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ORIGINAL PAPER

COMPOST UTILISATION IN HEAVY METAL IMMOBILISATION PROCESS EVALUATED BY BIOCONCENTRATION FACTORS*

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Abstract

Many human activities lead to soil contamination by heavy metals and polluted areas must undergo the remediation process. Among various techniques, immobilisation with organic amendments is gaining popularity, as it reduces the bioavailability of heavy metal. The main aim of this study has been to evaluate the applicability of compost as stabilisation material to reduce metal bioavailability. Another goal has been to determine practical applicability of developed factors as a reliable and helpful indicator of metal-soil-plant interactions. The transfer of metals in environment was described by the bioconcentration factors (BCF_T, BCF_A) and the contamination level coefficient (CCL). Bioconcentration factors were calculated on the basis of total and bioavailable amounts of heavy metals. The investigations were carried out under greenhouse conditions with two soils (light and medium) with and without biowaste compost amendment and two test plants (winter barley and white mustard) at a simulated contamination with Cu (doses of 25 and 50 mg kg^{-1}) and Zn (doses of 100 and 200 mg kg^{-1}). It was demonstrated that the proposed BCF_T, BCF_A and CCL are reliable and helpful indicators of phytotoxicity. Regardless of the experimental factors, BCF_T ranged from 0.1 to 1.5, BCF_A from 0.5 to 8.8 and CCL from 1.0 to 4.0. Additionally, the presented threshold of toxicity, determined on the basis of the above indicators, facilitates the interpretation of metal-soil-plant interactions. The results proved that compost is a valuable organic amendment, which can significantly reduce metal bioavailability. Moreover, the proposed bioconcentration factor (BCF_T, BCF_A) and the contamination level coefficient (CCL) seem to be useful tools to assess soil contamination in relation to environment phytotoxicity. The two-sided *F*-Snedecor test and Student's *t*-test were applied as statistical tools for calculation and interpretation of the results. The visualisation of data was achieved via violin plots.

Keywords: biowaste compost, bioavailability, immobilization, bioconcentration factors, heavy metals.

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INTRODUCTION

While the behaviour of heavy metals in environment as well as their toxicity have been thoroughly documented in a significant number of publications, they continue to be priority and popular issues. The undiminishing interest in the behaviour and presence of heavy metals in the environment is related to some problems, such as:

- despite the awareness of the threat connected with heavy metals, there is a continuous increase in the total polluted area;
- the dangerous effects of heavy metals on living organisms are due to the fact that these elements are readily incorporated into food chains;
- technologies are being developed to remediate water and soil polluted by heavy metals.

Heavy metals are widely distributed in the earth's crust, and most of them occur naturally in soil parent material. However, a much more important source of heavy metals is created by anthropogenic activities, primarily associated with industrial processes, disposal of domestic and industry waste, use of agrochemicals, traffic and metal mining (Perez-Estaban et al. 2014, Singh, Prasad 2015, Bolan et al. 2016, Huang et al. 2016). These factors, related with the civilisation progress, result in considerable soil contamination.

A commonly applied indicator of the degree of heavy metal pollution in various constituents of the environment is connected with the total heavy metal content. However, it is well established that the total content of a metal in soil does not constitute a reliable indicator of the potential risk of toxicity (Jakubus 2013, Kelepertzis et al. 2015, Hu et al. 2017). The chemical forms of heavy metals are of much greater importance, as they facilitate the evaluation of their potential mobility, bioavailability and toxicity in the environment. Currently it is believed that the amounts of bioavailable metals found in the environment indicate their potential phytotoxic effect (Hernandez-Soriano, Jimenez-Lopez 2012) and they are the key indicator in their ecological and geochemical evaluation (Huang et al. 2016).

Bioavailability of metals in soils can be examined using chemical extraction and bioassay tests. Chemical extraction tests include single or sequential extraction, while bioassays involve plants, animals or microorganisms. Single extraction facilitates the separation of the tested environmental matrix into soluble and insoluble fractions in a given extracting solution. Bioassay tests are based on a phytotoxicity test, which is defined as detrimental effects on various physiological processes of plants caused by specific substances.

According to the EU directives (Directive 2010/75/EU), polluted areas must undergo remediation process. There is a wide variety of remediation techniques, e.g. the chemical method based on immobilisation is popular because of the relatively rapid and cost-effective manner to reclaim polluted sites (SINGH, PRASAD 2015, SONG et al. 2017). The primary objectives of remediating contaminated sites is to reduce metal bioavailability by *in situ* immobilisation using selected amendments. PARK et al. (2011) stated that bioavailability can be minimised through chemical and biological immobilisation of metals using a range of inorganic and organic compounds. Various types of organic materials including organic waste (food scraps or yard trimmings), sewage sludge, compost, manure, biochar are commonly used in remediation of soils contaminated with heavy metals (KUMPIENE 2010, SINGH, PRASAD 2015, BOLAN et al. 2016, HUANG et al. 2016). However, applied organic amendments may differently influence metal bioavailability in soil. Several studies have demonstrated that an addition of organic materials to soil has a moderate effect on the mobility of metals (MOHAMED et al. 2010). On the basis of both the literature review by BOLAN et al. (2014) and studies conducted by PEREZ-ESTEBAN et al. (2014) or BALDANTONI et al. (2016), the positive effect is mostly indicated, expressed by the reduction of metal bioavailability due to adsorption on solid surfaces and complexation with humic substances. Irrespective of various opinions concerning organic matter interactions with heavy metals, the polluted area during and after remediation processes should be continuously monitored in terms of their heavy metal state. First of all, mobility and bioavailability of heavy metals in the environment cannot be ignored, hence it must be controlled. In order to achieve this goal, common methods (chemical and biological) may be applied, as it was mentioned above. However, in this paper we would like to propose another approach, a more sophisticated view and interpretation of the discussed problem, based on the combined chemical and biological techniques and expressed as bioconcentration factors.

The bioconcentration factor (BCF) is a popular indicator used to evaluate heavy metal toxicity as well as their translocations from soil to plants (BOIM et al. 2016, CHRZAN 2016, JAKUBUS, TATUŚKO 2016, CHOWANIAK et al. 2017, GAUTAM et al. 2017, LU et al. 2017, WOŁEJKO et al. 2017). Additionally, HU et al. (2017) stated that the bioaccumulation values rather than the total content of heavy metals should be taken into consideration. Usually, the bioconcentration factor based on the total amount of heavy metals (BCF_T) in soil and plant tissue concentrations is applied in practise. In this study, apart from the typical BCF_T, another factor calculated on bioavailable amounts of heavy metals is presented. As it was proven above, bioavailability plays a more important role in plant–soil toxicity and its assessment. Taking into account the above, BCF_A as the ratio of the metal content in plant shoots to the bioavailable amount of metals in soil, compost or their mixture is introduced. Moreover, we have an additional conceptualisation idea to achieve a reliable state of the plant contamination level is given. To this end, the coefficient of contamination level (CCL) was calculated, illustrating the ratio of metal concentrations in plant shoots cultivated on amended, heavy metal polluted sites to the metal content in plant shoots cultivated on control sites. One can hypothesise that the application of both bioconcentration factors (BCF_T, BCF_A) and the coefficient of contamination level (CCL) can describe precisely migration of heavy metals from soil to plants, their uptake with plant yield and the potential toxic effect. To verify our concept, the conditions of soil contaminated by heavy metals and during the stabilisation process (compost application) were simulated. Moreover, on the basis of statistical analysis of the experimental results, the toxicity threshold is proposed.

This study was undertaken with the objective to: 1) evaluate applicability of compost as a stabilisation material to reduce metal bioavailability, and 2) determine practical applicability of $BCF_{T,}$ BCF_A and CCL as reliable and helpful indicators of the state of the environment for the toxicity of heavy metals.

MATERIAL AND METHODS

Soil samples and organic amendments

Two different soils from the Wielkopolska Province (Poland) were selected for analysis during this study. The first soil belonged to the light agronomic category and was classified as *Haplic Luvisols*; the other was in the medium agronomic category and classified as *Haplic Cambisols* according to WRB-FAO (2014). Organic amendment as mature compost made of household domestic waste and green plants with cow manure was used as stabilisation material. Table 1 shows basic properties of these soils and compost.

In order to obtain an unambiguous and precise response to the objectives set at work, a simple scheme of the experiment based on soil (light and

Table 1

Selected properties of soils and compost used in experiment

Properties	Light soil	Medium soil	Compost
pH*	4.90	5.70	5.50
EC (µS cm^{-1})	85.4	101	358
CEC (cmol kg^{-1})	53.4	60.8	87.6
Ntot (g kg^{-1})	0.16	1.20	2.70
TOC (g kg^{-1})	6.58	15.75	189.0
Cu tot (mg kg^{-1})	3.8	6.98	41.4
Zn tot (mg kg^{-1})	15.7	28.0	237.3
Cu DTPA (mg kg^{-1})	0.46	1.74	4.66
Zn DTPA (mg kg^{-1})	1.32	6.74	57.0

* pH in 1 mol L^{-1} KCl for soils and pH in H_2O for compost

medium), compost and a mixture of soil and compost (1/1 = w/w of dry matter) was adopted. Accordingly, five different treatments were prepared: with light soil (T1), medium soil (T3), compost (T5) and mixtures of each soil with compost (T2 for light soil + compost and T4 for medium soil + compost). Copper and zinc were applied into the light soil, medium soil, compost and to a mixture of soils with compost in the form of $Cu(NO_3)_2$ and $Zn(NO_3)_2$. The copper dose was 25 mg Cu kg^{-1} (T1Cu, T2Cu) and zinc dose was 100 mg Zn kg^{-1} (T1Zn, T2Zn). The treatments of medium soil (T3) and as its mixture with compost (T4) were amended by 50 mg Cu kg^{-1} (T3Cu, T4Cu) and 200 mg Zn kg^{-1} (T3Zn, T4Zn). Simultaneously, the compost was contaminated with 25 and 50 mg kg^{-1} of copper as well as 100 and 200 mg kg^{-1} of zinc (T5/25, T5/50, T5/100, T5/200). The selected doses were dictated by the threshold values provided in the *Regulation of the Minister of the Environment* (2016).

Greenhous experiment

The experiment was carried out in 3 replications (3 pots) for each treatment under greenhouse conditions. Temperature and humidity inside the greenhouse were automatically controlled. The moisture of soils, compost and their mixtures was kept at 60% of water field capacity.

To evaluate the response of plants in the different treatments, the ISO 11269-2:2012 (ISO 2012) test was performed on two plants: winter barley (*Hordeum vulgare* L.) cv. Rosita and white mustard (*Sinapis alba* L.) cv. Bondera. Plants were cultivated simultaneously. They were grown in 0.5 L polyethylene pots filled with 500 g of either type of soil, compost and their mixtures with and without an addition of Cu and Zn. Ten seeds of the plants were sown in separate pots (separate replications) and plants were harvested after 21 days after sowing. All harvested plants from a pot were cut at the ground level and evaluated together. At the same time, soil samples were collected. Plant material was dried at 50°C, weighed and next ground to powder. Soil material was air-dried and sieved to <2 mm for analysis.

Soil, compost and plant analysis

Total metal content (Cu tot and Zn tot) in soil samples, compost and their mixture was determined using acid digestion with *aqua regia* (ISO 1995). Bioavailable amounts of Cu and Zn in soil samples, composts and their mixture were assessed with DTPA solution (0.005 mol L^{-1} DTPA + + 0.1 mol L^{-1} TEA + 0.01 mol L^{-1} $CaCl_2$, pH 7.3). The details of the experimental protocols are available elsewhere (Quevauviller et al. 1998). The chemical analyses of compost were conducted on dried samples. Total organic carbon (TOC) and total nitrogen (N_{tot}) in soil samples, composts and their mixture were determined using Vario Max CNS.

For plant samples, the digestion of ground matter was accomplished by a dry ashing procedure at 500°C for 5 h, followed by ash dissolution in 5 mL of 6 mol L^{-1} HCl. Solutions were filtered and made up to 10 mL. Concentrations of Cu and Zn in the extracts were determined using atomic absorption spectrophotometry (ASA) in a Varian Spectra AA 220 FS apparatus. All the assays identifying nutrient amounts in the tested samples were performed in three replications.

On the basis of the Cu and Zn content in the growing medium and cultivated plants, the bioconcentration factor for total heavy metal amounts (BCF_T), the bioconcentation factor for heavy metal available amounts extracted by DTPA solution (BCF_A) and the coefficient of contamination level (CCL) were described.

The BCF_T is defined as the ratio of the metal content in plant shoots to total metal content in soils, compost or their mixture. It is the theoretical ability of a plant to take up and transport metals to the harvestable aerial parts:

$$BCF_T = \frac{\text{metal in plant shoots}}{\text{total metal content}}.$$

The BCF_A is defined as the ratio of the metal content in plant shoots to bioavailable metal amounts in soils, compost or their mixture. It is the practical ability of a plant to take up and transport metals to the harvestable aerial parts:

$$BCF_A = \frac{\text{metal in plant shoots}}{\text{bioavailable metal amount}}.$$

The CCL is defined as the ratio of the metal content in plant shoots cultivated on sites amended with metals to metal concentrations in plant shoots cultivated on control sites. It should be helpful in the assessment of actual site contamination by heavy metals:

$$CCL = \frac{\text{metal in plant shoots in contaminated site}}{\text{metals in plant shoots in the control}}.$$

Statistical analysis

At the first stage, our goal was to compare plants (winter barley and white mustard) in terms of average BCF_T content on soils with the addition of metal (copper or zinc). In the first step, using the two-sided *F*-Snedecor test, the zero hypothesis H_0 was discussed, referring to the uniformity of variance for the two compared plants. When there were no grounds for rejecting the H_0 hypothesis, further analyses assumed that homogeneity of variance was fulfilled. In such cases, to compare the average BCF_T content for barley with the average BCF_T content in mustard, Student's *t*-test was adequate for comparisons of two means with an unknown standard deviation of population and small sample size. When the H_0 hypothesis concerning the homogeneity of variance of both populations was rejected, an approximate Student's *t*-test was used in further analyses to compare mean BCF_T content.

Data presentation was achieved via violin plots. Inside the violin a standard boxplot is included. The white dot denotes the median of the observation, while the bottom and top of the black box determine the quartiles, the corresponding lower quartile Q1 and the upper quartile Q3. The ends of the boxplot whiskers determine the minimum and maximum values of the examined trait. If the height of the violin is greater than the height of the box with a whisker, it means that there were outliers in the sample. The width of the violin at point x gives the intensity of the observation with the value of the test trait close to x.

RESULT AND DISCUSSION

Several technologies can be suitable for the reclamation of soils that are polluted by heavy metals. Nevertheless, many of them are very costly or take long to achieve lasting effects (Lu et al. 2017). Immobilisation, included in the group of chemical methods of remediation, is a very promising and popular technique, especially when it employs waste materials which have been converted to valuable organic fertilisers. The use of composted biowaste is a very important strategy to help comply with the Landfill Directive (1999/31/EC) and to the "end-of-waste" policy in Europe (Saveyn, Eder 2014). Moreover, application of such organic soil amendments also fulfils the postulate of the Thematic Strategy for Soil Protection (2006). Thus, the use of composted organic waste amendments is consistent with the remediation/immobilisation idea, because they are inexpensive sorbents and have become particularly important sources of organic matter with significant amounts of essential nutrients (Jakubus 2013, Sciubba et al. 2014, Alvarenga et al. 2015, Singh, Prasad 2015).

Owing to large amounts of organic matter, composts added to soil can change the mobility and bioavailability of heavy metals in the soil environment as well as the toxic effects on plants. These actions are attributed to various processes including adsorption, complexation, precipitation and redox reactions (Huang et al. 2016, Song et al. 2017). The intensity of these interactions between compost and metals may be verified using single extraction and bioassays, as supplementary methods. For this reason, in the present research, both DTPA extraction and phytotoxity test were performed in addition to the assessment of heavy metal content. Zhu et al. (2012) particularly stressed the suitability of the DTPA solution, because it has been found to be more effective in removing soluble metal-organic complexes that are potentially bioavailable. In phytotoxicty tests, white mustard and winter barley were used because these plants have become very popular recenlty, and they are excellent model plants for metal uptake (Flores-Meza 2008, cited after Hernandez-Soriano, Jimenez-Lopez 2012, Song et al. 2017). In the present study, the ISO 11269-2:2012 (ISO 2012) test was also carried out, and the results were applied to calculate three different factors: BCF_T,

BCF_A, CCL. These factors were used to evaluate the tolerance of winter barley and white mustard to heavy metals present in the soils, compost and their mixtures. It was found that under simulated soil contamination with Cu and Zn, the above factors were first of all affected by the compost and cultivated plant. Generally, in most cases, the metal applied was of less important, although higher values of the indicators were obtained for copper. Regardless of the experimental factors, BCF_T ranged from 0.1 to 1.5, BCF_A from 0.5 to 8.8 and CCL from 1.0 to 4.0, respectively (Figure 1). The highest values of BCF_T and BCF_A were found for white mustard cultivated on uncontaminated light soil (T1). Winter barley accumulated significantly less Cu and Zn. The results justify the claim that the application of compost considerably decreased the BCF_T and BCF_A values for winter barley and white mustard. The influence of compost was also observed in the case of the CCL values, and the pattern of change was the same as above. Boim et al. (2016), Mahmoud, Ghoneim (2016) and Gautam et al. (2017) reported similar trends concerning the bioconcentration factor. Unfortunately, it is difficult to compare our results with the literature data because various authors (Chrzan 2016, Jakubus, Tatuśko 2016, Xie et al. 2016, Lu et al. 2017, Wołejko et al. 2017) present higher or lower values of BCF_T for Zn ranging between 0.44 and 1.34 and for Cu from 0.12 to 0.8.

As it was mentioned above, the factor (BCF) is a popular indicator, although it is only occasionally used as a helpful tool to diagnose the environmental condition and to interpret the findings. In this study, additional factors such as BCF_A and CCL were developed. In order to assess reliably the practical applicability of these factors, it was decided to take into consideration only the conditions of contaminated soils both with and without compost addition. The basic concept was to evaluate the response of individual plant species to the simulated conditions of contaminated soils. Figures 2-4 serve as an illustration of the data from the experiment. They present changes and differences in the discussed indicators' values depending on the plant and metal applied. Figure 2 shows the violin plots of the BCF_T contents. The actual values of the bioconcentration factor BCF_T for winter barley (left) and white mustard (right) observed in the experiment are marked on the vertical axis. The width of the violin at value x on the vertical axis corresponds to the intensity of the observation with the BCF_T content close to value *x*. When analysing the data of the experiment, irrespective of the plants and applied metal, four different threshold ranges can be distinguished: < 0.1, 0.1- 0.5, 0.5 – 1.0 and > 1.0. A higher percentage of observations (67-94% for winter barley and 39 - 78% for white mustard) was in the 2nd range. Outliers can be observed for winter barley with the addition of Cu,. A minor yet considerable percentage was found for white mustard (from 17 to 61%) cultivated on soils contaminated with Cu and Zn, and for winter barley grown on soils contaminated with Cu (17%) and without applied metals (67%). These findings indicate that the range of BCF_T between 0.1 - 0.5 shows normal concentration levels of metals in plant

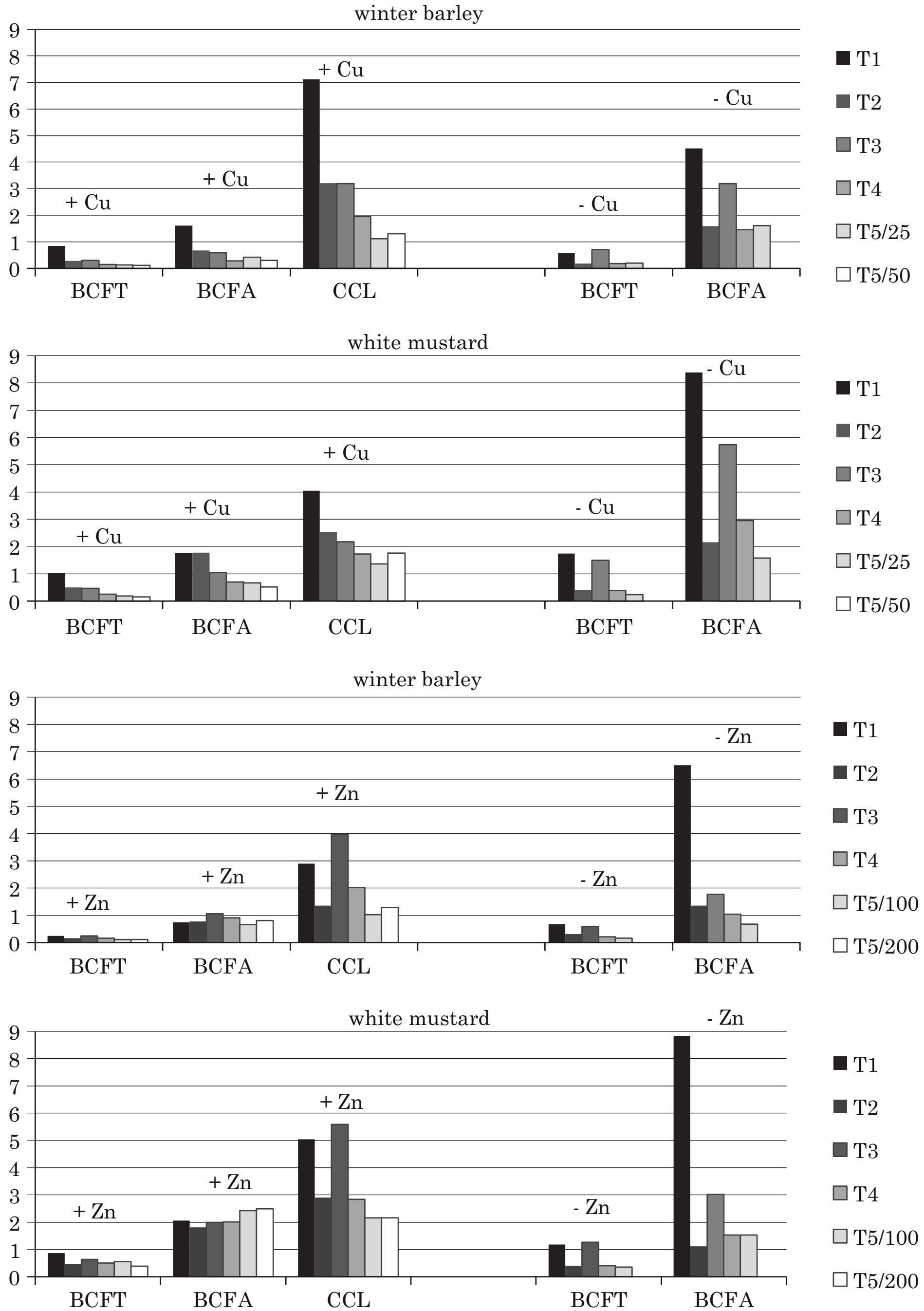

Fig. 1. The mean values of BCF_T, BCF_A and CCL in relation to plant and metal
T1 …T5/200 – descriptions see at Material and Methods

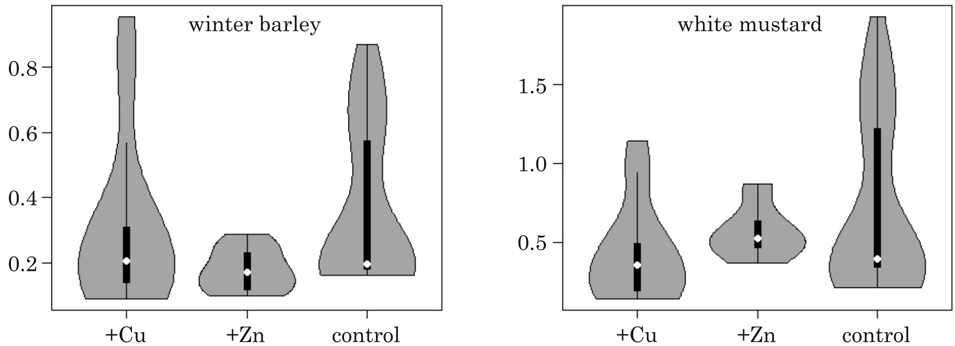

Fig. 2. Violin graph of BCF_T values in relation to the plants and metals in soils with and without compost addition. The actual observed values of the bioconcentration factor BCF_T for winter barley (left) and white mustard (right) are marked on the vertical axis

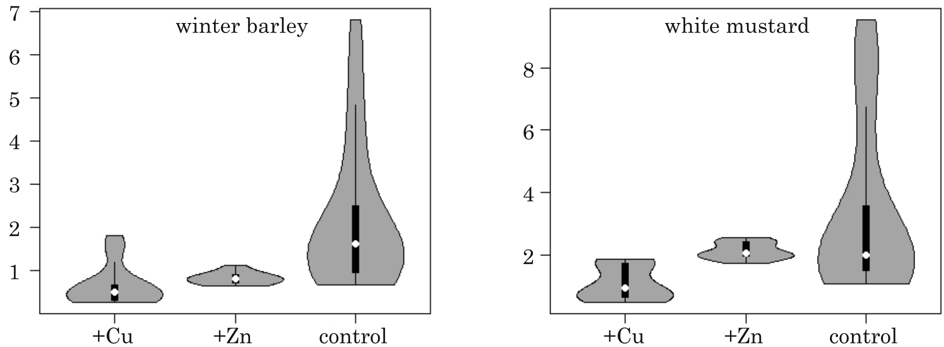

Fig. 3. Violin graph of BCF_A values in relation to the plants and metals in soils with and without compost addition. The actual observed values of the bioconcentration factor BCF_A for winter barley (left) and white mustard (right) are marked on the vertical axis

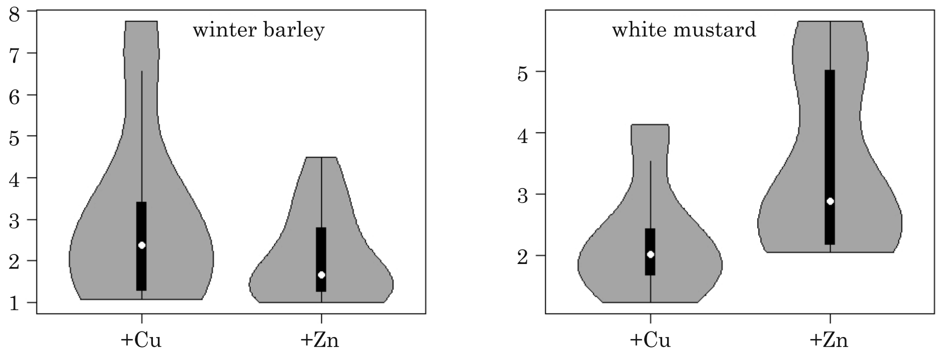

Fig. 4. Violin graph of CCL values in relation to the plants and metals in soils with and without compost addition. The actual observed values of the bioconcentration factor CCL for winter barley (left) and white mustard (right) are marked on the vertical axis

tissues. The next range between 0.5 to 1.0 presumably indicates slightly elevated levels of metals in plant tissues.

The discussed problem of metal-soil-plant relations was also presented with different formulas expressed by BCF_A (Figure 3). In our opinion, this factor is more sophisticated and sensitive to environmental factors, which in this study were represented by various plants and applied metals. It can be concluded from data presented in Figure 3 that the values of BCF_A obtained for plants cultivated in contaminated soil were different from those of BCF_A determined for plants grown in soils without applied metals. Moreover, the compost applied caused a significant reduction in the metal bioavailability, which resulted in lower BCT_A values.

Furthermore, the differences between BCF_A for both test plants were also obvious. To provide more reliable interpretation of this issue, thresholds were defined as follows: < 1, 1-2, 2-3 and > 3. On the basis of per cent shares of observations, the highest share was determined for winter barley (83-89%) in the range below 1. Additionally, 50% of white mustard cultivated in soil with the Cu application showed BCF_A values below 1. For another 50% of total plants grown under the same conditions, the BCF_A values were found in the range between 1 to 2. The above thresholds were also characteristic for 42% observations of winter barley cultivated in soil without any metal addition and for white mustard both grown in soil without any metal addition (47%) and with Zn doses (33%). The highest percentage of observations (67%) was for white mustard cultivated in soil contaminated with Zn and these represented the BCF_A values in the range between 2-3 (Figure 3). Such a division into threshold ranges, observed for the plants and contamination simulated with Cu and Zn salts, facilitates the evaluation of metal toxicity. In our opinion, supported by the results presented above, the values of BCF_A below 1 show natural concentrations of metals in plant tissues, between 1 and 2 they indicate slightly elevated levels of metals, between 2 and 3 – medium elevated levels of metals in plant tissues, and above 3 – highly elevated levels of metals in plant tissues.

The CCL proposed in this study somehow is the recapitulation of previous findings. As data presented in Figure 4 show, there are only small differences between two plants. To interpret these findings, we must take into account four thresholds, such as: < 1, 1-2, 2-3 and > 3. The highest percentage of observations, i.e. from 44 to 56% (in the range of 1 to 2), was assessed for both winter barley cultivated in soils contaminated with Zn and Cu and white mustard grown in soil enriched with Cu doses. One exception was found for white mustard planted in soil with Zn doses applied, where 67% of observations corresponded to the CCL values in the range of 2-3 (Figure 4). Basically, the range of 1-2 CCL values gives information about the natural level of heavy metals, without any metal contamination of plant tissues. The range of 2-3 can show elevated contamination of plants by heavy metals (in our study with Zn). The threshold above 3 for the CCL value is of interest because the percentage of observations was considerable and accounted for 40%, 22% 17% and 33%, respectively, for winter barley cultivated in soil amended with Cu and Zn and for white mustard grown in soil contaminated with Cu and Zn (Figure 4). It can be concluded that these data indicate high plant contamination with metals.

As it was mentioned above, the BCF values strongly depend on the plant species, and this study proves it. Irrespective of the applied metal and its dose, the BCF_T and BCF_A values were always significantly lower for winter barley. Most frequently, monocotyledonous plants assimilate lesser amounts of micronutrients, including Cu and Zn, in comparison to dicotyledonous plants. However, when interpreting the results, the micronutrient requirements of individual plants should be considered. As winter barley is a cereal and therefore has high demand for Cu, this also explains why it accumulates higher Cu amounts than white mustard (described by the CCL values), grown on light and medium soils without compost amendments (Table 2).

Table 2

Comparison of plants (winter barley and white mustard) with respect to values of BCF_T (BCF_A, CCL) in soils with metal (Cu or Zn) and compost

Treatment		BCF_T	BCF_A	CCL		BCF_T	BCF_A	CCL
T1*	Cu25	0.059	0.114	0.001↑	Zn100	0.000↓	0.000↓	0.000↓
T2		0.000↓	0.000↓	0.055		0.000↓	0.000↓	0.001↓
T5		0.003↓	0.000↓	0.011↓		0.000↓	0.000↓	0.000↓
T3	Cu50	0.000↓	0.000↓	0.011↑	Zn200	0.000↓	0.000↓	0.004↓
T4		0.010↓	0.010↓	0.056		0.000↓	0.000↓	0.000↓
T5		0.010↓	0.001↓	0.003↓		0.000↓	0.000↓	0.000↓

Values p of the statistical analysis consisting of Student's t-tests. The value 0.000 denotes p-value <0.001, p-values <0.01 denote the significance at level $\alpha = 0.01$, and 0.01 < p-values <0.05 denote the significance at level $\alpha = 0.05$.

* T1 – light soil, T2 – mixture of light soil with compost, T3 – medium soil, T4 – mixture of medium soil with compost, T5 – compost.

The downwards arrow ↓ – BCF_T, BCF_A, CCL values for winter barley are statistically significantly lower than for white mustard.

The upwards arrow ↑ – BCF_T, BCF_A, CCL values for winter barley are statistically significantly higher than for white mustard.

The absence of an arrow means that the plants did not differ in BCF_T, BCF_A, CCL values.

Table 2 presents the values of probabilities p (p-values) to test the hypothesis with the t-Student test. In order to achieve better data interpretation, beyond usual results from a statistical analysis, arrows are shown. Accordingly, tables we have contain more information, that is not only p values and resultant significance levels ($\alpha = 0.05$ *, $\alpha = 0.01$ **), but also the information (indicated by arrows) on how they differ (whether these values are significantly smaller or larger). For example, in Table 2, we have the value p, that is $p = 0.003$, as well as a downward arrow, which means that the value of BCF_T, for example, was much lower for barley than for mustard. The downward arrows indicate that the values of BCF_T as well as BCF_A

or CCL for winter barley were statistically lower than for white mustard. The reverse effect of Cu accumulation by winter barley was observed as a consequence of using compost alone as a growing medium, where the CCL values were higher for white mustard. A similar pattern of the CCL values was found for whiter barley and white mustard planted in soils, compost and their mixture contaminated with Zn (Table 2).

The significant influence of compost amendment on BCF_T, BCF_A and CCL values was revealed in Tables 3 and 4. Table 3 and Table 4 contain

Table 3

Significance of compost effect on BCF_T, BCF_A values in relation to tested plants cultivated in uncontaminated soils

Treatment	Winter barley		White mustard	
	BCF_T	BCF_A	BCF_T	BCF_A
T1* versus T2	0.001↓	0.001↓	0.001↓	0.001↓
T3 versus T4	0.001↓	0.001↓	0.001↓	0.007↓

* descriptions see at Table 2

p-values of probabilities from Student's *t*-tests. The downward arrows in Table 3 and Table 4 mean statistically lower values of BCF_T (BCF_A or CCL) for winter barley and white mustard cultivated in soil-compost mixture compared to soil without compost. The lowest rate of Cu and Zn bioconcetration expressed by both BCF_T and BCF_A was found for the soils fertilised with compost without simulated contamination (Table 3) as well as with such contamination (Table 4). Due to the high compost capacity (87.6 cmol kg^{-1}, Table 1), this material plays an important role in the heavy metal immobilisation process. The data in Tables 2 and 4 indicate such an influence. Regardless of the metal doses and the type of soil, the strength of compost–metal interactions was the same. In most cases, the values of $BCF_{T,}$ BCF_A and CCL were significantly lower when compost was applied. This was manifested by the values of the above factors, which showed that both winter barley and white mustard accumulated smaller amounts of copper and zinc in comparison to the plants cultivated without compost addition into the soil. A lack of compost-metal interactions was only visible in the treatments with light soil amended with Zn at doses of 100 mg kg^{-1} (winter barley) and 200 mg kg^{-1} (white mustard), and also Cu applied at 25 mg kg^{-1} (white mustard). According to Song et al. (2017), reduction and stabilisation of pollutants in soil usually diminish phytotoxicity. Additionally, Mayel et al. (2014) proved significant influence of compost on heavy metal sorption and desorption processes. The lowest values of Pb desorption factors were observed by the cited authors in both clay loam soil and sandy loam soil amended with compost, and the study presented in this paper confirmed it. The results demonstrated a positive effect of compost, independently of soil, on the reduction of heavy metal mobility, which was attributed to a lesser metal uptake by plants. The influence of organic matter on metal mobility

Table 4

Significance of compost effect on BCF_T, BCF_A, CCL values in relation to tested plants and applied metal

Winter barley								White mustard							
Relation*	BCF_T	BCF_A	CCL	relation	BCF_T	BCF_A	CCL	relation	BCF_T	BCF_A	CCL	relation	BCF_T	BCF_A	CCL
T1Cu versus T2Cu	0.001↓	0.000↓	0.001↓	T1Zn versus T2Zn	0.002↓	0.290	0.001↓	T1Cu versus T2Cu	0.000↓	0.436	0.001↓	T1Zn versus T2Zn	0.000↓	0.016↓	0.001↓
T3Cu versus T4Cu	0.000↓	0.000↓	0.001↓	T3Zn versus T4Zn	0.004↓	0.015↓	0.002↓	T3Cu versus T4Cu	0.000↓	0.014↓	0.021↓	T3Zn versus T4Zn	0.001↓	0.467	0.001↓

* T1Cu – light soil contaminated with Cu at 25 mg kg^{-1}
T2Cu – mixture of light soil with compost contaminated with Cu at 25 mg kg^{-1}
T1Zn – light soil contaminated with Zn at 100 mg kg^{-1}
T2Zn – mixture of light soil with compost contaminated with Zn at 100 mg kg^{-1}
T3Cu – medium soil contaminated with Cu at 50 mg kg^{-1}
T4Cu – mixture of medium soil with compost contaminated with Cu at 50 mg kg^{-1}
T1Zn – medium soil contaminated with Zn at 200 mg kg^{-1}
T2Zn – mixture of medium soil with compost contaminated with Zn at 200 mg kg^{-1}

depends greatly on its properties, for example the degree of humification, the ratio between soluble low molecular weight organic acids (which act mainly as metal carriers) and high molecular weight compounds (which tend to retain metals) (Kumpiene 2010). The applied compost was very well matured, completely humified and biologically stable organic matter. These characteristics are very important in the heavy metal immobilisation process, mainly owing to strong adsorption with humic compounds. Additionally, we must consider the potential decomposition process to increase dissolved organic matter, which complexes metals, reducing their bioavailability to plants. The slightly acid or neutral pH reaction of the growing medium provides favourable conditions for these interactions between metals and compost organic compounds.

CONCLUSIONS

In conclusion, the results of this research recommend the use of biowaste compost as immobilisation amendment of soil contaminated with heavy metals as a reasonable solution to reduce bioavailability of heavy metals in soil. The proposed bioconcentration factor calculated on the basis of total and bioavailable amounts of heavy metals ($BCF_{T,}$ BCF_A) as well as the contamination level coefficient (CCL) seem to be useful tools to assess soil contamination in relation to environment phytotoxicity. In our opinion the presented thresholds for the discussed factors simplify the interpretation of results with respect to soil environment protection. We realise that these factors are new and need additional extended research. Taking into consideration the fact that the bioaccumulation values more valuable than total content in assessment of heavy metal pollution, the research should be continued on this issue, especially on BCF_A practical applications. This factor seems to be more sensitive regarding even small changes in soil contamination with heavy metals and their plant uptake. In further studies, a multi-factor analysis of variance can be made to check the interactions between compost, soil and metals.

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