(Ahead of print) EFFECT OF ORGANIC AMENDMENT ON SOIL CHARACTERISTICS AND THE UPTAKE OF NICKEL BY ODONTARRHENA CHALCIDICA

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Abstract

A comparative research on the impact of organic amendments on the soil characteristic and uptake of heavy metals, micro and macroelements of Odontarrhena chalcidica has been carried out. Experiments have been implemented in controlled conditions. The serpentine soil used in this experiment was sampled from the vicinity of the village Kazak, Bulgaria. The pot experiment was a randomized complete block design containing 9 treatments and three replications (27 pots). The treatments consisted of a control (no organic meliorants), and compost, vermicompost, biochar and activated carbon (added at 2.5% and 5.0%, respectively, recalculated based on dry soil weight). The application of organic additives to the soil influences the physicochemical properties and leads to an increase in organic matter and the content of macroelements (P, K, Ca and Mg) and trace elements (Fe, Mn, Zn) in the soil. The application of organic additives to the soil affects the uptake of Ni, micro and macroelements by Odontarrhena chalcidica. Plant biomass increased significantly, and there was a clear correlation between the amount of supplement applied and the increase in biomass. Organic additives had a positive effect on yield, influenced by the type of additive and dose. Ni yield was 3 times higher in the variants with 5% compost and 2.5% vermicompost input. The application of biochar resulted in a twofold decrease in Ni yield, while the application of activated carbon resulted in a marginal increase.

Key words: phytomining, activated carbon, biochar, compost, vermicompost, serpentine soils, *O. chalcidica*

INTRODUCTION

Phytomining relies on hyperaccumulation by plants to extract metals from the soils and accumulate them in aboveground plant tissues. High biomass yield and metal concentration in plant tissues ensure the success of the phytomining process (Zhang et al., 2014; Novo et al., 2017). It is widely believed that an increase in mobile soil metal content leads to high bioaccumulation in plant tissues; therefore, an increase in labile forms of metals favors phytomining. This can be achieved either by changes in soil pH (e.g., an increase in pH mobilizes the arsenic oxyanions, whereas a decrease in pH mobilizes most metal cations) (Zheng et al., 2019) or by the addition chelating agents (such as EDTA) of

(Nedelkoska & Doran, 2000). Greater biomass be obtained by adding various can amendments, e.g. inorganic fertilizers and organic amendments (such as compost, biochar and animal waste). Most of the literature related to the use of organic additives in soil phytoremediation focuses on phytostabilization, and relatively few studies in phytoextraction of metals from soils, mainly with metal-accumulating plant species and rarely with hyperaccumulators.

The aim of the present study was to establish the influence of organic soil additives on Ni uptake by *Odontarrhena chalcidica*, as well as the possibilities of their use for Ni phytoextraction from Bulgarian serpentine soils.

MATERIALS AND METHODS

Vegetation experiments were conducted on serpentine soils from the area of Kazak. The experiments were set in 0.25 l containers with dimensions 7x7x8 cm, in 9 variants with 3 repetitions according to the following scheme. 1. Control soil (no addition). 2. Soil + compost (2.5%). 3. Soil + compost (5%). 4. Soil + vermicompost (2.5%). 5. Soil + vermicompost (5.0%). 6. Soil + biochar (2.5%). 7. Soil + Biochar (5.0%). 8. Soil + activated carbon (2.5%) and 9. Soil + activated carbon (5.0%).

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, 5 seeds of the test plant Odontarrhena chalcidica were sown in each pot. The containers with the plants were placed in a growth chamber. Harvesting was done 90 days after sowing. The plants were cut one inch above the soil, washed with distilled water and placed in labeled paper envelopes. After removal of plant roots, soils were air-dried and sieved through a sieve (< 2 mm) and stored in paper envelopes for subsequent analyses. Soil pH (ISO 10390), 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 heavy metal content, micro and macroelements in plant samples (aboveground mass) was determined by the microwave mineralization method (EPA method 3052).

The SPSS for Windows program was used in the statistical processing of the data. Correlation-regression analysis was performed between pH, organic matter content, and mobile forms of nickel in soil and the concentration of the elements in the tested plant.

RESULTS AND DISCUSSION

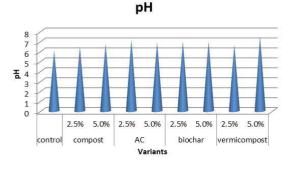
The effect of the applied organic amendments on the main soil parameters is shown in Figure 1. The results show that the chemical and physical properties of soil are affected by the type and amount of soil amendments.

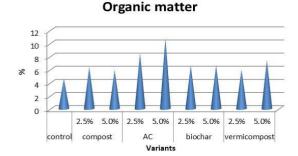
The application of organic additives leads to an increase in soil pH (Fig. 1), and the increase depends on the amount of additive applied. In general, the application of additives results in a change in soil pH to slightly alkaline values. In serpentine soils, an increase in pH can lead to a decrease in the Ni content of the aerial parts of hyperaccumulator plants.

The amount of exchangeable ions was determined by extraction with 0.1 M BaCl₂ solution. Addition of organic additives resulted in an increase in the amount of exchangeable cations Ca-BaCl₂, Mg-BaCl₂ and K-BaCl₂.

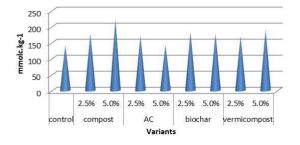
The amounts of Ca-BaCl₂ varied significantly between treatments. being influenced by the type of additive and dose. The highest Ca values were recorded for the 5% vermicompost application, while for Mg-BaCl₂ the highest values were recorded for the 5% compost application. The amount of exchangeable ions increased in direct proportion with increasing dose of compost applied. A similar but less pronounced relationship observed for was the vermicompost variants. However, the opposite trend is observed for activated carbon and biochar.

In serpentine soils the Ca:Mg ratio is usually low and less than 1 (Ca:Mg < 1). A low Ca:Mg ratio is often associated as one of the limiting growth factors of serpentine soils (Brooks, 1987), but hyperaccumulators are naturally adapted to low Ca:Mg ratios. The application of organic amendments influences the Ca:Mg ratio. The Ca:Mg ratio was less than 1 in the control and the treatments with compost, activated carbon and biochar inputs, except for vermicompost inputs (Ca:Mg ratio > 1). The Ca:Mg ratio remained almost unchanged with compost and activated carbon, decreased with biochar and increased to 1.22-1.34 with vermicompost. The Ca:Mg ratio values depend on the extent to which the Mg content increases relative to Ca. In the biochar variant, Mg increases more than Ca, whereas the opposite trend is observed with vermicompost.

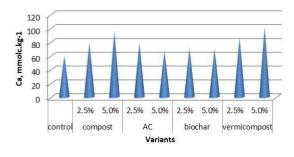


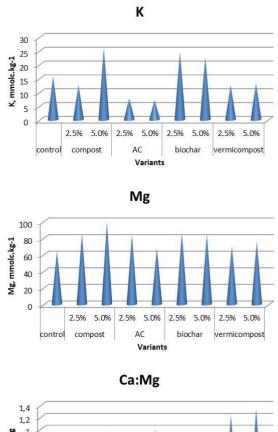












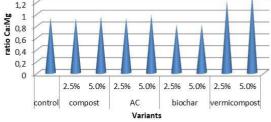


Fig. 1. Effect of organic additives on pH, CEC (mmolc.kg⁻¹), organic matter (%) and concentrations of Ca, Mg and K (mmollc.kg⁻¹) and Ca:Mg ratio

The amounts of K-BaCl₂ varied significantly between treatments, influenced by the type of additive and dose. The highest K values were recorded for the biochar and 5% compost inputs and the lowest for the activated carbon inputs. Lower values compared to the control were also found for vermicompost application. The amount of exchangeable K ions increased in direct proportion with increasing dose of compost applied. In the other treatments, the amount of additive did not have a significant effect on the exchangeable K ion content.

The application of organic additives resulted in an increase in soil CEC, with the dose having an influence. As the amount of compost and vermicompost applied increased to 5%, the CEC increased to 21.99 mmolc.kg⁻¹ and 18.98 mmolc.kg⁻¹, respectively, compared to the control (13.99 mmolc.kg⁻¹). Application of activated carbon and biochar also resulted in an increase in soil CEC, but this increase was more pronounced at the lower rate (2.5%).

Organic matter plays an important role in soil due to its high cation exchange capacity as well as its ability to form complex compounds. Soils treated with compost, vermicompost, activated carbon and biochar had significantly higher organic matter content than the control. The results show that organic matter increased proportionally with the amount of applied activated carbon and vermicompost. No such trend was observed for compost and biochar.

The organic matter content reached 35.97 and 7.43% when 2.5% and 5% vermicompost was applied. The application of activated carbon resulted in an increase in organic matter content to 8.49 and 10.78% (Fig. 1).

The mobile forms of metals were measured using DTPA extraction (diethylene triamine pentaacetic acid). The amount of Ni extracted from the DTPA solution represents Ni that is exchangeable and complexed to the surfaces of organic matter and clay minerals, and is therefore available for uptake by plants (Echevarria, 2018).

The application of organic additives leads to a decrease in the bioavailable Ni (DTPA) content of the soil, and this decrease is more pronounced at 5% additive application in all treatments. The most significant decrease was observed with the application of 5% vermicompost (77.4 mg.kg⁻¹) (Fig. 2).

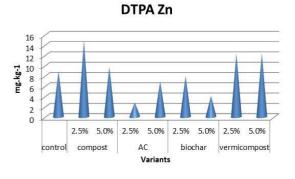
A significant negative correlation was found between organic matter content and DTPA-Ni (r=0.71). Soils with higher organic matter content had lower DTPA-Ni values. The lower DTPA-Ni content may be explained by the higher metal holding capacity of soils as a result of increasing organic matter content, where greater binding of cations to negatively charged surface or organic ligands is observed. The results from this study are consistent with Alvarez-Lopez et al. (2016), who reported a decrease in extractable Ni after compost addition.

The mobile forms of Ni are affected by soil texture, pH, electrical conductivity (EC), and organic matter (Chardot et al., 2007; Kabata Pendias, 2011). Chardot et al. (2007) reported a significant positive correlation of Ni-DTPA with exchangeable Ca and CEC, as well as with pH, clay content, total Mg and Fe, exchangeable Mg and free Fe oxides. However, Chardot et al. (2007) and Alves et al. (2011) found no significant correlation between DTPA-extractable Ni and soil carbon content.

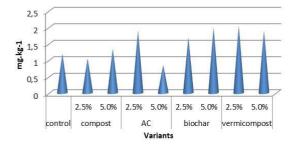
The results show that DTPA-Ni decreases with higher CEC and Ca values. The addition of compost leads to a decrease of available Ni in the soil, which is consistent with the results of Thuringer (2020). This can be partly, but not entirely, explained by soil dilution as well as the formation of organometallic insoluble complexes that reduce the bioavailability of metals in soil (Vangronsveld et al., 2009). The effect of compost addition on the available forms of Ni in soil reinforces the need to optimize the amount of addition to maximize biomass productivity, and minimize the reduction of plant-available forms of the metal, as well as prevent reduced uptake and accumulation of the metal in plants.

Soils treated with compost and vermicompost contained significantly more P than control soils (Fig. 2). The amount of applied amendments also had an effect. The application of compost and vermicompost resulted in an increase in soil P content, with this increase being more pronounced when 5% compost (4.25 mg.kg⁻¹) and 5% vermicompost (4.85 mg.kg⁻¹) were applied. The application of activated carbon at both rates resulted in a

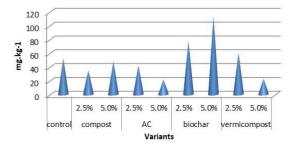
decrease in P content, while the application of 2.5% biochar resulted in a slight increase to 3.0 mg.kg⁻¹ and a decrease at 5% amendment.



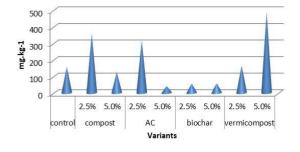


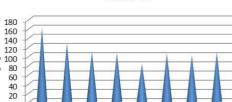




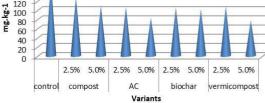




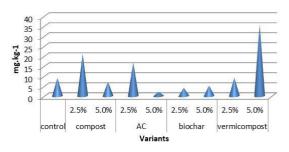




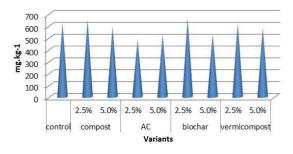
DTPA Ni



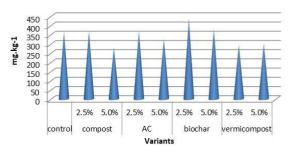












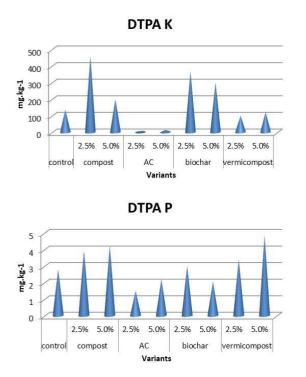


Fig. 2. Influence of organic additives on DTPA mobile forms of elements in soils

The increase in DTPA-extracted P after compost and vermicompost application may be due to the release of humic acids during decomposition of organic matter, resulting in the conversion of non-digestible forms of phosphorus to digestible forms.

DTPA-extracted K increased significantly with compost and biochar application, and this increase was more pronounced with the 2.5% amendment (Fig. 2). Activated carbon application resulted in a significant decrease in K to 13.7mg.kg⁻¹, while vermicompost application resulted in a slight decrease in K to 99.4mg.kg⁻¹ and 121.6mg.kg⁻¹.

The application of 2.5% compost, biochar and vermicompost did not affect the amount of Ca in the soil, while the application of 5% supplement resulted in a decrease in Ca content. The application of activated carbon resulted in a decrease in Ca content to 479.8mg.kg⁻¹ and 522.6mg.kg⁻¹, respectively.

The application of vermicompost resulted in a slight decrease in soil Mg content, with no effect of the amount of additive applied. Biochar resulted in an increase in Mg content, with this increase being more pronounced when a 2.5% additive was applied. The application of 2.5% compost and activated charcoal did not affect the amount of Mg in the soil, while a decrease was observed with the application of 5%.

The application of biochar resulted in an increase in soil Mn content compared to the control, and this increase was more pronounced with the application of 5% amendment (489mg.kg⁻¹). The application of 2.5% compost and vermicompost resulted in an increase in Mn content, while a decrease was observed with 5% amendment.

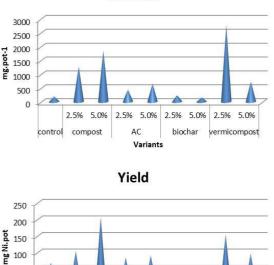
The Fe mobility was significantly affected by the applied amendments (Fig. 2). The application of biochar resulted in an increase in soil Fe content, this increase being more pronounced at 5% amendment (113.1mg.kg⁻¹). Application of 5% activated carbon and 5% vermicompost resulted in a decrease in Fe content to 21.5 mg.kg⁻¹ and 22.55mg.kg⁻¹, respectively.

The application of compost, biochar and vermicompost resulted in an increase in soil Cu content, while the opposite trend was observed for activated carbon.

The application of vermicompost resulted in an increase in the mobile forms of zinc up to 12.35mg.kg⁻¹, while the application of activated carbon and biochar resulted in a Zn decrease. The application of compost leads to an increase in the zinc content of the soil, with a more significant increase in the 2.5% compost application option.

Fig. 3 shows the effect of organic amendments on plant development. The application of organic amendments has a pronounced beneficial effect on plant development and leads to an increase in aboveground plant mass. When 5% activated charcoal and 5% biochar were applied, the biomass increased to 665 mg and 766 mg, respectively. However, the most significant increase was with the application of compost, leading to an increase in plant biomass to 1281 mg (2.5% compost) and to 1852 mg (5%

compost). Increasing the compost rate from 2.5% to 5% resulted in a significant increase in plant biomass, consistent with the results of Alvarez- Lopez et al. (2016).



Biomass

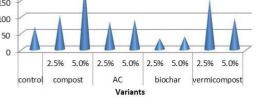


Fig. 3. Effect of organic amendments on biomass and yield of Ni

Fig. 4 presents the results obtained for the Ni, micro and macroelement contents in the above ground mass of the tested plant *O*. *chalcidica*.

The Ni content in the aboveground of the control plants reached mass 3504.4mg.kg⁻¹, which significantly exceeded the threshold value of 1000mg.kg⁻¹ set for Ni hyperaccumulation. The amount of Ca taken up by the plants reached 40004.2mg.kg⁻¹. Bani et al. (2009) found that Ca and Ni could be antagonists and the higher Ca content in leaves could be explained by the lower Ni concentrations in O. chalcidica leaves. The Ni:Ca ratio was lower than unity (0.088), and the Ni:Mg ratio was lower than unity (0.12).

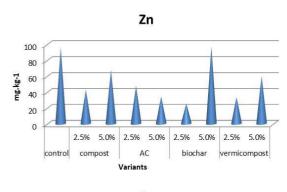
Although Mg content is high in soils, Mg accumulates to a significantly lesser extent compared to Ni and Ca. The Mg content reaches in aboveground mass up to 29412.7mg.kg⁻¹. A correlation was found between Ca and Ni uptake (r=0.82) and Mg and Ni uptake (r=0.73) in O. chalcidica leaves, similar to other authors (Broadhurst & Chaney, 2016; Thuringer, 2020).

The Ca/Mg ratio in the aboveground mass was > 1. Plants from serpentine soils have the ability to maintain a ratio greater than 1 despite the low Ca content of the soil (and unfavourable Ca/Mg ratio for plant growth). Plants probably have very efficient Ca uptake systems or the ability to exclude Mg despite high soil Mg concentrations. Ni hyperaccumulators have been found to uptake large amounts of Ca from soils and due to the repeated collection and removal of biomass during phytoremoval, agrotechniques such as Ca fertilization are necessary to maintain soil fertility to appropriate levels (Chaney et al., 2007). Some authors recommend adding gypsum to serpentine soils to overcome this limitation (Cabello-Conejo et al., 2013), but organic amendments (such as the compost and vermicompost used here) could also be used for this purpose.

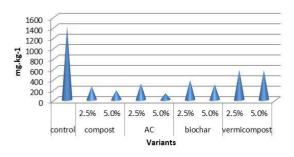
The P content of the control plants reached 1720.6mg.kg⁻¹ for P and 22127.0mg.kg⁻¹ for K. Potassium is a key plant nutrient whose most important role is the maintenance of water balance in plants (osmoregulation). Although K is low in serpentine soils, it accumulates in the aboveground mass of hyperaccumulator plants.

The Fe content reached up to 1422.7mg.kg⁻¹ in the tested control plants. Similar values for the Fe content in the aboveground mass of *O. chalcidica* was also found by other authors (Bani et al., 2014; Broadhurst & Chaney, 2016; Xhaferri et al., 2018).

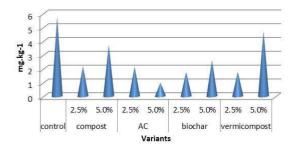
The Mn content of the control reached up to 672.6mg.kg⁻¹, Co up to 160.1mg.kg⁻¹, Zn up to 95.7mg.kg⁻¹ and Cu up to 5.7mg.kg⁻¹.



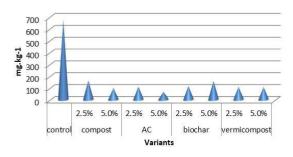


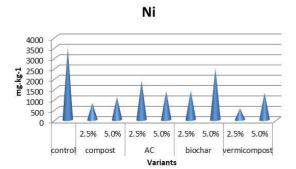


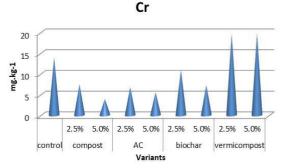




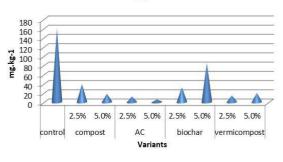




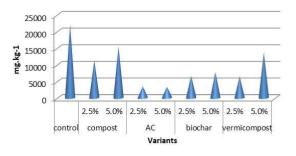


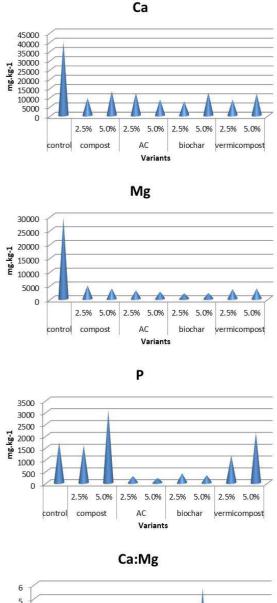


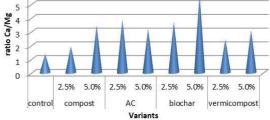


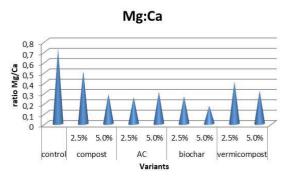


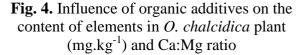












The low Cr content of *O. chalcidica* confirms the findings of Brooks (1987) that serpentine plants without exception contain only traces of Cr. Despite high total soil Cr, the amounts of Cr accumulated by *O. chalcidica* only 13.9 mg.kg⁻¹. Plants from serpentine soils have been found to generally contain <15mg.kg⁻¹ Cr (Reeves, 1992).

Ni uptake efficiency was calculated the bioaccumulation factor using (BAF=Cshoots/Csoils) BAF values indicate that Ni in plant tissue of O. chalcidica is up to 2.2 times higher than in soil. These values show once again the ability of O. chalcidica to accumulate Ni in its aboveground mass. Plants also assimilate significant amounts of P and Ca despite their low content in soil. The results confirm that the tested plant is а hyperaccumulator and can be used for Ni phytoextraction.

The application of organic amendments significantly influenced Ni uptake by the tested plants. Fig. 4 shows also the results obtained for the effect of the introduced additives on the Ni, heavy metals, micro and macroelement content of the above-ground mass of *O. chalcidica*. The Ni content of the tested plants was higher in the control compared to the variants with additive application. Application of organic additives resulted in lower Ni content in the plants, influenced by the type of additive and dose. The application of a higher dose of the additives resulted in a smaller decrease in the Ni content of the plants. In

plants where compost was applied to the soil, the Ni content varied from 810.0mg.kg⁻¹ (2.5% compost) to 1112.3mg.kg⁻¹ (5% compost). Similar results were obtained when vermicompost was applied. Slightly higher values were found for the activated charcoal and biochar input treatments, which reached 1901.3mg.kg⁻¹ (5% activated charcoal) and 2505.0mg.kg⁻¹ (5% biochar). Our results are in agreement with the results of Bani et al. (2007), who suggest that the cause is the dilution process in a closed system. Similar results were obtained by Alvarez-Lopez et al. (2016), who found that the addition of compost significantly reduced the amount of Ni compared to plants growing in untreated soils. The authors found that increasing the compost rate (from 5% to 10%) was accompanied by a further decrease in the Ni concentration in the above-ground mass.

A correlation was found between Ni content in the above-ground mass and Ni-DTPA.

The uptake of micro and macro elements by the test plant was influenced by the applied organic additives. In most cases, there was a tendency for their content to decrease after the application of the organic additives (Fig. 4).

The application of organic additives leads to a decrease in the content of the trace elements zinc, Cu, Fe and Mn in the plants. The trend is similar for the macroelements K, Ca and Mg. According to Alvarez-Lopez et al. (2016), above-ground Ca content decreased in plants grown in NPK-treated compared to untreated soils, while the addition of compost at 5% and 10% resulted in a significant increase in Ca content, which was not confirmed by our results.

The application of organic amendments also affected Ca:Mg and Mg:Ca ratios. The Ca:Mg ratio in both the control and all treatments with organic supplementation was greater than 1. The addition of compost increased the ratio from 1.36 in the control to 3.45, activated carbon to 3.22, biochar to 5.72 and vermicompost to 3.11.

The Mg:Ca ratio decreased from 0.74 in the control to 0.29 when 5% compost was applied, to 0.31 in the 5% activated carbon option, to 0.17 when biochar was applied and to 0.32 when 5% vermicompost was applied.

The Ca/Mg ratio of soil has been shown to be a relatively important factor in the growth and uptake of Ni by hyperaccumulators. Ghasemi & Ghaderian (2009) studied the effect of Ca/Mg ratio on hyperaccumulator A. inflatum and found that the plant has higher tolerance at low Ca/Mg ratio. The same authors found an increase of Ni in plants with Ca/Mg increasing ratio in hydroponic solutions. Similar results were obtained in this study that showed that the application of organic additives in the amount of 5% leads to an increase in the Ca/Mg ratio in the soil in which the uptake of Ni by plants increases. Opposite results were obtained by Bani et al. (2014) who found a decrease in Ni concentration in the above ground mass with increasing Ca/Mg ratio in compost amended soil. Similarly, Brooks et al. (1987) showed that the presence of high soil Ca concentrations resulted in lower Ni uptake bv Α. serpyllifolium. For this reason, when applying organic amendments (compost, vermicompost, etc.) it is important to regulate the Ca/Mg ratio in the soil. to obtain an optimal balance between plant growth and Ni accumulation in above-ground for optimum the mass phytoremoval. The addition of compost and vermicompost can increase the organic matter content and fertility of serpentine soils, but it is also important to optimize the amount of additions to achieve optimum plant growth and metal extraction. Field studies also need to be conducted to evaluate the effect and potential benefits of compost and vermicompost application to the soil for Ni phytoextraction.

Ni yield was also calculated. Maximizing the yield of hyperaccumulator crops is essential in phytomining. This study shows that application of organic amendments can improve soil fertility and hence plant

growth on serpentine soils. The application of organic additives has a significant effect on the yield of Ni from plants, as the type of additive and the dose have an influence. On the one hand, a decrease in the Ni content of the aboveground plant mass was found when they were applied to the soil, but on the other hand, a significant increase in plant biomass was recorded. Phytoextracted Ni increased after the addition of the organic additives due to the stimulation of plant growth. The results obtained after calculating the yield showed that the organic additives had a positive effect on the yield. In the variant with 5% compost application, the Ni yield was 3 times higher compared to the control. Similar results were obtained with 2.5% vermicompost application. Biochar application resulted in a twofold decrease in Ni yield, while activated carbon application resulted in a 1.2-1.3-fold increase (Fig. 3).Similar results were obtained by Bani et al. (2007), who found that when O. chalcidica over a period of 6 months, the phytoextraction yield of Ni was 84.8 mg Ni in untreated plants, 399 mg Ni in swine manure treatment; 265 mg Ni in chicken manure treatment and 152 mg Ni in NPK treatment. Significant yield increases were also found by Thuringer (2020) in NPK and compost treated soils, who found that compost addition had a higher growth promoting effect than inorganic fertilization.

The application of organic amendments (compost, vermicompost, activated carbon, and biochar) has a positive effect on improving the quality of serpentine soils. All tested organic additives are a source of essential nutrients (which are deficient in serpentine soils), improve soil structure and porosity and water holding capacity, which may explain the positive effect of additives on plant yields. The addition of compost, vermicompost, biochar and activated carbon improves soil physicochemical properties such as nutrient content, CEC, organic matter content and improves the Ca/Mg ratio in the soil.

Phytoextracted Ni increases after the

addition of organic amendments due to the stimulation of plant growth. In the treatments with 5% compost and 2.5% vermicompost application, plant Ni yield was 3 times higher compared to the control. However, field studies need to be conducted to evaluate the effect and potential benefits of compost and vermicompost application to soil on Ni yield.

CONCLUSION

On the basis of the obtained results the following more important conclusions can be drawn:

1. The application of organic additives to the soil influences the physicochemical properties and leads to an increase in organic matter and the content of macroelements (P, K, Ca and Mg) and trace elements (Fe, Mn, Zn) in the soil, and this increase depends on the type and composition of the organic additive.

2. Organic additives reduce the amount of DTPA-extracted mobile forms of Ni, with the decrease being proportional to the amount of additive applied.

3. The amount of biomass is affected by the type and amount of organic additives used. Plant biomass increased significantly, with a clear correlation between the amount of additive applied and the increase in biomass.

4. The application of organic additives to the soil affects the uptake of Ni, micro and macro elements of *O. chalcidica*. There was a distinct trend of decreasing Ni content in the test plant after the application of all the four additives in the soil.

5. Organic additives have a positive effect on yield as influenced by additive type and dose. Ni yield was 3 times higher in the treatments with 5% compost and 2.5% vermicompost application. Biochar application resulted in a twofold decrease in Ni yield, while activated carbon application resulted in a 1.2-1.3-fold increase.

6. It is necessary to optimize the amount of additions, adjust the Ca/Mg ratio in the soil to obtain an optimal balance between

plant growth and Ni accumulation in the above ground mass for optimum phyto yield. Field studies also need to be conducted to evaluate the effect and potential benefits of compost and vermicompost application to the soil for Ni phytomining.

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