, according to ISO 11466) and DTPA-extracted mobile forms of heavy metals, micro- and macroelements in soils (ISO 14870) were determined. The microwave mineralization method determined the heavy metal content and micro and macroelements in plant samples (above-ground mass) (EPA method 3052). The quantitative measures were carried out by ICP (Jobin Yvon Emission - JY 38 S, France). The SPSS for Windows program was used in the statistical processing of the data.

## RESULTS AND DISCUSSION

The main characteristics and the content of micro and macro elements in the soil and organic additives used are presented in Table 1. The soils are characterised by a neutral reaction (pH=7.2), high organic matter content (7.1 %) and medium to high nutrient (P, K) availability. The total Zn, Pb, and Cd contents are high (3075.0 $mg.kg^{-1}$ Zn, 2314.0 $mg.kg^{-1}$ Pb and 53.8 $mg.kg^{-1}$ Cd, respectively) and exceed the maximum allowable concentrations (320 $mg.kg^{-1}$ Zn, 100 $mg.kg^{-1}$ Pb, 2.0 $mg.kg^{-1}$ Cd) (Table 1). The soil additives are characterised by slight acid reactions, high organic matter, and low heavy metal content (Table 1).

The effect of the applied organic amendments on the main soil parameters is shown in Figure 1. The results show that soil's chemical and physical properties are affected by the type and amount of soil amendments.

Applying biochar increased soil pH relative to the control (Fig. 1), with the increase depending on the amount of additive used. The increase in soil pH can Pb to adsorption and precipitation of heavy metals (Cd as $CdCO_3$, Cu as $Cu(OH)_2$ and Pb as $Pb_5(PO_4)_3OH$ (Cao et al., 2011), their immobilisation (Beesley & Marmiroli, 2011) and a decrease in the heavy metal content in the above-ground mass of the plant.

**Table 1.** Physico-chemical properties of the soil, biochar and vermicompost used in the study

| Parameter | Soil | Biochar | Vermi-compost |
|---|---|---|---|
| pH | 7.2 | 6.7 | 6.4 |
| EC, µS.cm$^{-1}$ | 165.6 | 88.4 | 3.5 |
| Organic matter,% | 7.1 | 89.8 | 33.9 |
| P, mg.kg$^{-1}$ | 884.3 | 1653 | 1755 |
| K, mg.kg$^{-1}$ | 4127.5 | 33125 | 6497.5 |
| Ca, mg.kg$^{-1}$ | 30175 | 26250 | 19388 |
| Mg, mg.kg$^{-1}$ | 11163 | 29610 | 910.3 |
| Pb, mg.kg$^{-1}$ | 2314 | 2.9 | 32.5 |
| Cu, mg.kg$^{-1}$ | 200.7 | 23 | 215.4 |
| Zn, mg.kg$^{-1}$ | 3075.0 | 37.5 | 53.3 |
| Cd, mg.kg$^{-1}$ | 53.8 | 0.54 | 0.96 |
| Fe, mg.kg$^{-1}$ | 26475 | 336.1 | 5945 |
| Mn, mg.kg$^{-1}$ | 818.5 | 54.2 | 423.3 |
| Hg, ng.g$^{-1}$ | 327.9 | 24.9 | 721.1 |

MPCsoil (pH 6.0-7.4) - 320 mg.kg$^{-1}$ Zn, 100 mg.kg$^{-1}$ Pb, 2.0 mg.kg$^{-1}$ Cd, Hg-1.5 mg.kg$^{-1}$

The application of 10% vermicompost resulted in a decrease in soil pH, which is consistent with the results of Atiyeh et al. (2002), who found that an increase in the amount of vermicompost applied to the soil resulted in a decrease in soil pH.

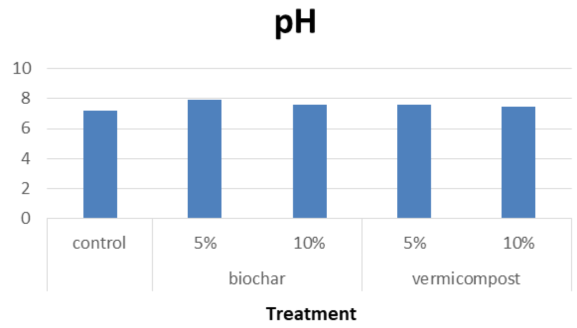
The electrical conductivity (EC) of the control soils (165.6 µS.cm$^{-1}$) for the soils of the NFMW-Plovdiv area) increased after the application of the organic amendments, and the amount of amendment applied also had an effect. This increase was more pronounced after using 5% and 10% vermicompost (519 and 1041 µS.cm$^{-1}$).

Although using vermicompost resulted in a 5-fold increase in EC, this did not result in soil salinisation. Biochar application also increased soil electrical conductivity, but the effect was less pronounced (206-236 µS.cm$^{-1}$).

The introduction of organic additives increases the amount of exchangeable cations Ca-BaCl$_2$, Mg-BaCl$_2$ and K-BaCl$_2$. The amounts of exchangeable cations varied significantly between treatments, influenced by the type of additive and the dose. The highest concentrations of K, Ca and Mg were recorded when 10% vermicompost was applied (Fig. 1). However, biochar application decreased the amount of exchangeable forms of K and did not significantly affect exchangeable Mg. The application of vermicompost increased soil CEC, influenced by the dose. As the amount of vermicompost applied increased, the CEC increased to 233.4 mmolc.kg$^{-1}$ and to 270.8 mmolc.kg$^{-1}$, respectively, relative to the control (211.5 mmolc.kg$^{-1}$). The application of 10 % biochar resulted in a marginal increase in soil CEC (217.1 mmolc.kg$^{-1}$)

Applying biochar and vermicompost increased soil organic matter content, with no significant difference, despite biochar's higher organic matter content (89.8%) (Table 1). The amount of organic matter in soils increased proportionally with the additive applied.

The application of 5% vermicompost and 5% biochar resulted in a slight increase in the content of mobile forms of Cd (to 29.5 mg.kg$^{-1}$ and 29.2 mg.kg$^{-1}$, respectively, compared to the control 28.6), while the higher dose of the additives resulted in a slight decrease (to 27.9 mg.kg$^{-1}$ and 26.9 mg.kg$^{-1}$, respectively). The decrease in the mobile forms of Cd extracted with DTPA may be due to the high cationic capacity of organic matter and the ability to bind Cd from the soil. A similar trend was observed for Zn. Applying 5% vermicompost and 5% biochar resulted in a slight increase in the mobile forms of Zn to 297.3 mg.kg$^{-1}$ and 305.1 mg.kg$^{-1}$, respectively. In contrast, applying the higher dose of the additives had no significant effect (Fig. 1). The application of organic additives decreased the mobile Pb content. This effect was more pronounced with applying the higher dose of the additives (10%) (to 760 mg.kg$^{-1}$ and 711 mg.kg$^{-1}$, respectively, compared to the control of 962 mg.kg$^{-1}$).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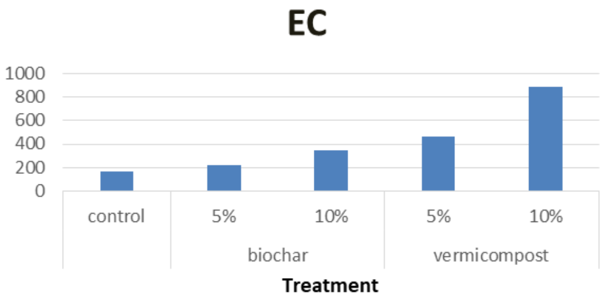
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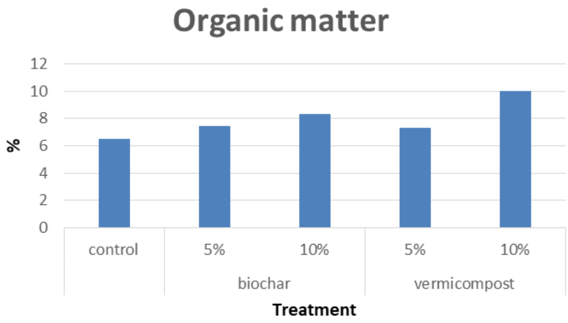
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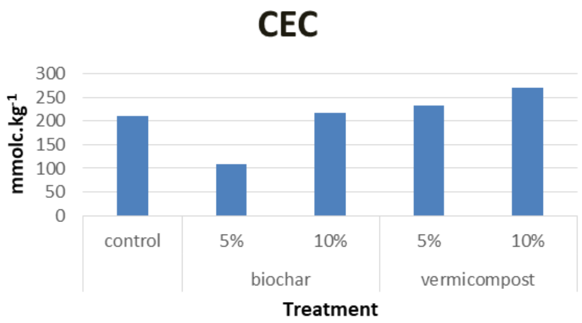
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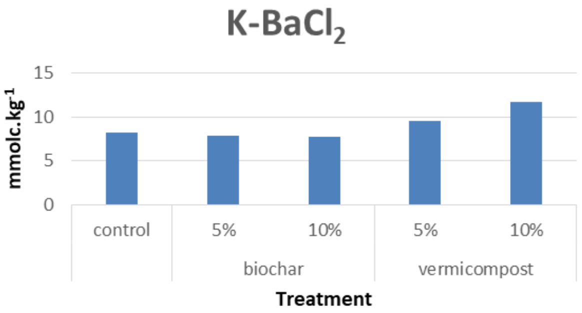
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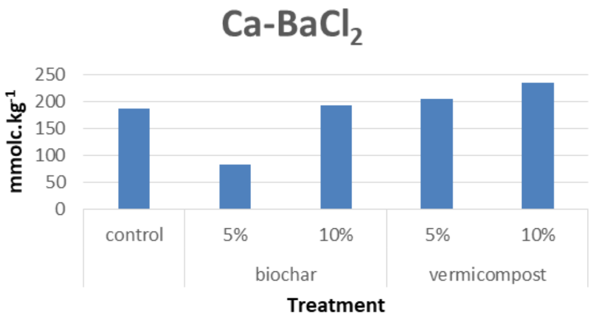
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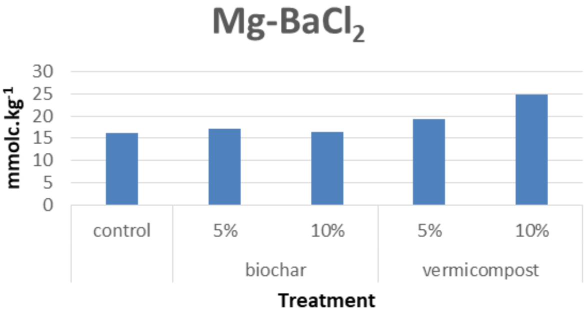
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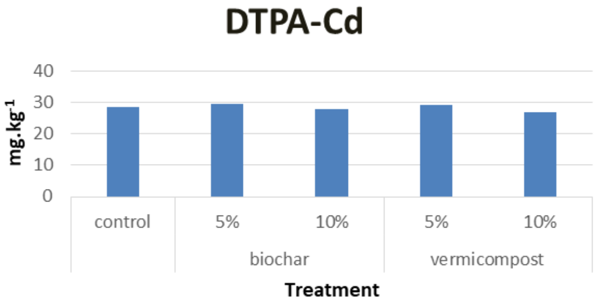
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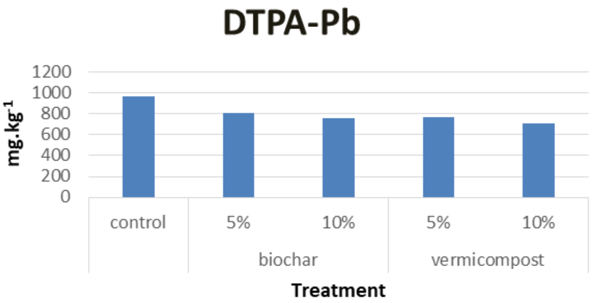
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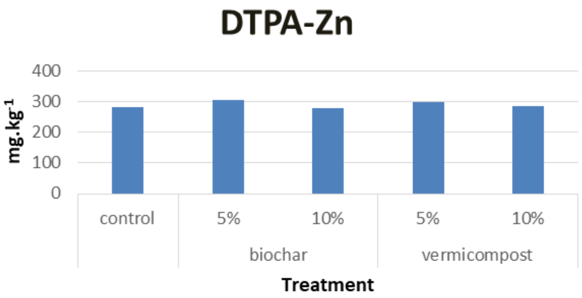
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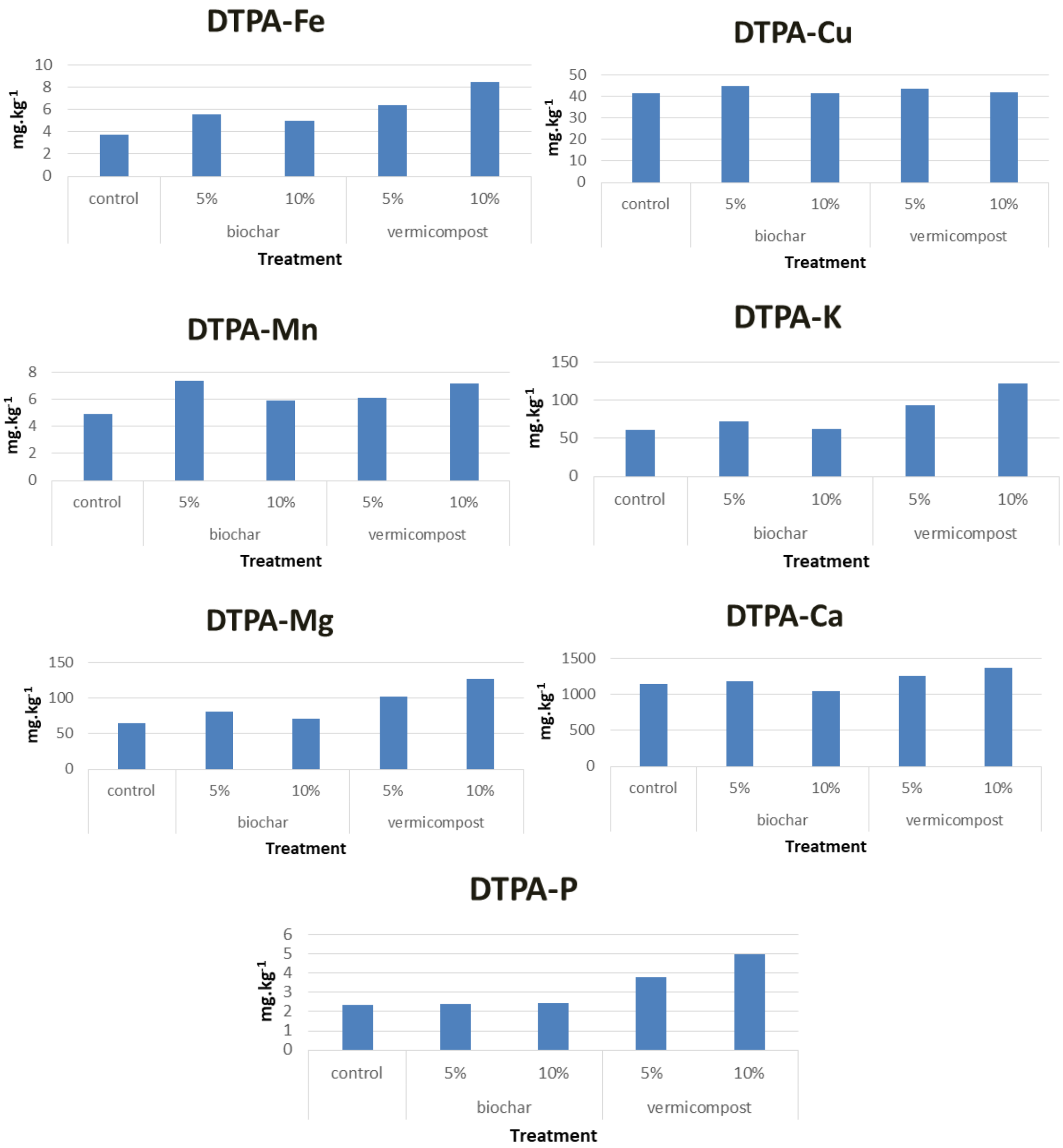

**Fig.1.** Effect of organic additives on pH, CEC, organic matter and DTPA mobile forms of elements in soils

The application of vermicompost and biochar resulted in an increase in the amount of the mobile Mn and Fe but did not significantly affect the content of DTPA-extracted Cu.

The application of organic amendments to the soil led to an increase in the content of the significant nutrient macroelements (P, K), the extent of this increase depending on the composition of the organic amendments (Figure 1). The application of vermicompost led to an increase in the content of DTPA-Ca and DTPA-Mg, which was more pronounced when 10% vermicompost was applied. Biochar application resulted in a slight increase in Ca and Mg content, with a more significant effect found when 5% biochar was used.

Fig. 2 shows the results obtained for the heavy metal, micro and macro element contents of the above-ground mass of *S. Plumbizincicola*.

The Cd content in the aerial mass of the plants reached 912.5 mg.kg$^{-1}$, significantly above the threshold value set for Cd hyperaccumulation (100 mg.kg$^{-1}$). The results obtained indicate the ability of *S. Plumbizincicola* to accumulate Zn in the above-ground mass (5440 mg.kg$^{-1}$). Still, the values are lower than the threshold value set for Zn hyperaccumulation (10000 mg.kg$^{-1}$). The potential ability of *S. Plumbizincicola* to extract Cd and Zn from contaminated soils has been demonstrated in greenhouse and field experiments (Wu et al., 2012a, Wu et al., 2013; from mine tailings and wastewater. The Cd and Zn contents were as high as 1,470 mg.kg$^{-1}$ and 14,600 mg.kg$^{-1}$, respectively. A probable reason for the lower Cd and Zn accumulation in this study is due to the different soil characteristics under *S. plumbizincicola* cultivation. In Zhejiang Province (China), the soils are sandy, acidic, and highly leached, whereas the soils of the NFMW area are alluvial soil with a high carbonate content. The results confirmed that the metal uptake efficiency of *S. Plumbizincicola* is significantly higher in acidic than in alkaline soils (Li et al., 2014).

Hyperaccumulators are tolerant to soil contamination with heavy metals. This is likely due to defense mechanisms including metal exclusion, active excretion, limited distribution of toxic metals in sensitive tissues, binding of metals to cell walls, chelation by organic molecules, and compartmentalization in vacuoles (Vogeli-Lange & Wagner 1990). Vacuolar sequestration and binding to cell walls may play a significant role in the metal hyperaccumulation. These mechanisms are involved in metal tolerance, transport, and storage in plants (Frey et al., 2000). Cao et al. (2014) found a preferential localization of Zn in the epidermis of young and mature leaves of *S. Plumbizincicola*, as well as higher Zn concentrations in the mesophyll of young leaves, which plays an essential role in Zn tolerance and hyperaccumulation. For Cd, it was found to be localized in the cell walls and the organelle fraction for both young and mature leaves of *S. Plumbizincicola*.

The Pb content reaches 194.3 mg.kg$^{-1}$ in the leaves. The translocation of Pb from the roots to the above-ground mass in plants is generally low. When Pb enters the plant's roots, it immediately interacts with phosphates, carbonates and bicarbonates contained in high concentrations in the intercellular spaces. As a result of this interaction, Pb precipitates as phosphates or carbonates and does not reach the xylem for translocation (Yang et al., 2014). The obtained results agree with Wu et al.'s (2012a) finding that *S. Plumbizincicola* has a low capacity to accumulate Pb despite a higher content of the mobile Pb in the soil.

The Cu and Mn contents in the above-ground mass of *S. Plumbizincicola* reached 6.8 mg/kg and 22.6 mg/kg, respectively, values lower than the critical values for plants (20-100 mg.kg$^{-1}$ Cu and 400-2000 mg.kg$^{-1}$ Mn) (Kabata Pendias, 2001).

The Fe content reached 248.8 mg.kg$^{-1}$ and was within the normal range (50-250 mg.kg$^{-1}$).

The Ca content in the above-ground mass of *S. Plumbizincicola* reached 31918.8 mg.kg$^{-1}$. According to Proctor (1971), Ca is one of the elements influencing the lowering of the toxicity of heavy metals. Plants growing on contaminated soils probably have a mechanism that allows them to absorb more Ca to compensate for the toxic action of various toxic metals. Broadhurst et al. (2013) reported exceptional Ca uptake in the leaves of the hyperaccumulator species. Plants of the family Crassulaceae are known to be calcitrophic species (Rössner and Popp 1986), meaning they contain a large amount of water-soluble Ca (White, 2003).

The content of K reached 8150 mg.kg$^{-1}$, P - up to 981,3 mg.kg$^{-1}$ and Mg - up to 3148,8 mg.kg$^{-1}$ in the above-ground mass of *S. Plumbizincicola.*

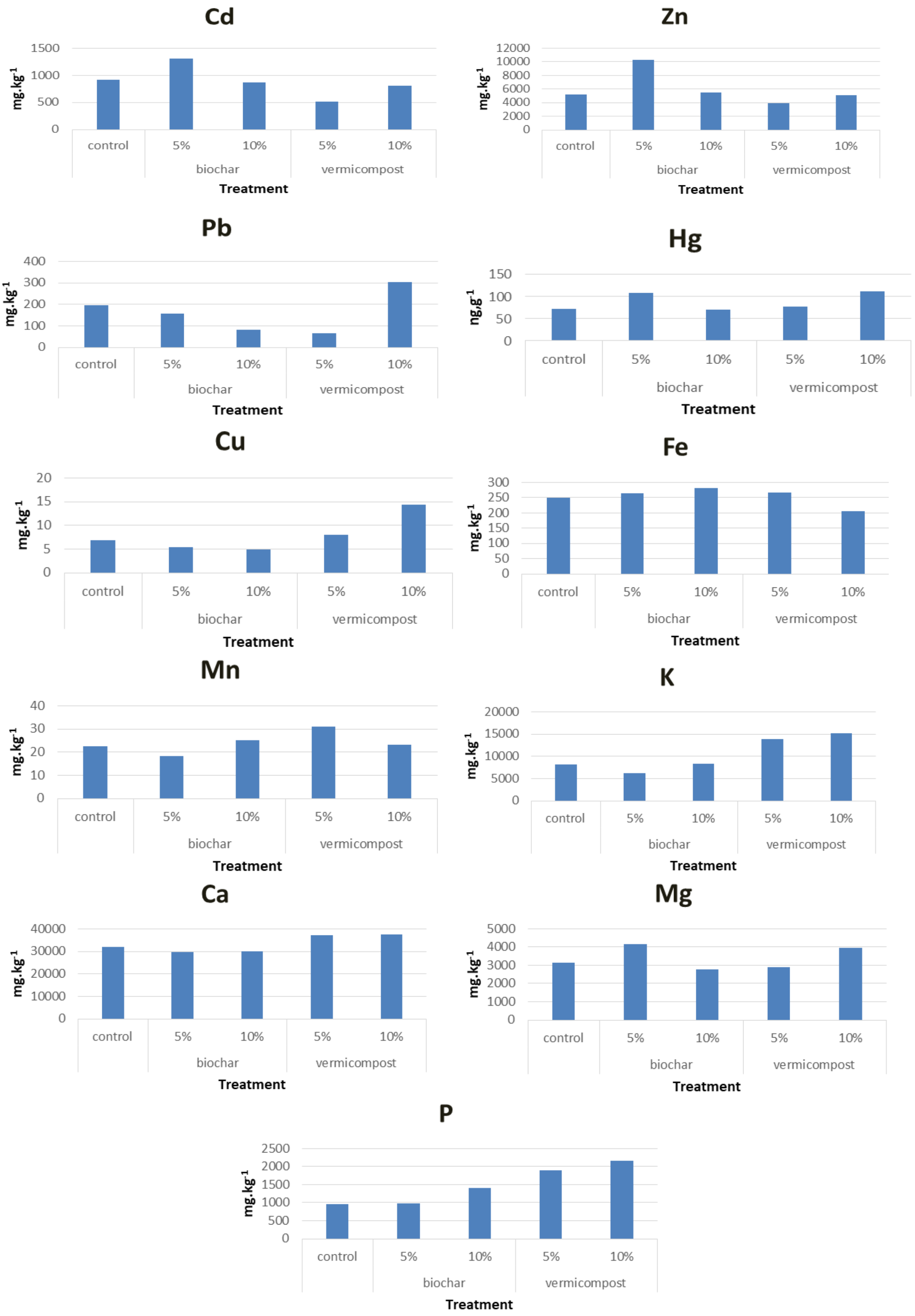

**Fig. 2.** Influence of organic additives on the content of elements in *Sedum Plumbizincicola*

Fig. 2 presents the results obtained on the effect of the introduced additives on the content of heavy metals and micro and macro elements in the above-ground mass of *S. Plumbizincicola*. The application of 5% vermicompost resulted in a decrease in the range of Pb from 194.3 mg.kg$^{-1}$ in control to 65.5 mg.kg$^{-1}$, Zn from 5191.9 mg.kg$^{-1}$ to 3967.1 mg.kg$^{-1}$ and Cd from 912.5 mg.kg$^{-1}$ to 519.0 mg.kg$^{-1}$ (Fig. 2). Application of 10% vermicompost had no significant effect on Zn and Cd contents compared to the control, while an increase was observed for Pb and Hg.

Applying 5% biochar increased Zn content to 10325.8 mg.kg$^{-1}$, Cd to 1309.6 mg.kg$^{-1}$ and Hg to 107.0 ng.g$^{--1}$, while a decrease was observed in Pb to 155.3 mg.kg$^{-1}$. Application of a higher dose of biochar did not significantly affect plants' Cd and Zn contents. The amount of the mobile forms of heavy metals can explain the results obtained. The dilution effect of the increase in plant biomass also influenced the heavy metal content.

Biochar application decreased Cu uptake by *S. Plumbizincicola*, which was more pronounced at 10% biochar application (4.84 mg.kg$^{-1}$). Vermicompost application increased Cu content from 6.8 mg.kg$^{-1}$ in control to 14.4 mg.kg$^{-1}$.

The application of biochar resulted in an increase in the Fe content of the above-ground of *S. Plumbizincicola*. As the amount of supplement applied increased, the amount of Fe increased from 248.8 mg.kg$^{-1}$ in control to 264.6 mg.kg$^{-1}$ and 282.2 mg.kg$^{-1}$. The application of 5% vermicompost also increased content to 267.4 mg.kg$^{-1}$, while a decrease was observed in the 10% vermicompost variant (205.7 mg.kg$^{-1}$).

An increase in Mn content in above-ground mass was found only in the variants with an application of 10% biochar (25.1 mg.kg$^{-1}$) and 5% vermicompost (31.1 mg.kg$^{-1}$) compared to control (22.6 mg.kg$^{-1}$)

The application of vermicompost resulted in a significant increase in the P and K content of the above-ground mass of *S. Plumbizincicola* compared to the control. Application of a higher dose (10% vermicompost) resulted in a significant increase in leaf P and K contents (P from 959.4 mg.kg$^{-1}$ in control to 2168.1 mg.kg$^{-1}$ in 10% vermicompost, K from 8150 mg.kg$^{-1}$ to 15255.2 mg.kg$^{-1}$ and 5964 mg.kg$^{-1}$, respectively). The increase in P and K may be due to the effect of the additive used on the uptake and/or movement of these elements by the plant. Vermicompost contains significant amounts of P and K, resulting in an increase in their content in the soil and their uptake by plants. However, the application of 5% biochar resulted in a decrease in the range of K and P in the above-ground mass of the plants (Fig. 2). The application of biochar resulted in a reduction of Ca content from 31918.8 mg.kg$^{-1}$ in control to 29770.7 mg.kg$^{-1}$ in the 5% biochar variant. In contrast, an increase was observed with the application of vermicompost at both rates (Fig. 2). The effect of organic additives on Mg uptake from *S. Plumbizincicola* leaves was not unidirectional. Applying 5% biochar and 10% vermicompost increased leaf Mg content, while using 5% vermicompost and 10% biochar resulted in a decrease in Mg content.

An increase in Mg content was found only in the treatments with the application of 5% biochar 4148.2 mg.kg$^{-1}$ and 10% vermicompost 3965.4 mg.kg$^{-1}$ compared to the control (3148.8 mg.kg$^{-1}$).

Notably, the effect of ameliorants on Ca and Mg uptake by *S. Plumbizincicola* leaves was significantly less pronounced compared to that of K and P. Similar results were obtained by Gallardo-Lara and Nogales (1987), who reported that no significant changes in Ca and Mg contents were observed after the application of organic meliorants. Applying biochar and vermicompost to the soil increased the mobile forms of Ca and Mg (Fig. 2), and this increase did not correspond to Ca and Mg content of *S. Plumbizincicola* shoots compared to the control. The increased plant biomass can explain the results obtained due to the application of organic amendments to the soil. The formation of more significant biomass decreases the amount of micro and macro elements taken up by some plants (a dilution effect in the organic matrix occurs) (Singh et al., 2016).

Figure 3 shows the effect of organic amendments on plant growth and development. The biomass produced is significantly influenced by the additives used. The application of vermicompost has pronounced a beneficial impact on plant development and increases above-ground mass. Increasing the vermicompost dose from 5 % to 10 % increased the plant biomass from 25.0 g to 51.7 g fresh weight. The biomass increased to 19.0 g when 10% biochar was added, while a significant decrease (11.4 g) was observed when 5% was added.

The yield of Cd and Zn from the plants was also calculated. The application of organic additives significantly affects Cd and Zn yield from plants, as the type of additive and the dose have an influence. The Cd and Zn yield in the variant with 10% vermicompost application was 2 to 3 times higher than the control. The application of 5% vermicompost resulted in a decrease in Cd and Zn yield. Applying 10% biochar resulted in a negligible increase in Cd and Zn yields.

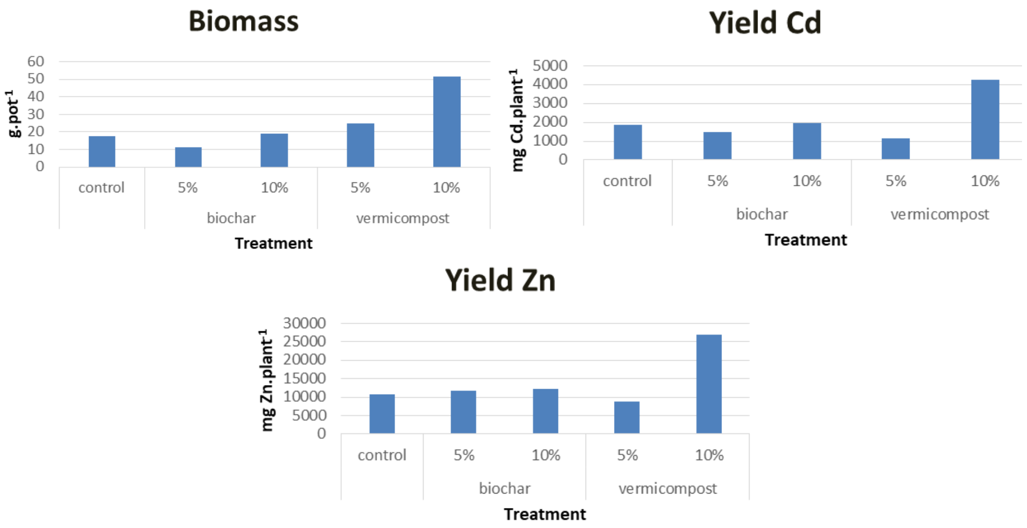

**Fig.3**. Effect of organic amendments on the biomass and yield of Zn and Cd

## CONCLUSION

Based on the obtained results, the following more important conclusions can be drawn:

1. Introduction of organic additives into the soil influences the physicochemical properties and leads to an increase in organic matter, electrical conductivity and the content of macroelements (P, K, Ca and Mg) and trace elements (Fe, Mn, Zn) in the soil, this increase depends on the type and composition of the organic additive.
2. The effect of organic additives on the mobile forms of Cd and Zn is similar. Applying 5% vermicompost and 5% biochar resulted in a slight increase in the content of Cd and Zn mobile forms. In contrast, the higher dose of the additives resulted in a slight decrease in Cd content and had no significant effect on Zn. The addition of vermicompost and biochar increased the amount of mobile Mn, Fe, Ca, Mg, P, and K, lowering the content of mobile Pb, but did not significantly affect the content of DTPA Cu.
3. Applying 5% biochar increased Zn, Cd and Hg content and decreased Pb content in *S. Plumbizincicola shoots*, while 10% biochar did not significantly affect Cd and Zn content.

4. Application of 5% vermicompost resulted in a lowering of Pb, Zn and Cd contents in *S. Plumbizincicola shoots*, while 10% vermicompost increased Pb and Hg and had no significant effect on Zn and Cd contents.

5. Organic additives affect the content of micro and macro elements in the above-ground mass of *S. Plumbizincicola*, with the type of additive and dose having an influence. Applying 10% biochar resulted in an increase in Fe, Mn, K and P contents and a decrease in Cu, Ca and Mg contents. Application of 10% vermicompost resulted in an increase in Cu, Mn, K, Ca, Mg and P and a decrease in Fe.

6. The amount of biomass is affected by the type and amount of organic additives used. The application of vermicompost has a pronounced beneficial effect on plant development and increases above-ground mass. A positive result is also observed when 10% biochar is applied.

7. 5. Organic additives affect yield, influenced by the type of additive and dose. Cd and Zn yields were up to 3 times higher in the variant with 10% vermicompost application. Application of 10% biochar resulted in a negligible increase.

8. It is necessary to optimize the amount of additions to obtain an optimum balance between plant growth and the accumulation of Cd and Zn in the *S. Plumbizincicola* shoots, for optimum phytoextraction. The field studies must also be conducted to evaluate the effect and potential benefits of the vermicompost application to the soil for Cd and Zn phytoextraction.

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