Hindawi Publishing Corporation
Applied and Environmental Soil Science
Volume 2014, Article ID 506142, 10 pages
http://dx.doi.org/10.1155/2014/506142

*Research Article*

# The Effect of *Trichoderma* on Heavy Metal Mobility and Uptake by *Miscanthus giganteus*, *Salix* sp., *Phalaris arundinacea*, and *Panicum virgatum*

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Received 6 October 2013; Revised 11 December 2013; Accepted 12 December 2013; Published 9 February 2014

Academic Editor: Teodoro Miano

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The effect of land application of biomaterials based on two strains of *Trichoderma* fungus on phytoremediation processes was studied. Six metals (Cd, Cr, Cu, Pb, Zn, and Ni) were analysed in soil and soil leachate as well as in plant tissues. The translocation index ($T_i$) and metal bioconcentration factors (BCF) calculated for the inoculated plants were increased compared to the noninoculated control, except for Pb and *Salix* sp. Simultaneously, the mobilisation of metals in soil solution as an effect of biomaterials was noted. The highest values of $T_i$—339% (for Cr), 190% (for Ni), and 110% (for Cu)—were achieved for the combination *Miscanteus giganteus* and *Trichoderma* MSO1. The results indicated that the application of fungus has positive effects on increasing the biomass, soil parameters (C, N, and P), and solubility of heavy metals in soil and therefore in enhancing phytoextraction for *Miscanthus giganteus* L., *Panicum virgatum* L., *Phalaris arundinacea* L., and *Salix* sp.

## 1. Introduction

Successful plant cover and high biomass production with a high metals concentration are prerequisites for each phytoremediation process. The phytoextraction technique has been described as a promising technique for the remediation of heavy metal contaminated agricultural land [1–3]. Generally, two kinds of plants are used: hyperaccumulators with a very high heavy metal accumulation potential and low biomass productivity and fast growing species, which have lower phytoextraction ability than hyperaccumulators, but whose total biomass production is significantly higher. Hence, to improve the metal accumulation capacities and uptake speed of nonhyperaccumulators, the addition of chelating agents has been proposed [4]. However, large quantities of highly contaminated plant material require disposal [5]. Some authors suggest that the combination of soil remediation by fast growing species with their consequent energetic utilisation seems to be a suitable technological process [6, 7]. Next to willow and poplar, there are several lignocellulosic herbaceous species, including grasses such as *Miscanthus*, reed canary grass, or switchgrass.

Areas degraded by heavy metals are often poor in organic matter and characterised by a low abundance of microorganisms; therefore, in order to accelerate the biogeochemical cycles, microorganisms (bacteria or fungi) should also be applied to soil [8]. Soil microorganisms can help in the absorption of plant nutrients, increase the performance of plants, and, consequently, improve the physical and chemical properties of contaminated soil. The presence of soil microorganisms in the rhizosphere of plants can intensify the phytoremediation process by increasing phytostimulation or rhizodegradation [9, 10].

Some of the fungi which can be used in the phytoremediation process are species of the genus *Trichoderma*, extremely common in nature, with the potential for colonisation of very different backgrounds. These beneficial fungi belong to the most resistant microorganisms for natural or synthetic chemicals and toxins and are able to reduce some of them. In addition, many species of *Trichoderma* are able to support

plant growth and development. There are reports that *Trichoderma* can stimulate plant growth by up to 300%, as well as protect them from stress factors, especially pathogenic organisms. *Trichoderma* sp. produces organic acids such as gluconic acid, fumaric acid, and citric acid, which can decrease the pH of the soil and allow for the dissolution of phosphate, as well as macro- and micronutrients such as iron, manganese, and magnesium, which are necessary for plant metabolism. *Trichoderma* fungi are characterised by the ability to modify the rhizosphere microflora of plants through the intensive colonisation of roots, and with strong fungi that are aggressive against pathogens [7, 9, 11–13].

Therefore, the objective of this study was to access the impact of fungal biopreparations on the intensity phytoremediation. The results allowed us to determine the degree to which *Trichoderma* mainly improved the process of phytoextraction in the energy crops of *Miscanthus giganteus* L., *Salix* spp., *Phalaris arundinacea* L., and *Panicum virgatum* L.

## 2. Materials and Methods

*2.1. Soil Characterisation.* The soil was collected from an industrial area located near a smelter in Czestochowa (South Poland, Silesia Region). The top 20 cm soil (represented upper layers O and A horizons) was collected, air-dried for 3 weeks, and then sieved through a 2 mm mesh. A series of initial analyses was carried out for the purpose of soil characterisation. The granulometric data indicate that it was medium sandy loam. A more physicochemical characteristic of soil used in the experiment is shown in Table 1.

*2.2. Fungal Strains.* The soil fungal species were isolated from two types of soil: clean forestry soil from the Wielkopolska Region (West Poland) and degraded soil within the area of smelter Czestochowa (South Poland). For isolation, the dilution plate method was used: 1 mL of a $10^3$ dilution was plated on Sabouraud dextrose agar containing gentamycin-chloramphenicol (Scharlau). The identification of species was based primarily on the macroscopic and microscopic morphology. The *Trichoderma* strain isolated from Wielkopolska was described as MS01 and that from Czestochowa as MS02. Both strains were cultivated on sterile wheat grain under specific conditions. Bioactive substrates were prepared shortly before the experiment in order to preserve fungal properties.

*2.3. Mesocosms Experiment.* The experiment was performed in 10 $dm^3$ plastic pots containing soil, additives, and different plant species. The soil in 16 pots was mixed with previously prepared biopreparations, where the weight percent was 5%: 8 pots contained *Trichoderma* MS01, 8 pots contained *Trichoderma* MS02, and the other 4 pots were controls. The pots were next placed in a phytotronic chamber and left for two weeks to determine the geochemical equilibrium. After mixing the soil with biomaterials, a physiochemical analysis of sample mixtures was made. After determining the geochemical equilibrium in mixtures of soil with biopreparations (ca. 2 weeks), the plants were planted (one genus for five pots). Plants from the following genera were used: *Miscanthus giganteus* L., *Salix* spp., *Phalaris arundinacea* L., and *Panicum virgatum* L.

The experiment lasted 21 weeks. During this time, the pots were watered and soil filtrates were collected at 3 week intervals. Plants were cultivated in a phytotronic chamber at 23°C during the day and 14°C at night. The photoperiod was 16 hours light and 8 dark. No additional fertiliser was used.

In order to determine the migration of heavy metals in the soil profile, the contents of Zn, Cd, and Pb in collected filtrates were determined.

At the end of experiment, the aerial parts of plants were beheaded at a height of 5 cm above the soil surface; then the plants were weighed. After drying at 105°C, the plant material was ground in an electric grinder. Plant roots were rinsed with distilled water, weighed, dried at 105°C, and crushed.

After completion of the experiment, soil material was collected from rhizosphere zones of plants. Samples for physicochemical analysis were brought to an air-dried state and sieved through a sieve with a diameter of 2 mm.

*2.4. Physicochemical Analyses.* Soil samples were analysed for pH, CEC, total carbon, total N, and available P. The pH values (pH in $H_2O$ and pH in 1 M KCl) were measured by a pH-meter CPO-401 Elmeton according to PN-ISO 10390:1997 [14]. Cation exchange capacity (CEC) was measured using the method of Kappen. Total carbon (TC) was determined after dry combustion by a Multi N/C 2100S Analyzer according to PN-ISO 10694:2002 [15]. Total N was measured using the Kjeldahl method by BUCHI Digestion Unit K-435 and BUCHI Distillation Unit K-355 according to PN-ISO 11261:2002 [16], and available P was measured using the Egner-Riehm method by DR/4000V Spectrophotometer HACH according to PN-R-04023:1996 [17].

To determine the total heavy metals (Cd, Cr, Cu, Ni, Pb, and Zn) content of soil and biomass samples mineralised in ultrapure nitric acid by SpeedWave MWS-2 Berghof according to PN-ISO 11047:2001 [18], the mineralised samples and filtrated samples were analysed by atomic emission spectrometry with inductively coupled plasma (ICP AES).

*2.5. Mathematic Formulas.* To evaluate the mobility of heavy metals from the soil into the plants and the ability to translocate the metals to the harvestable aerial part, the following factors were used [9, 19–21]:

(i) bioconcentration factor—BCF,

$$\mathrm{BCF} = \frac{\text{metal concentration in plant shots, mg kg}^{-1}}{\text{metal concentration in soil, mg kg}^{-1}}, \quad (1)$$

the bioconcentration (BCF) is a measure of the ability of a plant to take up and transport metals to the shoots;

(ii) translocation index—$T_i$,

$$T_i = \frac{\text{metal concentration in plant shots, mg kg}^{-1}}{\text{metal concentration in plant roots, mg kg}^{-1}} \times 100\,[\%], \quad (2)$$

Table 1: Physiochemical characteristics of soil (G) and soil mixed with biopreparations (MSO1 and MSO2) used in the experiment.

| Parameter | Unit | Value | | |
|---|---|---|---|---|
| | | G | GMSO1 | GMSO2 |
| pH in $H_2O$ | — | $7.88 \pm 0.01^a$ | $7.59 \pm 0.02^a$ | $7.75 \pm 0.42^a$ |
| pH in 1 M KCl | — | $7.4 \pm 0.01^a$ | $7.23 \pm 0.03^a$ | $7.48 \pm 0.06^a$ |
| CEC (cation exchange capacity) | cmol $kg^{-1}$ d.m. | $19 \pm 0.014^a$ | $20 \pm 0.85^a$ | $27 \pm 0.42^b$ |
| TC (total carbon) | mg $kg^{-1}$ d.m. | $17450 \pm 0.95^a$ | $22035 \pm 1.65^b$ | $21540 \pm 2.05^b$ |
| Total nitrogen | mg $kg^{-1}$ d.m. | $481 \pm 4.9^a$ | $963 \pm 4.9^c$ | $837 \pm 4.9^c$ |
| C/N | | 36 | 23 | 25 |
| Available phosphorus | mg $P_2O_5$ $kg^{-1}$ d.m. | $15 \pm 1.4^a$ | $101 \pm 1.4^c$ | $95 \pm 0.71^c$ |
| Cd | mg $kg^{-1}$ d.m. | $0.927 \pm 0.09^a$ | $0.791 \pm 0.005^a$ | $0.673 \pm 0.006^b$ |
| Cr | mg $kg^{-1}$ d.m. | $26.32 \pm 2.83^a$ | $22.16 \pm 1.74^a$ | $20.82 \pm 0.78^b$ |
| Cu | mg $kg^{-1}$ d.m. | $29.44 \pm 0.01^a$ | $21.48 \pm 1.1^b$ | $19.12 \pm 0.16^b$ |
| Ni | mg $kg^{-1}$ d.m. | $16.81 \pm 1.7^a$ | $13.95 \pm 1.32^a$ | $12.56 \pm 0.72^b$ |
| Pb | mg $kg^{-1}$ d.m. | $46.57 \pm 5.25^a$ | $40.39 \pm 1.46^a$ | $42.89 \pm 1.74^a$ |
| Zn | mg $kg^{-1}$ d.m. | $112.0 \pm 9.83^a$ | $97.39 \pm 3.5^a$ | $99.43 \pm 1.37^a$ |

The data for GMSO1 and GMSO2 was measured after two weeks. Means in rows not sharing the same small letters are statistically significantly different within experimental groups ($P < 0.05$).

the translocation index ($T_i$) determines the ability of plants to translocate heavy metals from roots to aerial parts.

*2.6. Statistic Evaluation.* The data were subjected to ANOVA test and Tukey's test (Statistica 6.0 program).

## 3. Results and Discussion

*3.1. Changes in the Physiochemical Character of Soil as an Effect of Trichoderma Treatment.* The soil used for the experiment was poor in both organic matter and basic chemical components, such as N or P. Industrial activity caused a significant change in plant cover or litter, so a loss of soil structure was observed. The O horizon was practically not formed. The data analysed after 2 weeks indicated that the addition of fungal biopreparations based on two different strains of *Trichoderma* (MSO1 and MSO2) caused a significant increase in N and P values and improved the $C/N$ ratio. On the other hand, it did not significantly affect the change in the value of soil pH, C, or CEC (Table 1). Moreover, the content of heavy metals (Cd, Cr, Cu, Ni, Pb, and Zn) decreased in both treatments, mainly due to the so-called "dilution effect." During the first 4 weeks of experiment, we did not observe the increasing solubility of heavy metals in leachates (Figures 1, 2, and 3).

After 21 weeks of pot experiments, some differences in soil quality as an effect of *Trichoderma* treatment were noted (Table 3). Soil pH and CECs lightly decreased, independent of plant species or additives. However, in the case of receiving the values of TC, total nitrogen, and available phosphorus, the higher concentration of these elements was maintained in all mixtures where fungal preparations were applied, irrespective of the test. This caused the better growth and development of plants and resulted in greater biomass (Table 2). The most positive effect on increasing biomass was observed in the case of *Panicum virgatum* (21-fold increase compared to control).

Table 2: The biomass of *Miscanthus giganthus*, *Salix* spp., *Phalaris arundinacea*, and *Pancium virgatum* calculated as an average value of gram dry matter per pot.

| Value | Supplement | Biomass (g d.m.) |
|---|---|---|
| MG | Control | 8.66 ± 1.12 |
| | MSO1 | 20.49 ± 2.34 |
| | MSO2 | 18.27 ± 1.29 |
| S | Control | 11.47 ± 1.51 |
| | MSO1 | 32.05 ± 3.42 |
| | MSO2 | 20.65 ± 1.88 |
| PA | Control | 2.81 ± 0.23 |
| | MSO1 | 7.86 ± 0.28 |
| | MSO2 | 7.32 ± 0.23 |
| PV | Control | 0.32 ± 0.1 |
| | MSO1 | 7.0 ± 0.34 |
| | MSO2 | 2.92 ± 0.28 |

The content of studied heavy metals in the soil was smaller in the replicates containing *Trichoderma*-based materials, as compared to the control, except for the combinations of *P. arundinacea* and MSO2, where a significant increase in all of the studied metals was noted (Table 3). However, when we compare the initial and final values of metals, we can notice that, in some cases, the final value was higher (bold values in Table 3).

There has been increasing researcher interest in the change in traditional heavy metal hyperaccumulators (which usually produce low crops) for more intensive growing plants [6]. One of the promising solutions is nonfood crops, specifically grown for energy production and known as biomass crops. They include grass and trees which would be a significant change in land-use from arable agriculture. The presented results confirm that such plants can grow on degraded soil that is characterised by low nutrient and higher

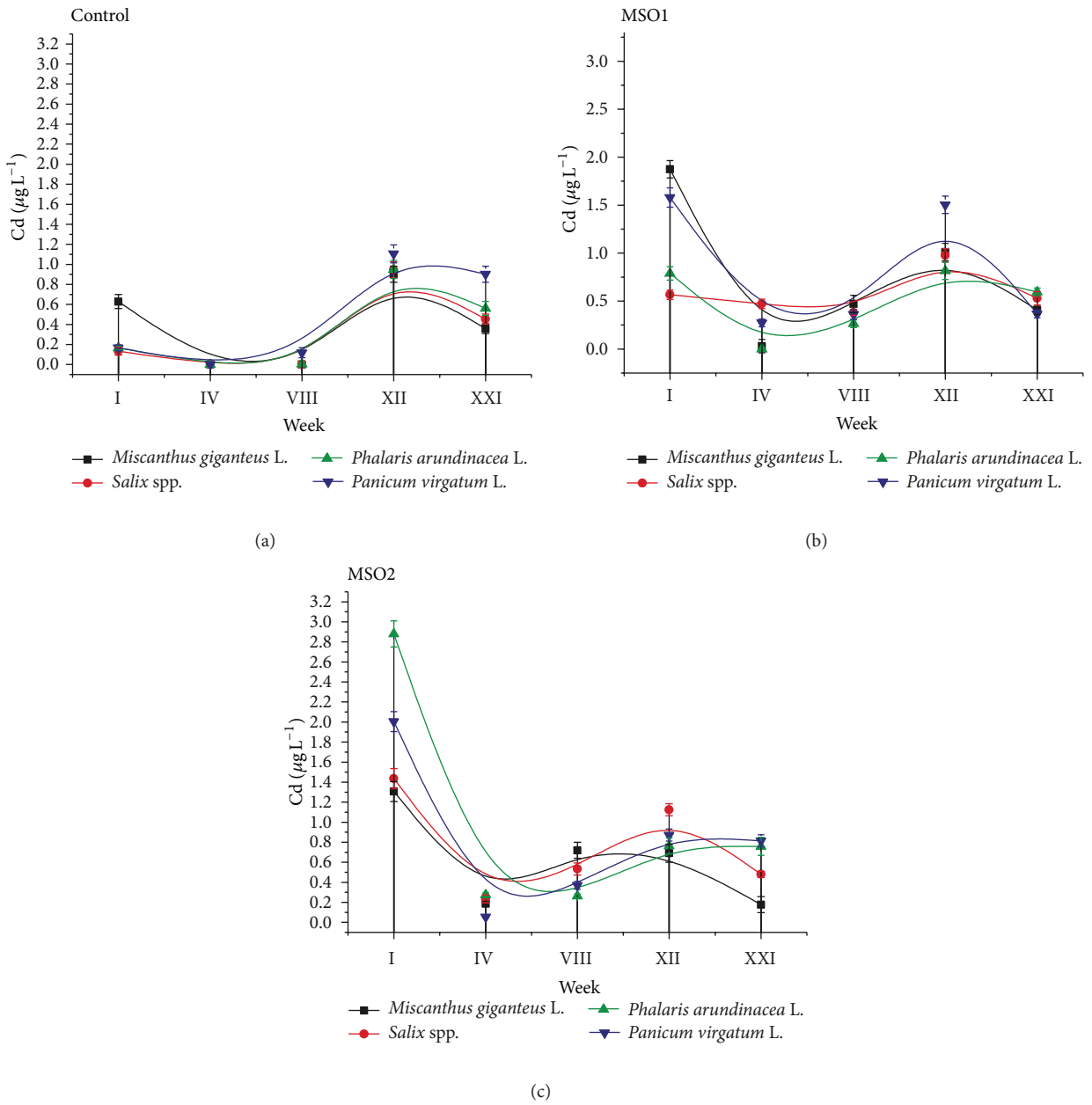



Figure 1: The content of Cd ions in soil leachate after the application of *Trichoderma*-based materials (MSO1 and MSO2).

metal contents and collect and even accumulate heavy metals [22].

### 3.2. The Effect of Trichoderma on Cd, Pb, and Zn Solubility

*3.2.1. Solubility of Cd.* In the first week of the process in the soil solution, in nontreated soil, the highest Cd concentration amounting to 0.63 $\mu g\,L^{-1}$ was observed for *M. giganteus*, while for other plants it did not exceed 0.2 $\mu g\,L^{-1}$. After the 8th week, the concentration of Cd increased and reached the maximum value of 1.1 $\mu g\,L^{-1}$ for *P. virgatum* in the 12th week. Next, a slight decrease in metal content for all plants was observed (Figure 1(a)). The addition of *Trichoderma*-based materials to the soil increased the Cd content in soil solution, independent of plant species (Figures 2(b) and 2(c)). In samples with MSO1 strain in the first week of the process, the highest Cd concentration of 1.8 $\mu g\,L^{-1}$ was recorded for *M. giganteus*, while in samples with strain MSO2 the highest concentration was for *P. arundinacea* (2.88 $\mu g\,L^{-1}$). During the 21st week process, the contents of Cd in soil solution in pots with different plant species decreased to the level of the control. Only in pots with *P. virgatum* and MSO1 were there an increase in the twelfth week, reaching a value of 1.5 $\mu g\,L^{-1}$, and then a decrease at the end of the process, reaching a minimum value of 0.37 $\mu g\,L^{-1}$ (Figures 1(b) and 1(c)).

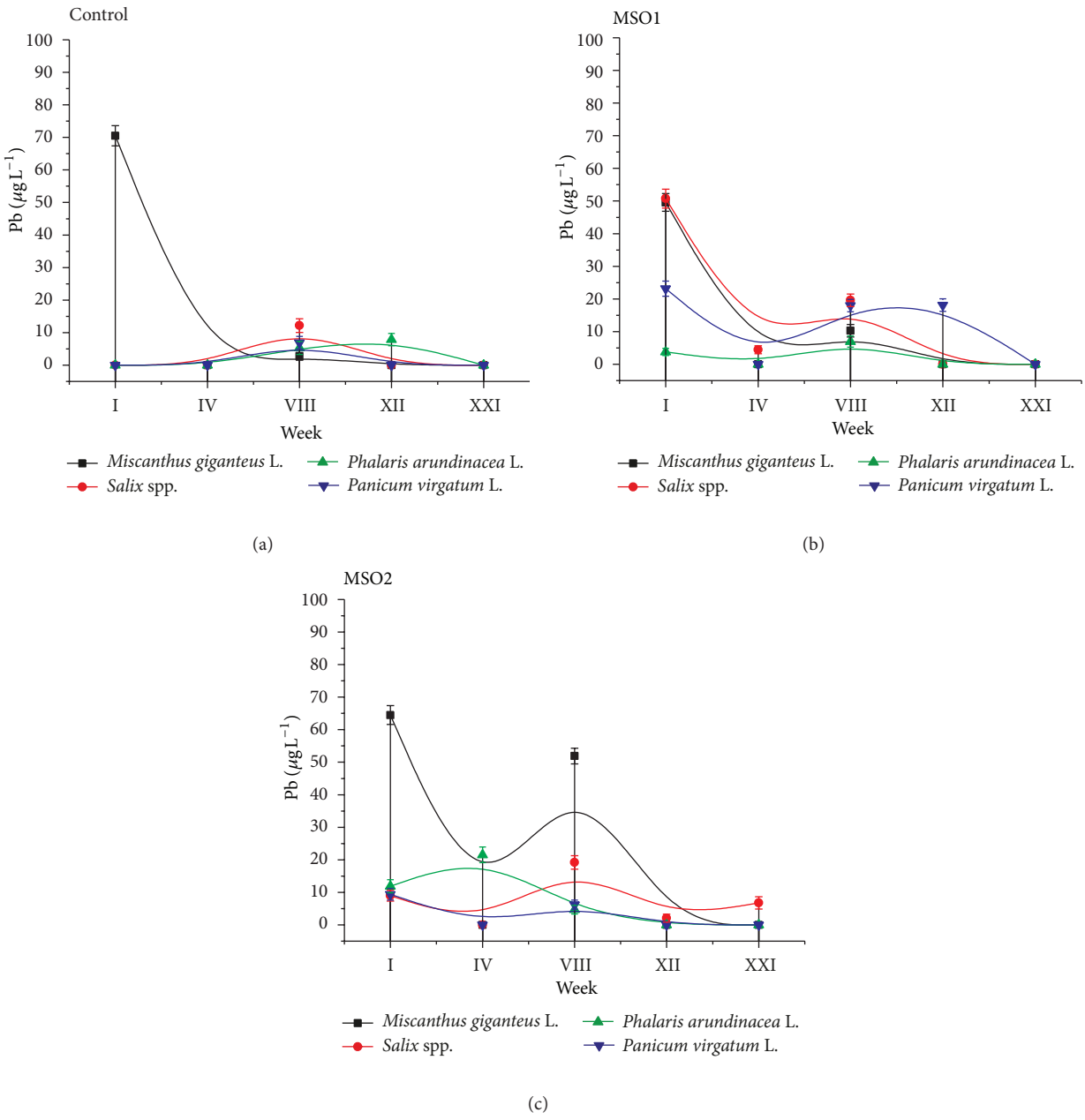



Figure 2: The content of Pb ions in soil leachate after the application of *Trichoderma*-based materials (MSO1 and MSO2).

*3.2.2. Solubility of Pb.* The content of Pb in the solution of the nontreated soil showed that during the 21st week of the process, the concentration of Pb remained at a similar level. The maximum content of Pb, amounting to 70 $\mu g\,L^{-1}$, was recorded at the beginning of the process for *M. giganteus* (Figure 2(a)). The addition of both fungal strains caused the initial increase of Pb concentration in the soil solution to be more visible in the case of MSO2. From the 4th week of the process, the content of Pb decreased and was maintained at similar level and did not exceed 20 $\mu g\,L^{-1}$. On the contrary, the samples from pots with *M. giganteus* and MSO2 showed a more significant fluctuation. In the first week of the process, there was a maximum concentration of Pb amounting to 64 $\mu g\,L^{-1}$, a decrease, and then an increase of Pb to 52 $\mu g\,L^{-1}$ (in the 8th week). After this time, there was a further decrease in the concentration of Pb to 0 $\mu g\,L^{-1}$ in the 21st week of the process (Figures 2(b) and 2(c)).

*3.2.3. Solubility of Zn.* The amount of Zn in soil solutions in the nontreated soil of all plants decreased during the process (Figure 3(a)). In the case of *Salix* and *P. virgatum,* after the 4th week, the concentration of metal slightly increased, although a renewed decrease in its content was noted. The maximum concentration of Zn was reported for *M. giganteus* in the first week of the process (62 $\mu g\,L^{-1}$), while the minimum for *P.*

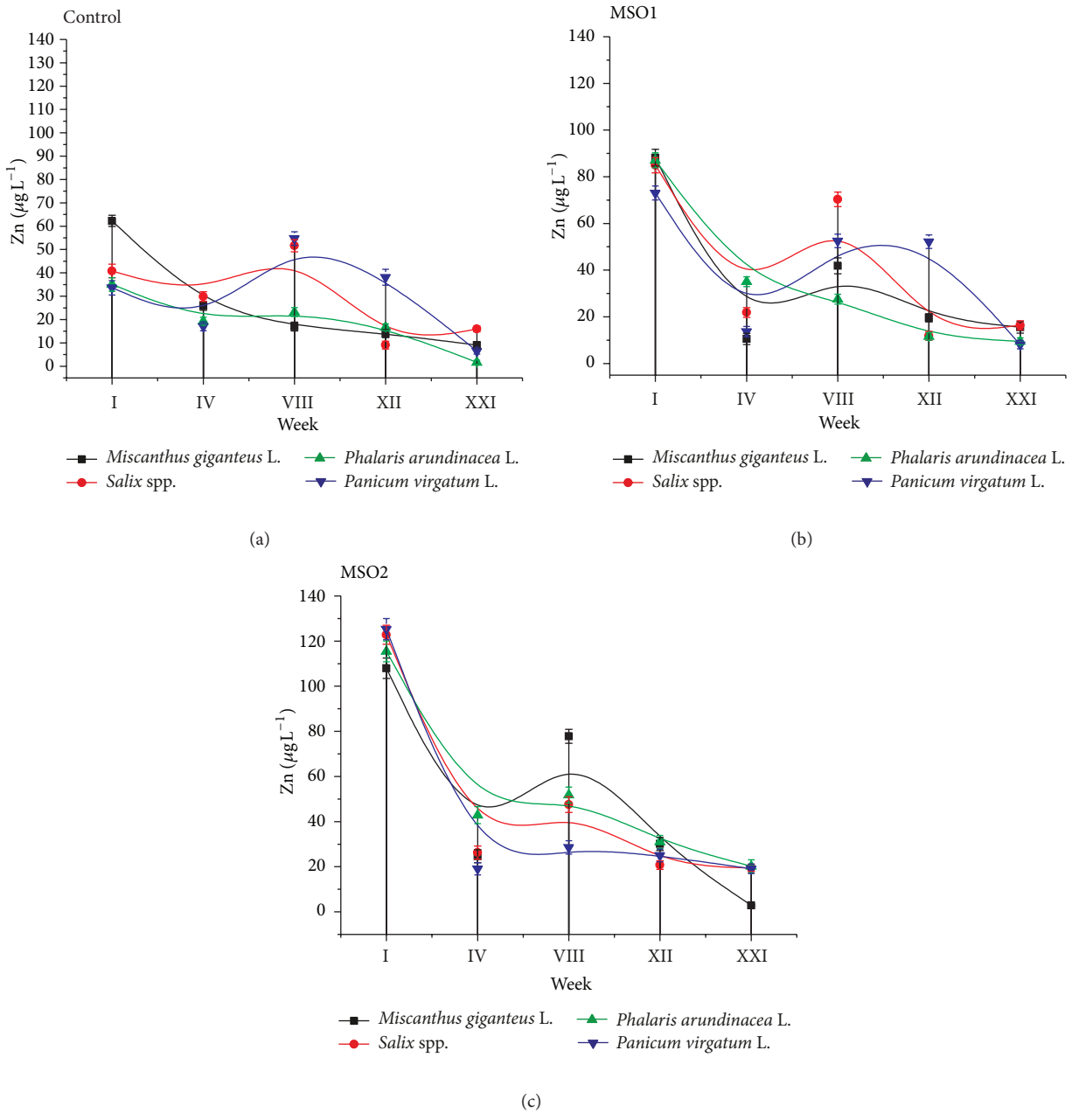



Figure 3: The content of Zn ions in soil leachate after the application of *Trichoderma*-based materials (MSO1 and MSO2).

*arundinacea* was in the 21st week (1.67 $\mu g\,L^{-1}$). *Trichoderma* strains (both MSO1 and MSO2) similarly affect the concentration of Zn in the soil solution of all studied plant species (Figure 3(b) and 3(c)). In the first week, the maximum metal content was recorded. After that time, the concentration of Zn in all samples, independent of fungal strain, decreased. After the 4th week, there was a slight increase in Zn in the soil solution, and after the 8th week there was a decrease until the 21st week of the process in which the concentration of Zn did not exceed 20 $\mu g\,L^{-1}$ in all samples.

Natural low molecular weight organic acids (NLMWOA) such as citric acid, oxalic acid, or malic acid and humic substances (HS) belong to natural chelating agents. These compounds play an important role in the metals' solubility indirectly by their effects on microbial activity, rhizosphere physical properties, and root growth dynamics and, directly, through acidification, chelation, precipitation, and oxidation-reduction reactions in the rhizosphere [4]. Also, many species of soil fungi including *Trichoderma* are also able to dissolve through the release of chelating compounds of organic acids. The fungus releasing organic acids causes acidification of the environment, which helps increase the mobility of heavy metals [23–25]. Our study confirms these reports. In addition both *Trichoderma* MSO1 and MSO2 introduced to the soil caused the mobilisation of Cd, Pb, and Zn at the beginning of the process and resulted in increased leaching of heavy

Table 3: Physiochemical characteristics of soil after the end of the experiment.

| Parameter | Unit | Value | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | MG | MG + MSO1 | MG + MSO2 | S | S + MSO1 | S + MSO2 | PA | PA + MSO1 | PA + MSO2 | PV | PV + MSO1 | PV + MSO2 |
| pH in $H_2O$ | — | 8.1 ± 0.02 | 7.8 ± 0.01 | 8 ± 0.06 | 8.01 ± 0.01 | 7.8 ± 0.04 | 7.65 ± 0.15 | 8.01 ± 0.01 | 7.88 ± 0.16 | 7.89 ± 0.02 | 7.86 ± 0.02 | 7.92 ± 0.07 | 7.91 ± 0.09 |
| pH in 1 M KCl | — | 7.54 ± 0.01 | 7.3 ± 0.04 | 7.44 ± 0.01 | 7.4 ± 0.02 | 7.33 ± 0.06 | 7.32 ± 0.06 | 7.57 ± 0.01 | 7.42 ± 0.05 | 7.42 ± 0.01 | 7.52 ± 0.02 | 7.33 ± 0.01 | 7.36 ± 0.06 |
| CEC | cmol $kg^{-1}$ d.m. | 26 ± 0.14 | 15 ± 2.05 | 26 ± 0.07 | 25 ± 0.01 | 18 ± 1.77 | 22 ± 1.06 | 27 ± 0.57 | 20 ± 1.16 | 26 ± 2.05 | 29 ± 0.01 | 19 ± 2.62 | 24 ± 2.05 |
| TC | mg $kg^{-1}$ d.m. | 17620 ± 1.55 | 23065 ± 1.51 | 20200 ± 2.82 | 17155 ± 4.45 | 20365 ± 1.08 | 19890 ± 1.11 | 18635 ± 3.88 | 21005 ± 2.17 | 19495 ± 5.16 | 20375 ± 3.54 | 21035 ± 1.83 | 20245 ± 2.26 |
| Total nitrogen | mg $kg^{-1}$ d.m. | 525 ± 0.98 | 984 ± 0.94 | 865 ± 2.72 | 553 ± 0.98 | 872 ± 4.95 | 907 ± 3.46 | 399 ± 0.98 | 847 ± 0.89 | 798 ± 5.93 | 655 ± 4.95 | 1180 ± 4.95 | 847 ± 6.92 |
| Available phosphorus | mg $P_2O_5$ $kg^{-1}$ d.m. | 60 ± 6.36 | 92 ± 2.75 | 104 ± 3.54 | 58 ± 4.95 | 85 ± 1.41 | 148 ± 0.92 | 38 ± 5.65 | 77 ± 2.89 | 96 ± 1.69 | 61 ± 1.41 | 141 ± 1.06 | 152 ± 3.04 |
| Cd | mg $kg^{-1}$ d.m. | 0.839 ± 0.01 | 0.768 ± 0.001 | **0.800 ± 0.13** | 0.698 ± 0.02 | 0.628 ± 0.05 | 0.667 ± 0.08 | 0.681 ± 0.02 | 0.580 ± 0.05 | **0.768 ± 0.07** | 0.874 ± 0.01 | **0.805 ± 0.02** | **0.867 ± 0.07** |
| Cr | mg $kg^{-1}$ d.m. | 19.16 ± 0.08 | 17.13 ± 0.47 | 18.70 ± 1.21 | 15.96 ± 0.19 | 14.30 ± 0.56 | 13.69 ± 0.26 | 13.35 ± 0.18 | 12.80 ± 1.46 | **21.11 ± 2.6** | 19.61 ± 0.69 | 18.14 ± 0.21 | 18.78 ± 1.24 |
| Cu | mg $kg^{-1}$ d.m. | 29.31 ± 0.74 | **27.31 ± 0.09** | **28.29 ± 2.98** | 26.42 ± 0.18 | **23.55 ± 2.4** | **23.55 ± 3.24** | 22.02 ± 0.05 | 21.54 ± 0.85 | **27.81 ± 1.69** | 31.03 ± 0.38 | **29.19 ± 2.01** | **31.93 ± 1.85** |
| Ni | mg $kg^{-1}$ d.m. | 16.60 ± 0.07 | 14.19 ± 0.89 | **15.15 ± 2.55** | 13.91 ± 0.29 | 11.57 ± 1.1 | 13.51 ± 3.75 | 11.90 ± 1.18 | 10.73 ± 1.79 | 15.96 ± 1.87 | 19.93 ± 0.18 | **17.59 ± 2.68** | 16.74 ± 1.96 |
| Pb | mg $kg^{-1}$ d.m. | 32.09 ± 0.16 | 29.87 ± 1.65 | 26.07 ± 3.36 | 24.23 ± 0.06 | 24.53 ± 1.68 | 23.58 ± 2.81 | 21.66 ± 0.55 | 21.51 ± 1.87 | 26.59 ± 2.35 | 28.61 ± 0.08 | 32.76 ± 1.18 | 29.76 ± 0.38 |
| Zn | mg $kg^{-1}$ d.m. | 114.5 ± 1.06 | **107.3 ± 1.84** | **114.4 ± 2.05** | 98.09 ± 0.73 | 87.56 ± 2.34 | 87.87 ± 3.36 | 86.78 ± 0.42 | 83.34 ± 1.25 | **111.5 ± 1.41** | 124.3 ± 0.94 | **116.6 ± 3.25** | **116.0 ± 1.78** |

(MG: *M. giganteus*, S: *Salix*, PA: *P. arundinacea*, and PV: *P. virgatum*).

Table 4: Bioconcentration factor (BCF) and translocation index ($T_i$).

| Value | Supplement | BCF | | | | | |
|---|---|---|---|---|---|---|---|
| | | Cd ↑ | Cr ↑ | Cu ↑ | Ni ↑ | Pb ↓ | Zn ↑ |
| MG | Control | 0.18 ± 0.02 | 0.52 ± 0.02 | 0.19 ± 0.01 | 0.33 ± 0.01 | 0.036 ± 0.001 | 0.25 ± 0.02 |
| | MSO1 | 0.25 ± 0.03 | 0.75 ± 0.03 | 0.27 ± 0.02 | 0.41 ± 0.04 | 0.038 ± 0.002 | 0.30 ± 0.02 |
| | MSO2 | 0.41 ± 0.01 | 0.83 ± 0.08 | 0.32 ± 0.01 | 0.38 ± 0.03 | 0.017 ± 0.001 | 0.36 ± 0.03 |
| S | Control | 6.64 ± 0.02 | 0.14 ± 0.02 | 0.41 ± 0.01 | 0.14 ± 0.01 | 0.024 ± 0.001 | 1.89 ± 0.05 |
| | MSO1 | 2.75 ± 0.03 ↓ | 0.14 ± 0.01 | 0.55 ± 0.04 | 0.19 ± 0.02 | 0.022 ± 0.001 | 0.68 ± 0.05 ↓ |
| | MSO2 | 3.08 ± 0.06 ↓ | 0.40 ± 0.03 | 0.58 ± 0.03 | 0.23 ± 0.02 | 0.024 ± 0.002 | 0.52 ± 0.05 ↓ |
| PA | Control | 0.11 ± 0.01 | 0.44 ± 0.03 | 0.28 ± 0.02 | 0.25 ± 0.02 | 0.019 ± 0.001 | 0.38 ± 0.04 |
| | MSO1 | 0.21 ± 0.05 | 0.41 ± 0.04 ↓ | 0.39 ± 0.04 | 0.20 ± 0.01 ↓ | 0.011 ± 0.001 | 0.58 ± 0.05 |
| | MSO2 | 0.29 ± 0.02 | 0.35 ± 0.03 ↓ | 0.45 ± 0.03 | 0.26 ± 0.02 | 0.010 ± 0.001 | 0.74 ± 0.07 |
| PV | Control | 0.19 ± 0.04 | 0.18 ± 0.01 | 0.29 ± 0.01 | 0.06 ± 0.01 | 0.025 ± 0.001 | 0.24 ± 0.02 |
| | MSO1 | 0.29 ± 0.01 | 0.50 ± 0.04 | 0.36 ± 0.03 | 0.18 ± 0.01 | 0.016 ± 0.001 | 0.32 ± 0.02 |
| | MSO2 | 0.24 ± 0.02 | 0.44 ± 0.04 | 0.35 ± 0.02 | 0.12 ± 0.01 | 0.018 ± 0.001 | 0.21 ± 0.01 ↓ |
| Value | Supplement | $T_i$ % | | | | | |
| | | Cd ↑ | Cr ↑ | Cu ↑ | Ni ↑ | Pb ↓ | Zn ↑ |
| MG | Control | 18 ± 2 | 165 ± 7 | 61 ± 4 | 92 ± 5 | 28 ± 3 | 64 ± 5 |
| | MSO1 | 48 ± 4 | 339 ± 5 | 110 ± 6 | 190 ± 7 | 52 ± 5 ↑ | 97 ± 6 |
| | MSO2 | 37 ± 2 | 195 ± 6 | 67 ± 4 | 64 ± 5 ↓ | 6 ± 2 | 71 ± 5 |
| S | Control | 485 ± 5 | 13 ± 3 | 65 ± 4 | 14 ± 2 | 8 ± 1 | 235 ± 8 |
| | MSO1 | 279 ± 5 ↓ | 4 ± 1 ↓ | 80 ± 6 | 20 ± 3 | 6 ± 1 | 83 ± 6 ↓ |
| | MSO2 | 222 ± 4 ↓ | 31 ± 3 | 56 ± 4 ↓ | 18 ± 2 | 5 ± 2 | 47 ± 5 ↓ |
| PA | Control | 2 ± 1 | 17 ± 3 | 20 ± 2 | 9 ± 2 | 5 ± 1 | 25 ± 3 |
| | MSO1 | 9 ± 2 | 37 ± 4 | 46 ± 3 | 17 ± 2 | 2 ± 1 | 63 ± 1 |
| | MSO2 | 13 ± 3 | 34 ± 3 | 38 ± 2 | 18 ± 2 | 2 ± 1 | 80 ± 9 |
| PV | Control | 23 ± 4 | 37 ± 5 | 52 ± 4 | 12 ± 3 | 34 ± 3 | 28 ± 5 |
| | MSO1 | 28 ± 1 | 161 ± 6 | 69 ± 2 | 59 ± 5 | 23 ± 3 | 53 ± 4 |
| | MSO2 | 16 ± 3 ↓ | 109 ± 7 | 64 ± 1 | 19 ± 2 | 16 ± 3 | 33 ± 2 |

(MG: *M. giganteus*, S: *Salix*, PA: *P. arundinacea*, and PV: *P. virgatum*).

metals into the soil solution. However, the effect of soil acidification was not so clear, according to the results of pH measured at the end of the experiment—in most cases, the increase of pH value was noted. Similar results were noted during earlier experiments carried out in the vicinity of the zinc smelter in Silesia Region [26]. We suppose that there were several interactions between the activity of the fungus, activity naturally occurring in rhizosphere microbial communities, plant root exudates, and soil.

*3.3. Metal Uptake.* Bioaccumulation coefficients (= concentration of the metal in dried plants divided by the initial soil content of the same element) for Cd, Cr, Cu, Ni, Pb, and Zn as a function of the total metal content of the soil for the plant unit experiments are shown in Table 4. The addition of *Trichoderma* generally caused an increase in the BCF value, with the exception of the uptake of Pb (all plants); Cd, Cr, and Zn by *Salix*; and Cr and Ni by *P. arundinacea*. Mostly, the higher uptake of metals from the soil was recorded after MSO2 injection (the *Trichoderma* strain isolated from degraded terrains within Czestochowa mill zone). The opposite results were obtained for *P. virgatum*, where higher BCF values were obtained in the case of MSO1 injection. The highest increase of BCF value (as compared to control) was observed for Ni and *P. virgatum* with the addition of MSO1 and for Cr and for *Salix* with the addition of MSO2, respectively. On the contrary, the lowest rate of 0.014 BCF was calculated for Pb in the combination of *P. arundinacea* with MSO2.

The translocation index ($T_i$) results show that this index decreases with the addition of the soil biopreparations, both MSO1 and MSO2, but only for Pb (Table 4). For other metals, the $T_i$ index increases. The highest value of $T_i$ index, 339, was observed for Cr in a pot of *M. giganteus* with MSO1. The lowest value of translocation index (equal to 2) was calculated for Pb in samples of *P. arundinacea*. Other studies have shown that the plants that are most suitable for the process of phytoextraction of Cd, Cu, and Zn are *Salix*, *M. giganteus,* and *P. arundinacea*, and in the phytostabilisation process the above-mentioned metals perfectly suited *P. virgatum* [27]. Other authors [28] suggest that biomass crops (such as *Salix*) are able to absorb a low to moderate uptake of metal pollutants, which seems to be linked to the effects of dissolved organic carbon (DOC) mobilising the pollutants and thus increasing the uptake. This study did not confirm

these reports. The addition of organic substrates caused a decrease in extraction efficiency in the case of *Salix*. We observed the increase of $T_i$ value only for Ni. On the other hand, interestingly, the addition of MSO1 caused the increase of $T_i$ value for Pb and *M. giganteus*.

The biomass of soil fungi including *Trichoderma* plays an important role in the bioremediation of contaminated soils and can be applied in integrated pest management and phytoremediation [29]. Moreover, it can remove and concentrate the various ions, such as Pb, Cd, Cu, Zn, and Ni, and sorption was widely recognised as the main mechanism of uptake [30–32]. De Lima Freitas et al. [33] noted that *Trichoderma harzianum* growing on control cultures showed potential for the accumulation of polyphosphate and the presence of cadmium induced a reduction in the related polyphosphate content. The authors suggest that such behaviour can be applied to the removal of metals in sewage. However, experiments are mostly performed under *in vitro* conditions and all researchers emphasise the need to conduct research in the field to confirm *Trichoderma* practical use as a bioremediating agent. There are only a few studies showing that *Trichoderma* can be used in practice [34]. The main question is focusing on the behaviour of the organism in the presence of plants and roots activity. Some authors suggest that rhizosphere microorganisms may increase phytoextraction processes by promoting plant growth or an increase in heavy metal accumulation in plant tissues. Our study confirms that the inoculation of soil by *Trichoderma* had the effect of increasing values of coefficients of BCF and $T_i$ and consequently increasing the efficiency of phytoextraction. The same relationship was observed in other studies [9, 21]. We, however, reported two exceptions: the first was willow and the second was Pb, where both factors' values were reduced after the application of biomaterials. Additionally, for a few cases, we observed a higher content of metal at the end of experiment (compared to initial stages), suggesting that *Trichoderma* was able to stabilise the sorption of metals in its mycelium. This has also been documented for mycorrhizal fungi, which are well known from their ability in metal accumulation [35]. This may be due to the fact that mycorrhizal fungi also develop a large mycelial biomass under field conditions, in contrast to *Trichoderma* and other *Deuteromyces*, whose behaviour is similar to plant growth promoting bacteria (PGPB) commonly present in the rhizosphere.

## 4. Conclusion

The fungal amendments were effective (much like known chelating agents) in the mobilisation and extraction of selected heavy metals (Cd, Cr, Cu, Zn, and Ni) independently of the plant species used. The exception was Pb. This was confirmed by the calculation of the translocation index ($T_i$) and bioconcentration factor (BCF) based on the concentration of metals in soil and plant materials. The obtained results suggest that *Trichoderma*-based materials seem appropriate for the acceleration of phytoextraction programs in energetic crops. The substrate also improved the soil parameters, such as TC, N, and P and increased biomass. Finally, taking into consideration the biomass date by a mass balance, the amounts of metals removed from the soil were much higher, even in the case of Pb. On the other hand, an addition of organic matter amendments, such as compost, fertilisers, and wastes, is a common practice for the immobilisation of heavy metals and soil amelioration of contaminated terrains. Further research is needed to evaluate the long-term effects of *Trichoderma* on metal behaviour in the heterogeneous system under field conditions.

## Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

## Acknowledgments

The study was supported by the Czestochowa University of Technology internal Grants BS PB 401/304/11 and BS MN 401-317/11. The phytotron chamber was bought as part of the project: Equipment of Central Environmental Laboratory WKP_1/1.4.3/2/2005/61/180/365/2006/U. The authors wish to thank Dr. Anna Grobelak for her technical assistance and Mr. Jon Pross for language corrections. The author Karolina Rosikon received a grant for the project *DoktoRIS — Scholarship program for innovative Silesia* cofinanced by the European Union under the European Social Fund.

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