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REMEDIATION OF SOIL CONTAMINATED WITH CADMIUM

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Abstract

The search for the best solutions to restore soil balance is essential for attaining a stable and sustainable agricultural development worldwide. This research, which makes a contribution to these investigations, focuses on four substances (two innovative ones: basalt flour, brown algal extract, and two classic soil improvers: finely ground barley straw and compost) which can potentially alleviate the inhibitory effect of Cd^{2+} on the soil environment. The following were analyzed: the activity of acid phosphatase and alkaline phosphatase, counts of *Pseudomonas* sp., cellulolytic bacteria, copiotrophic bacteria and copiotrophic spore-forming bacteria, and the yield of spring barley. Cadmium (Cd^{2+}) was applied as $CdCl_2 \cdot 2.5H_2O$ in the following doses: 0, 4, 40, 80, 120, 160, and 200 mg Cd^{2+} kg^{-1} of soil. For a more complete assessment of the soil, its biochemical properties and the counts of microorganisms were scrutinized with the following indices: RS – soil resistance, R:S – rhizosphere effect and EF – fertilization effect of the contamination alleviating substances. It was found that alkaline phosphatase is more sensitive to cadmium contamination of the soil than acid phosphatase. Cadmium did not exert any inhibitory effect on the number of microorganisms present or the yield of spring barley. Cellulolytic bacteria were the least sensitive to stress associated with the accumulation of high cadmium doses in the soil, whereas copiotrophic bacteria were the most sensitive microorganisms to the above stressor. The ability of cadmium-polluted soil to restore homeostasis depended on the type of a soil improver and the level of soil contamination. Negative consequences of cadmium pollution were effectively mitigated by straw, but less so by brown algal extract and basalt flour.

Keywords: cadmium, alkaline phosphatase, acid phosphatase, soil microorganisms, soil remediation.

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INTRODUCTION

The growing concern about the state of ecosystems arises from the progressing industrialization, which results in large amounts of Cd^{2+} being released to soil (Wyszkowska et al. 2007). Subjected to an increasingly severe environmental pressure, soil loses its capacity to fulfil basic functions (Wyszkowska et al. 2006, Kucharski et al. 2011, Griffiths, Phillpot 2013). The main sources of cadmium in soil are sediments and phosphate fertilizers (Smith 2009), waste disposal, galvanization and the production of plastics and paint pigments (Cordero et al. 2004). Each year, an amount of 30.000 Mg of Cd^{2+} is released to the atmosphere, of which around 4.000-13.000 Mg originates from industrial operations. Cadmium pollution is stimulated by the substantial demand for Cd^{2+} all around the world (ATSDR 2005). The negative Cd^{2+} impact manifests itself in the biochemical and microbiological properties of soil ecosystems, as well as the quality and yields of plants (Ciećko et al. 2005, Wyszkowski, Wyszkowska 2009). Exposure to doses of this metal exceeding the upper plant tolerance threshold may eventually lead to plant death. It is possible to limit the availability of Cd^{2+} to plants by increasing the amounts of phosphorus, arsenic and silicon (Mohamed et al. 2012). Plants can also cope with this abiotic stress by raising the content of salicylic acid, jasmonic acid, nitrogen oxide and ethylene in cells. As the amount of ethylene in soil contaminated with Cd, Cu, Fe and Zn increases, so does the activity of ACC (1-aminocyclopropane-1-carboxylic acid), which is responsible for ethylene synthesis (Maksymiec et al. 2007). These compounds, induced by the activity of Cd^{2+}, are formed in response to Cd^{2+} toxicity (Wyszkowska et al. 2013*c*). The exposure to Cd^{2+} leads to alterations in the diversity and counts of microorganisms, but it also affects the enzymatic activity of the soil, which is a reliable manifestation of its biological status (Wyszkowska et al. 2013*b*). Among the enzymes released to soil by microorganisms, the important ones are those which are involved in the transformations of nitrogen, phosphorus and sulphur compounds (Jezierska-Tys, Rutkowska 2014). Apart from oxidoreductases, hydrolases play an important function in soil. The latter group includes acid phosphatase, alkaline phosphatase, urease, arylsulphatase and β-glucosidase (Wyszkowski, Wyszkowska 2009).

A wide range of soil quality parameters must be analyzed in order to determine the actual state of soil, hence only complex soil studies are justifiable (Griffiths, Phillpot 2013). Therefore, the following indices: RS – soil resistance, R:S – rhizosphere effect and EF – fertilization effect of an alleviating substance, were applied to process the results pertaining to the activity of acid and alkaline phosphatase as well as the counts of four microbial groups. In addition, the impact of Cd^{2+} on the growth and development of spring barley was analyzed. However, the main objective was to determine and compare the effectiveness of different substances that might neutralize the effect of pressure caused by cadmium deposits in soil.

MATERIAL AND METHODS

Soil used in the experiment originated from the Research and Teaching Centre in Tomaszkowo, situated in NE Poland (53.716° N, 20.4167° E). The soil samples were collected from the organic and humus horizon (0 and A levels) of leached brown soil (Eutric Cambisol). According to the grain size composition classification and to the US Department of Agriculture taxonomy, this is soil developed from loamy sand. Its grain-size composition is presented in Table 1.

Table 1

Some physicochemical properties of soil used in the experiment

Type of soil	Grain-size composition of soil % of fractions (d)			C_{org} (g kg^{-1})	pH_{KCl}	HAC	TEB	CEC	BS (%)
Loamy sand	sand 2.0≥d≥0.05mm	silt 0.05≥d>0.002	clay d ≤ 0.002			mmol (+) kg^{-1} of soil			
	75	20	5	6.4	5.8	14.75	48.67	63.42	76.75

Corg – organic carbon content per 1 kg of soil d.m., pH_{KCl} – soil reaction, HAC – hydrolytic acidity, TEB – sum of exchangeable bases, CEC – cation exchange capacity, BS – base cations

The experiment was carried out in a greenhouse at the University of Warmia and Mazury in Olsztyn (NE Poland), in five replications. The experimental variables were:

1) cadmium dose applied to soil in mg Cd^{2+} kg^{-1} DM soil: 0, 4, 40, 80, 120, 160 and 200;
2) soil improvers: basalt flour, Labimar 10S algal extract, finely ground straw of spring barley and compost;
3) soil use: soil uncropped or cropped with spring barley (*Hordeum vulgare* L.) duration of the experiment: 25, 50 days.

Before the experiment, soil samples (3.2 kg) were put into a polyethylene pot and mixed with mineral fertilizers (NPKMg). Afterwards, some soil batches were mixed with one of the four neutralizing substances and polluted with cadmium (as cadmium chloride). Thoroughly mixed soil was transferred to 3.5 dm^3 pots, and its moisture content was brought to 60% of capillary water capacity. All the experimental treatments received the same level of macro- and micronutrient fertilization, which equalled (in mg of pure element per kg of soil): N – 250 [$CO(NH_2)_2$], P – 50 (KH_2PO_4), K – 90 (KH_2PO_4), Mg – 20 ($MgSO_4 \cdot 7H_2O$), Cu – 5 ($CuSO_4 \cdot 5H_2O$), Zn – 5 ($ZnCl_2$), Mo – 5 ($NaMoO_4 \cdot 2H_2O$), Mn – 5 ($MnCl_2 \cdot 4H_2O$) and B – 0.33 (H_3BO_3). Basalt flour (Stomeb PPHU, Mietków, Poland) and finely ground barley straw were applied in doses 0 and 5 g kg^{-1} of soil, brown algal extract Labimar 10S (PUH Polger - Kido, Słupsk, Poland) in doses 0 and 1.56 cm^3 kg^{-1} of soil, and compost (Dun-Pol, Susk, Poland) in doses 0 and 3.2 g kg^{-1} of soil.

Spring barley cv. Rabel was sown in some of the pots. After sprouting, barley was thinned, leaving 15 plants per pot. The plant growing period was 50 days. After harvesting spring barley (BBCH 52, 20% of inflorescence emerged), dry matter yield was determined.

On days 25 and 50 of the experiment, soil samples were tested for the counts of copiotrophic bacteria and copiotrophic spore-forming bacteria on the ONTA and HATTORI medium (1983), while the counts of cellulolytic bacteria and *Pseudomonas* sp. were tested on the medium described by WYSZKOWSKA et al. (2007). Counts of microorganisms were determined with a colony counter. In the same samples, the activity of acid and alkaline phosphatases was determined using the ALEF and NANNPIERI'S method (1998). Disodium 4-nitrophenylphosphate (PNPNa) was the substrate used for determination of these enzymes. Extinction of 4-nitrophenol (PNP) was measured on a Perkin-Elmer Lambda 25 spectrophotometer (USA) at the 410 nm wavelength. The activity of acid and alkaline phosphatase was expressed in mmol PNP kg^{-1} DM of soil h^{-1}. Considering the activity of these enzymes, the resistance of soil (RS) index was used to investigate the impact of cadmium on the environment (ORWIN, WARDLE 2004). The results are expressed as the rhizosphere effect (R:S), that is the ratio of enzymatic activity and the count of microorganisms in soil sown with spring barley (R) to the same parameters in uncropped soil (S). The yield of spring barley was also determined.

The impact of each alleviating substance was determined according to the effect factor of the given substance calculated from the following formula:

$$EF = \frac{Ss}{Sc},$$

where: EF – coefficient of the fertilization effect of an alleviating substance EF (EF < 1 – an alleviating substance does not affect positively the enzymatic activity or the count of microorganisms, EF > 1 – an alleviating substance stimulates the analyzed soil parameters);

Ss – activity of enzymes or counts of microorganisms in soil with an alleviating substance;

Sc – activity of enzymes or counts of microorganisms in soil without an alleviating substance.

The results were statistically processed using Statistica 10.0 software (StatSoft Inc. 2012). Homogenous groups were distinguished with the Tukey's test at $P = 0.01$. Coefficients of the Pearson's simple correlation between the incremental doses of cadmium and the activity of phosphatases or the soil's microbiological properties were determined. The effect of each neutralizing substance was submitted to the principal component analysis (PCA), while the response of microorganisms to cadmium contamination of soil was analyzed with data clustering and a dendrogram according to the

Ward's method. The variation of all the investigated variables (η^2) was determined with an analysis of variance (Anova).

As the analysis of η^2 coefficient demonstrated that the duration of the experiment did not have any significant impact on the biochemical and microbiological properties of soil, the data are presented as means for 25 and 50 days.

RESULTS AND DISCUSSION

The results indicate that cadmium is an inhibitor of the microbial counts in soil. The response of microorganisms to soil contamination with Cd^{2+} is illustrated by a diagram derived from the cluster analysis performed with the Ward's method (Figure 1). Separate clusters were formed by cellulolytic bacteria and copiotrophic spore-forming bacteria, whereas copiotrophic bacteria and *Pseudomonas* sp. constituted another group with homogenous variance.

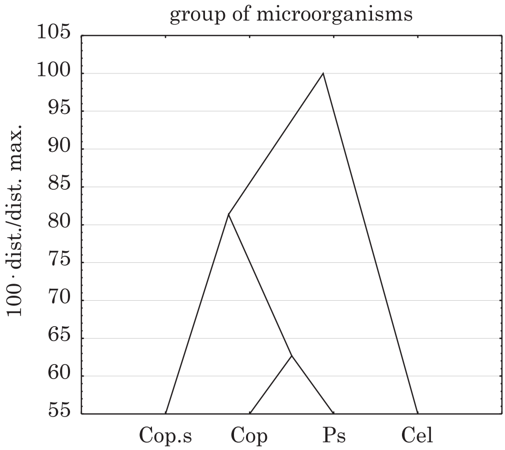

Fig. 1. Similarity of microbial responses to the contamination of soil with Cd^{2+}: Cel – cellulolytic bacteria, Ps. – *Pseudomonas* sp., Cop. – copiotrophic bacteria, Cop. s. – copiotrophic spore-forming bacteria

Biosorption or bioaccumulation by exopolysaccharide (EPS) produced by *Pseudomonas* sp. is one of the most important mechanisms contributing towards an acquisition of resistance to heavy metals by this genus (Kilic, Domez 2008). Copiotrophic bacteria are sensitive to heavy metals deposited in soil with the order of toxicity as follows: Cd > Ni > Cr (III) > Zn > Cu (Wyszkowska et al. 2013c). The activity and diversity of soil microorganisms may be modified by the quality and quantity of root secretions. In the soil with no addition of a soil improver, the beneficial impact of the plant on the microorganisms, according to the recorded R:S values, can be arranged as follows: cellulolytic bacteria, *Pseudomonas* sp. > copiotrophic, spore-forming bacteria > copiotrophic bacteria (Figure 2). The intensive growth of microor-

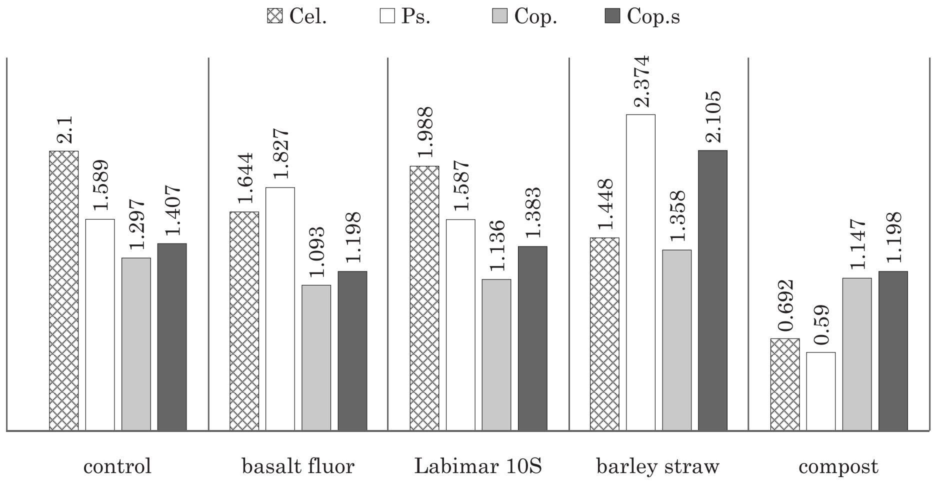

Fig. 2. The rhizosphere effect (R:S) of the counts of microorganisms in soil contaminated with Cd^{2+}: Cel. – cellulolytic bacteria, Ps. – *Pseudomonas* sp., Cop. – copiotrophic bacteria, Cop. s. – copiotrophic spore-forming bacteria

ganisms in the soil sown with barley stemmed from the fact that young roots were a source of simple amino acids, while the rhizosphere of adult plants supplied complex carbohydrates (HOULDEN et al. 2008). In the present study, only straw increased the average values of the rhizosphere effect for all groups of microorganisms except for cellulolytic bacteria.

Application of a neutralizing substance aimed at alleviating the inhibitory effect of Cd^{2+} on the biochemical properties and counts of selected microbial groups. It was found that this factor significantly influenced soil fertility, which manifested itself by high values of the η^2 coefficient, which determines the impact of soil improvers on the examined parameters (Table 2).

The effect of the neutralizing substances was analyzed with the PCA method and the EF - fertilization effect of each alleviating substance. The distribution of vectors around the axis representing the first factor, which describes 92.69% (Figure 3) and 40.95% (Figure 4) of the total data variance, indicates that the number of microorganisms was positively correlated with this variable. In the cropped soil, the number of cellulolytic bacteria was most important for the second factor, which describes 30.30% of variance (Figure 4). The PCA analysis revealed that compost exerted the most beneficial impact on microorganisms in bare soil (Figure 3). Within the range of treatments cropped with spring barley, straw stimulated the number of all microorganisms (Figure 4), whereas compost increased IF values for the co-

Table 2

The observed variability (%) determined by the value of coefficient η^2

The variable	Counts of microorganisms				Enzyme activity	
	cellulolytic bacteria	*Pseudomonas* sp.	copiotrophic bacteria	copiotrophic bacteria spore	Pac	Pal
D	5.796	33.327	33.327	16.658	32.745	43.495
T	5.084	0.344	0.344	0.016	1.308	12.327
S	17.696	27.684	27.684	25.007	22.738	7.936
C	7.836	5.838	5.838	12.767	20.276	0.518
D T	0.303	3.421	3.421	1.283	0.070	3.429
D S	1.022	3.886	3.886	6.406	6.439	2.436
T S	11.670	1.749	1.749	11.073	0.916	8.757
D C	1.747	2.136	2.136	2.466	3.156	0.016
T C	13.648	0.452	0.452	0.125	0.445	0.937
S C	28.196	0.971	0.971	5.984	2.514	1.273
D T S	1.202	4.753	4.753	3.242	2.074	12.590
D T C	0.172	0.658	0.658	0.660	0.102	0.062
D S C	1.204	3.194	3.194	7.250	2.361	4.768
T S C	2.798	6.625	6.625	5.164	1.463	0.266
D T S C	1.480	4.479	4.479	1.677	3.387	1.172
Error	0.148	0.483	0.483	0.224	0.007	0.019

D – dose, T – time of analysis, S – neutralizing substances, C – method of cultivation, Pac – acid phosphatase, Pal – alkaline phosphatase

unts of cellulolytic, copiotrophic and copiotrophic spore-forming bacteria. This is confirmed by the distances between the cases and the values of their coordinates.

The results of our analyses prove a strong negative effect of Cd^{2+} on the investigated enzymes. In both cropped and bare soil, there was a negative correlation between RS values for acid phosphatase (Table 3) and alkaline phosphatase (Table 4) and incremental Cd^{2+} doses. However, alkaline phosphatase was found to be more sensitive to soil contamination with cadmium. When a higher dose of Cd^{2+} was applied (200 mg Cd kg^{-1} of soil), in the soil samples without a neutralizing substance and of soil not sown with spring barley, the resistance of alkaline phosphatase decreased by 61% and of acid phosphatase by 38%. The results of our study on the impact of Cd^{2+} on the biochemical resistance of soil corresponds to the findings reported by Khan et al. (2007). They confirm the inhibitory impact of cadmium on the activity of alkaline phosphatase. The activity of this enzyme decreased by 7.8% after the application of 1.5 mg Cd kg^{-1} of soil, while increasing by as much as 41.5% after applying 5 mg Cd kg^{-1} of soil. Liu et al. (2007), who tested this metal in doses from 5 to 200 mg Cd kg^{-1} of soil, noted that cadmium inhibited

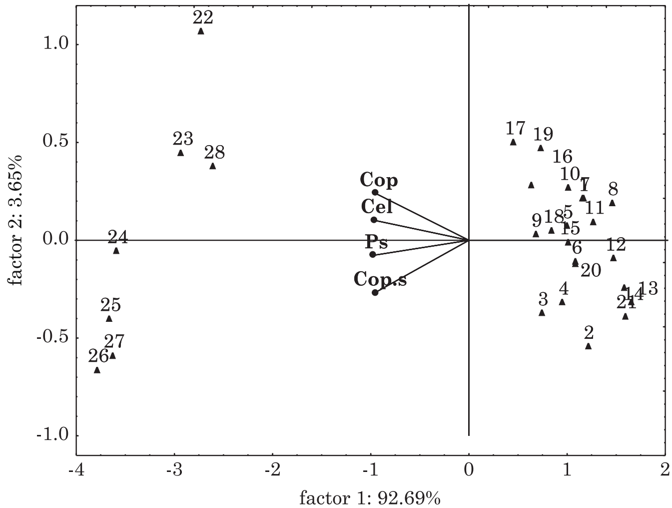

Fig. 3. Fertilization effect of an alleviating substance (EF) on counts of microorganisms – the PCA method (bare soil treatments). Vectors represent the analyzed variables: Ps – *Pseudomonas* sp.; Cel – cellulolytic bacteria; Cop. – copiotrophic bacteria; Cop. s. – copiotrophic spore-forming bacteria; with basalt flour: 1 – 0 mg Cd^{2+}, 2 – 4 mg Cd^{2+}, 3 – 40 mg Cd^{2+}, 4 – 80 mg Cd^{2+}, 5 – 120 mg Cd^{2+}, 6 – 160 mg Cd^{2+}, 7 – 200 mg Cd^{2+}, with algae: 8 – 0 mg Cd^{2+}, 9 – 4 mg Cd^{2+}, 10 – 40 mg Cd^{2+}, 11 – 80 mg Cd^{2+}, 12 – 120 mg Cd^{2+}, 13 – 160 mg Cd^{2+}, 14 – 200 mg Cd^{2+}, with barley straw: 15 – 0 mg Cd^{2+}, 16 – 4 mg Cd^{2+}, 17 – 40 mg Cd^{2+}, 18 – 80 mg Cd^{2+}, 19 – 120 mg Cd^{2+}, 20 – 160 mg Cd^{2+}, 21 – 200 mg Cd^{2+}, with compost: 22 – 0 mg Cd^{2+}, 23 – 4 mg Cd^{2+}, 24 – 40 mg Cd^{2+}, 25 – 80 mg Cd^{2+}, 26 – 120 mg Cd^{2+}, 27 – 160 mg Cd^{2+}, 28 – 200 mg Cd^{2+}

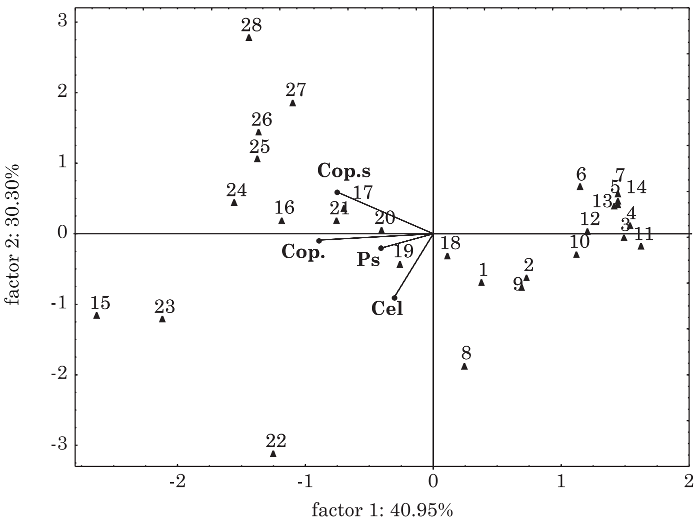

Fig. 4. Fertilization effect of an alleviating substance (EF) on counts of microorganisms – PCA method (barley cropped treatments)

Explanations under Figure 3

Table 3

Indicators of acid phosphatase resistance (RS) to soil contamination with Cd^{2+}, subject to the applied soil improver

Dose Cd (mg kg^{-1})	Control	Basalt flour	Labimar 10S	Barley straw	Compost
	unsown				
4	0.930^{b}	0.871^{b}	0.952^{a}	0.991^{a}	0.841^{b}
40	0.717^{d}	0.762^{cd}	0.754^{d}	0.760^{c}	0.759^{d}
80	0.661^{e}	0.673^{e}	0.757^{d}	0.683^{d}	0.746^{e}
120	0.621^{f}	0.660^{e}	0.728^{e}	0.650^{e}	0.733^{f}
160	0.599^{h}	0.560^{fg}	0.708^{f}	0.473^{f}	0.730^{g}
200	0.578^{i}	0.517^{g}	0.593^{i}	0.448^{g}	0.674^{i}
Average	0.684	0.674	0.749	0.668	0.747
r	-0.878^{*}	-0.981^{*}	-0.895^{*}	-0.961^{*}	-0.913^{*}
	sown				
4	0.957^{a}	0.998^{a}	0.932^{b}	0.844^{b}	0.897^{a}
40	0.736^{c}	0.807^{bc}	0.899^{c}	0.408^{h}	0.723^{h}
80	0.607^{g}	0.758^{c}	0.734^{e}	0.326^{ij}	0.783^{c}
120	0.583^{i}	0.695^{de}	0.678^{g}	0.331^{i}	0.676^{i}
160	0.552^{k}	0.676^{e}	0.631^{h}	0.322^{k}	0.650^{j}
200	0.549^{k}	0.630^{e}	0.594^{i}	0.308^{l}	0.653^{j}
Average	0.664	0.761^{ef}	0.745	0.423	0.730
r	-0.871^{*}	-0.924^{*}	-0.967^{*}	-0.739^{*}	-0.857^{*}

Same letters in columns are assigned to homogenous groups, r – the correlation coefficient, *significant for $P = 0.01$, $n = 17$

the activity of phosphatases. WYSZKOWSKA et al. (2007) and WYSZKOWSKA et al. (2013*c*) undertook an attempt to determine the sensitivity of the analyzed enzymes to Cd^{2+} and other metals. The order of toxicity was as follows: for acid phosphatase $Cr^{6+} > Ni^{2+} > Cu^{2+}$, $Cd^{2+} > Pb^{2+} > Zn^{2+}$, and for alkaline phosphatase $Cd^{2+} > Ni^{2+} > Cu^{2+} > Zn^{2+} > Cu^{2+} > Pb^{2+}$. It is worth emphasizing that cadmium behaves synergistically with zinc towards the alkaline phosphatase. It also shows high similarity to zinc ions, which means that it may replace the latter in numerous bio-complexes and therefore change biological activity (VIG et al. 2003).

The application of a soil improver to the soil did not entirely resolve the experimental hypothesis. The extract from brown algae (Labimar 10S) most favourably affected the resistance of acid phosphatase (Table 3) to Cd^{2+} in uncropped soil, increasing the RS value by 9.50%, whereas in soil sown with spring barley, basalt flour as a soil improver had a stimulating effect and increased the resistance of this enzyme by 15% (Table 3). Compost also led to higher RS values of acid phosphatase, in both cropped and bare soil. Basalt flour stimulated alkaline phosphatase, but a lesser effect was exerted by brown algal extract (Table 4).

Table 4

Indicators of alkaline phosphatase resistance (RS) to soil contamination with Cd^{2+}, subject to the applied soil improver

Dose Cd (mg kg^{-1})	Control	Basalt flour	Labimar 10S	Barley straw	Compost
	unsown				
4	0.767^{b}	0.801^{b}	0.797^{a}	0.913^{a}	0.561^{b}
40	0.473^{c}	0.514^{d}	0.531^{b}	0.272^{c}	0.509^{c}
80	0.362^{e}	0.396^{f}	0.408^{c}	0.225^{cd}	0.430^{e}
120	0.324^{g}	0.394^{f}	0.389^{cd}	0.184^{de}	0.363^{g}
160	0.317^{gh}	0.382^{g}	0.339^{cd}	0.184^{de}	0.331^{h}
200	0.299^{i}	0.332^{j}	0.328^{cd}	0.152^{e}	0.310^{j}
Average	0.424	0.470	0.465	0.322	0.417
r	-0.839^{*}	-0.839^{*}	-0.875^{*}	-0.739^{*}	-0.980^{*}
	sown				
4	0.888^{a}	0.841^{a}	0.817^{a}	0.941^{a}	0.760^{a}
40	0.416^{d}	0.564^{c}	0.555^{b}	0.372^{b}	0.494^{d}
80	0.349^{f}	0.405^{e}	0.389^{cd}	0.261^{c}	0.410^{f}
120	0.314^{h}	0.358^{h}	0.355^{cd}	0.228^{cd}	0.323^{i}
160	0.302^{i}	0.345^{i}	0.339^{cd}	0.205^{de}	0.294^{k}
200	0.302^{i}	0.304^{k}	0.299^{d}	0.190^{de}	0.297^{k}
Average	0.428	0.470	0.459	0.366	0.429
r	-0.761^{*}	-0.885^{*}	-0.883^{*}	-0.784^{*}	-0.887^{*}

Same letters in columns are assigned to homogenous groups,
r – the correlation coefficient, *significant for $P = 0.01$, $n = 17$

The above results are quite surprising because basalt dissolves slowly under natural conditions, which results in a long waiting period necessary for the positive effects of its application to be seen (Shammshuddin et al. 2011). Nevertheless, in the experiment by Anda et al. (2009), basalt slightly reduced soil pH, which might have indirectly resulted in Cd^{2+} exerting a weaker inhibitory effect on the activity of acid phosphatase. However, one should also bear in mind that the introduction of algae to soil is associated with limiting cadmium activity in soil at pH = 5.8. According to Munoz et al. (2006), cadmium may be accumulated by algae in soil at pH = 9. However, compared to other algae, brown algae and *Rhodophyta* are very effective in absorbing heavy metals. This is associated with the structure of their cell wall, which is composed of cellulose, alginic acid, polymer of mannuric acid and guluronic acid (M and G), sodium, potassium, magnesium, calcium salts and polysaccharides (Romera et al. 2006). Chaudhuri et al. (2003) suggested that the incorporation of organic matter into the soil limits the effects of Cd^{2+} accumulation on the activity of phosphatases. The effectiveness of compost may be associated with the fact that aerobic processes occurring during composting increase the complexing of heavy metals with organic matter. Similar to copper and zinc, Cd^{2+} is more strongly bound than nickel (Smith 2009).

WYSZKOWSKA et al. (2013a) emphasize that straw induces significantly higher resistance of both acid and alkaline phosphatase.

The cropping of soil with barley was found to be beneficial for soil. Spring barley stimulated the activity of alkaline phosphatase (Figure 5) and acid phosphatase (Figure 6), regardless of the addition of a neutralizing substance to soil. In the samples of soil without the application of this metal, straw caused an increase in R:S by 23% in relation to the control. In the case of acid phosphatase and alkaline phosphatase, the highest R:S values were in soil with barley straw, followed by brown algal extract, basalt flour and finally with compost. In general, the activity of soil enzymes is higher in cultivated soil. This effect is attributed to the positive role of substances produ-

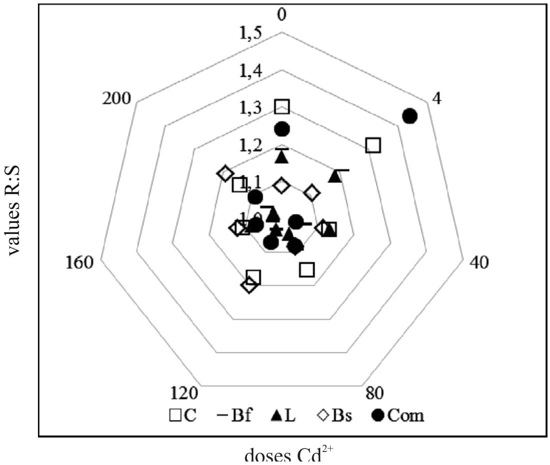

Fig. 5. The rhizosphere effect (R:S) of the activity of alkaline phosphatase in soil contaminated with Cd^{2+}: C – control, Bf – basalt flour, L – Labimar 10S, Bs – barley straw, Com – compost

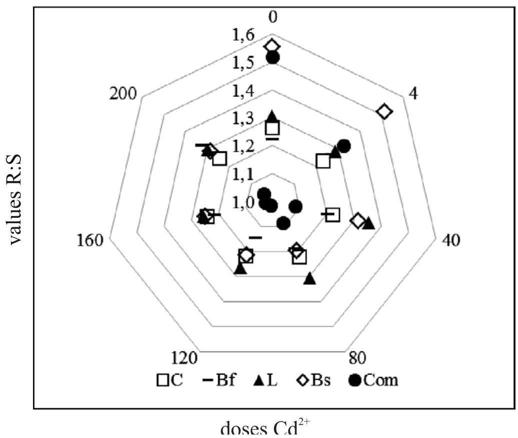

Fig. 6. The rhizosphere effect (R:S) of the activity of acid phosphatase in soil contaminated with Cd^{2+}: C – control, Bf – basalt flour, L – Labimar 10S, Bs – barley straw, Com – compost

ced by roots, which modify the consequences of inhibitory effects of heavy metals on the enzymatic activity of soil (WYSZKOWSKA et al. 2009, 2010).

The EF coefficient was calculated to evaluate the impact of a substance that would potentially neutralize the inhibitory effect of Cd^{2+}. This relation was analyzed with the PCA method (Figures 7, 8). For the activity of acid and alkaline phosphatases in both bare soil (Figure 7) and soil sown with spring barley (Figure 8), the distribution of vectors around the axis represen-

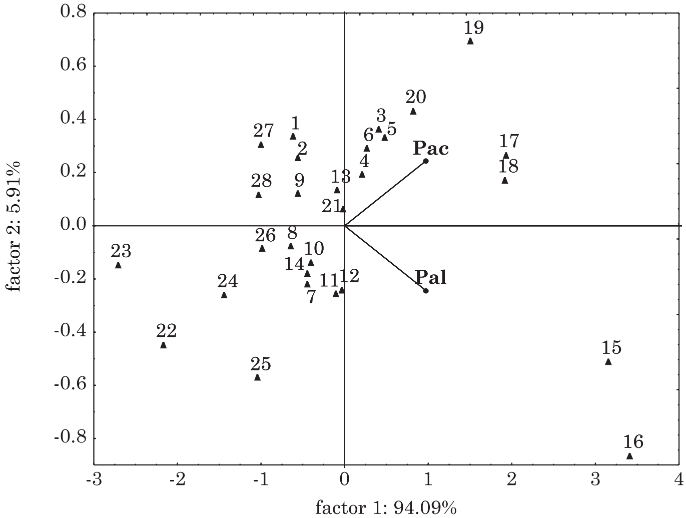

Fig. 7. Fertilization effect of an alleviating substance (EF) on acid phosphatase and alkaline phoshatase – PCA method (bare soil treatments). Vectors represent the analyzed variables: Pac – acid phosphatase; Pal – alkaline phosphatase; with basalt flour: 1 – 0 mg Cd^{2+}, 2 – 4 mg Cd^{2+}, 3 – 40 mg Cd^{2+}, 4 – 80 mg Cd^{2+}, 5 – 120 mg Cd^{2+}, 6 – 160 mg Cd^{2+}, 7 – 200 mg Cd^{2+}, with algae : 8 – 0 mg Cd^{2+}, 9 – 4 mg Cd^{2+}, 10 – 40 mg Cd^{2+}, 11 – 80 mg Cd^{2+}, 12 – 120 mg Cd^{2+}, 13 – 160 mg Cd^{2+}, 14 – 200 mg Cd^{2+}, with barley straw: 15 – 0 mg Cd^{2+}, 16 – 4 mg Cd^{2+}, 17 – 40 mg Cd^{2+}, 18 – 80 mg Cd^{2+}, 19 – 120 mg Cd^{2+}, 20 – 160 mg Cd^{2+}, 21 – 200 mg Cd^{2+}, with compost: 22 – 0 mg Cd^{2+}, 23 – 4 mg Cd^{2+}, 24 – 40 mg Cd^{2+}, 25 – 80 mg Cd^{2+}, 26 – 120 mg Cd^{2+}, 27 – 160 mg Cd^{2+}, 28 – 200 mg Cd^{2+}

ting the first factor that described 94.09% (Figure 7) and 96.64% (Figure 8) of total data variance means that the activity of the analyzed enzymes was positively correlated with this variable. Both in the samples of uncropped soil (Figure 7) and of soil sown with spring barley (Figure 8), there was one homogenous group with positive values of vectors representing the main component variables. The distribution of the cases defined by both PCA axes revealed the positive impact of the applied substances on the activity of the investigated enzymes. Regardless of the soil use, straw was the only substance that significantly effectively (Figures 7, 8) alleviated the inhibitory effect of Cd^{2+} on acid phosphatase and alkaline phosphatase. The other substances (basalt flour, brown algae extract and compost) did not meet the expectations. Of the four soil improvers tested in the experiment, the effectiveness of algae

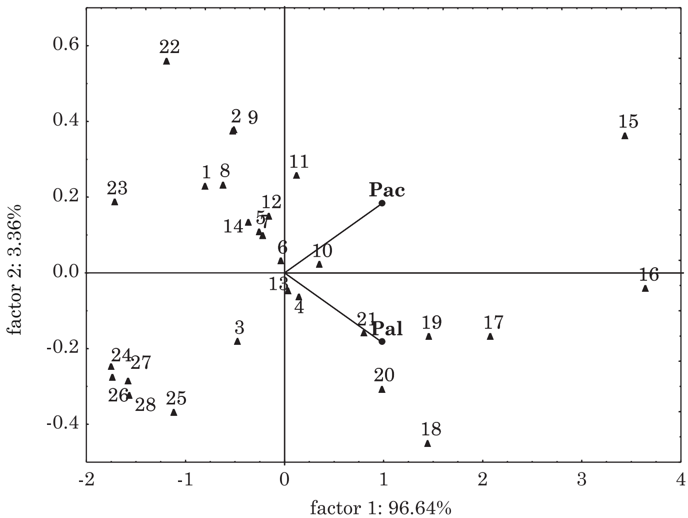

Fig. 8. Fertilization effect of an alleviating substance (EF) on acid phosphatase and alkaline phoshatase – PCA method (barley cropped soil treatments). Explanations under Figure 7

is the most disputable. Whereas it is acknowledged that biosorption of heavy metals by algae occurs *via* a combination of several active and passive mechanisms, which depend on the type and conditions of culture (CHOJNACKA et al. 2005), a question remains as to the selectivity of algae towards heavy metals. YOSHIDA et al. (2006) discovered that algal preferences for metals are as follows: Cu > Cd > Zn. Nevertheless, the adsorption capacity of microalgae in conjunction with the biomass of bacteria with similar properties makes them attractive biosorbents (MUNOZ et al. 2006). Undoubtedly, straw is an optimal source of organic carbon in the soil environment (WYSZKOWSKA et al. 2013*a*) and basalt flour may be effective in increasing plant yields together with an additional source of carbon (ANDA et al. 2013). Moreover, basalt flour, as a result of hydrolysis of silicic acid (H_4SiO_4), may lower the pH of the soil environment and therefore deteriorate the conditions for microorganisms (ANDA et al. 2009).

In order to accurately identify the unfavorable impact of cadmium on soil life, it is necessary to analyze as many parameters that determine soil health as possible. For this reason, our investigations included the impact of Cd^{2+} on spring barley yielding (Table 5). In the pots with an increased content of Cd^{2+}, the growth of barley was inhibited, its root system was malformed and chlorosis affected its leaves. Consistent with the assumed hypothesis, the neutralizing substances introduced into soil alleviated the consequences of stress induced by the increasing soil contamination with Cd^{2+}. The beneficial effect of all the alleviating substances was seen in the variants with an amount higher than 120 mg Cd^{2+} kg^{-1} of soil DM. When straw was used, barley yield increased by 13% in relation to the control without any alleviating substance. Straw was particularly effective in elimi-

Table 5

Impact of cadmium on the yield of spring barley (g pot^{-1})

Dose Cd (mg kg^{-1})	Control	Basalt flour	Labimar 10S	Barley straw	Compost
0	14.990^{a}	14.110^{a}	12.680^{a}	14.710^{a}	12.210^{a}
4	14.010^{a}	12.430^{a}	11.570^{a}	13.530^{ab}	11.590^{a}
40	8.250^{b}	5.860^{b}	6.710^{b}	10.400^{bc}	8.170^{b}
80	4.860^{c}	5.490^{b}	5.160^{bc}	7.770^{cd}	6.460^{bc}
120	4.650^{c}	4.990^{b}	5.070^{bc}	4.700^{de}	5.090^{cd}
160	4.150^{c}	4.440^{b}	4.850^{bc}	5.470^{de}	4.230^{cd}
200	2.780^{d}	4.110^{b}	3.800^{c}	3.870^{e}	3.280^{d}
Average	7.670	7.350	7.120	8.640	7.290
r	-0.900*	-0.830*	-0.870*	-0.950*	-0.960*

Same letters in columns are assigned to homogenous groups, r – the correlation coefficient,
* significant for $P = 0.01$, $n = 20$

nating the effects of Cd^{2+} in soil, at its lower doses, on barley yielding. Furthermore, similarly to previous studies, a dose of 60 mg Cd kg^{-1} of soil reduced the yield of the tested plant by half (Wyszkowski, Wyszkowska 2009). According to Mohamed et al. (2012), the inhibition of growth of a cultivated plant is associated with an unfavourable impact of cadmium on photosynthesis. Irfan et al. (2013) observed an inhibition of barley root growth as a result of cadmium absorption by root hair.

CONCLUSIONS

1. Soil contamination with Cd^{2+} disturbs the balance of soil, which is a complex ecosystem. Cadmium (Cd^{2+}) had an inhibitory impact on biochemical properties of soil. The negative effects of its deposition in soil were also reflected by decreased counts of microorganisms and lower spring barley yield.

2. Alkaline phosphatase was found to be a more sensitive indicator of the contamination of soil with Cd^{2+} than acid phosphatase. Cellulolytic bacteria were the least sensitive to stress associated with the inhibitory effect of this metal, whereas copiotrophic bacteria were the most sensitive ones. The sowing of soil with spring barley increased the enzymatic activity and counts of soil microorganisms.

3. The ability of the soil improvers to restore the homeostasis of cadmium-contaminated soil depended on the type of an alleviating substance and the level of soil contamination. The negative consequences of cadmium pollution were effectively mitigated by straw, but less so by brown algal extract and basalt flour.

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