doi: 10.17221/281/2014-SWR

Using Basalt Flour and Brown Algae to Improve Biological Properties of Soil Contaminated with Cadmium

Magdalena ZABOROWSKA, Jan KUCHARSKI and Jadwiga WYSZKOWSKA

Department of Microbiology, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

Abstract

Zaborowska M., Kucharski J., Wyszkowska J. (2015): Using basalt flour and brown algae to improve biological properties of soil contaminated with cadmium. Soil & Water Res., 10: 181–188.

In order to achieve homeostasis of soil, the potential of alleviating substances (two innovative: basalt flour and brown algae extract against two classic compounds: barley straw and compost) were analyzed in soil contaminated with cadmium. The studies thus determined the activity of urease, number of ammonification bacteria, nitrogen-immobilizing bacteria, *Arthrobacter* sp., *Azotobacter* sp., and spring barley yield. The analyzed parameters were presented as the following indices: RS – resistance of soil; EF – fertilization effect of an alleviating substance; and R:S – rhizosphere effect. Cadmium was applied as $CdCl_2 \cdot 2.5H_2O$ at the following doses: 0, 4, 40, 80, 120, 160, and 200 mg Cd^{2+}/kg of soil. Straw increased the values of most examined parameters, mainly at lower doses of cadmium. Among the cultivated plants, resistance was most stimulated by compost. Basalt flour and brown algae extract did not play a major role in the recovery of contaminated soil. Ammonification bacteria were the least sensitive to stress associated with the deposition of cadmium in soil, whereas *Azotobacter* sp. was the most sensitive. Urease was found to be a reliable indicator of soil condition.

Keywords: cadmium; fertilizing substances; microorganisms; soil; urease

Since the industrial revolution, cadmium, apart from other heavy metals, has become a serious threat to the homeostasis of soil ecosystems. The reason lies in the large demand for Cd^{2+} all over the world. The interest in this metal is based on using sediments, phosphorus fertilizers (Tejada 2009), waste deposition, galvanization, and the production of plastics and paint pigments (Cordero *et al.* 2004). The symptoms of a negative cadmium effect reflect not only the biochemical and microbiological soil properties, but also the quality and plant yielding (Čechmánková *et al.* 2011). Cadmium is an inhibitor of δ-aminolevulinic acid synthetase and protochlorophyllide reductase as well as the enzymes of chlorophyll biosynthesis pathway (MacFarlane & Burchett 2001). The response of plants to its toxicity is an induction of salicylic acid, jasmonic acid, nitrogen oxide, and ethylene oxide and an increase in ACC activity (1-aminocyclopropane-1-carboxylic acid) (Maksymiec *et al.* 2007). The reaction of microorganisms to soil contamination with heavy metals is a controversial issue as it is seen that microorganisms are becoming resistant to increasing pressure of these xenobiotics. On the other hand, even if the number of microorganisms is not decreasing, their diversity is reduced (Wang *et al.* 2007). Disruptions of the microbiological balance associated with cadmium deposition are a result of disturbances to the physiological functions of microbes, including protein denaturation and destruction of the cell membranes of microorganisms (Renella *et al.* 2006).

The idea that enzymatic activity is an accurate reflection of the biological status of soil is also changing (Wyszkowska *et al.* 2013b). Urease is a sensitive indicator of soil contamination with, for instance, cadmium. Apart from hydrolases such as acidic phosphatase, arylosulphatase, and β-glucosidase, urease has important functions in the soil ecosystem (Wyszkowski & Wyszkowska 2009; Kucharski *et al.* 2011). It is one of the most commonly determined soil enzymes as it significantly impacts transformation and pathways of urea in cultivated soil (Abalos

doi: 10.17221/281/2014-SWR

et al. 2012). In 27 EU countries (EMEP/EEA 2013), 24% nitrogen emission (for which urea is the source) has been recorded based on NH_3 determination.

Soil biochemical properties are used both as individual indices and in diverse mathematical transformations and statistical calculations. Since a comprehensive analysis with different soil parameters seems to be the most appropriate for evaluating soil fertility, indices of resistance of soil (RS) were calculated to provide an overview of its stability (Griffiths & Phillipot 2013). The impact of fertilization effect of substances with properties that potentially alleviate the results of cadmium deposition in soil (EF) and the impact of spring barley expressed in the values of rhizosphere effect (R:S) were also included. However, the superior objective was to analyze innovative procedures of soil fertilization to potentially reduce the scale of inhibition of the tested metal against the classic methods.

MATERIAL AND METHODS

Soil sampling and sample preparation. The studies were conducted based on a pot experiment carried out in five replications in a vegetation hall of the University of Warmia and Mazury in Olsztyn (north-eastern Poland). Soil that was used in the experiment originated from the Teaching and Research Centre in Tomaszkowo (north-eastern Poland, 53.7161°N, 20.4167°E). The soil material was sampled from the arable-humus layer of brown soil (Eutric Cambisol). According to the USDA classification, the soil was loamy sand, granulometric composition presented in Table 1.

The impact of four variable factors was evaluated: (1) the level of soil contamination with cadmium in mg Cd^{2+}/kg DM of soil: 0, 4, 40, 80, 120, 160, 200, (2) addition of fertilizing substances: basalt flour, Labimar 10S algae extract, finely ground straw of spring barley and compost, (3) soil use: unsown treatments and treatments sown with spring barley (*Hordeum vulgare* L.), and (4) duration of the studies: 25, 50 days.

The experiment was carried out in pots (3.5 dm^3), each filled with 3.2 kg of soil. Prior to the experiment set-up, the soil material had been prepared in a polyethylene vessel by contaminating it with cadmium ($CdCl_2$), adding NPKMg fertilizers, and potentially alleviating substances in respective experimental objects. After mixing and packing the pots with the prepared soil, the level of moisture was evened to 60% of capillary water capacity in all objects and one level of fertilization with macro- and microelements was applied, which were converted to pure component in mg/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).

Classic fertilizing substances were applied at the following doses: finely ground barley straw at 0 and 5 g/kg of soil and compost (Dun-Pol, Susk, Poland) at 0 and 3.2 g/kg of soil. The innovative compounds were applied in the following amounts: basalt flour (Stomeb PPHU, Mietków, Poland) at 0 and 5 g/kg of soil and brown algae extract Labimar 10S (P.U.H. Polger-Kido, Słupsk, Poland) at 0 and 1.56 cm^3/kg of soil.

The impact of substances was determined based on the impact coefficient of an alleviating substance that was calculated with the following formula:

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

where:

EF – coefficient of fertilization effect of an alleviating substance (EF < 1 – alleviating substance does not impact positively enzymatic activity or the number of microorganisms, EF > 1 – alleviating substance stimulates the analyzed soil parameters)

Ss – activity of enzymes or the number of microorganisms in soil with an alleviating substance

Sc – activity of enzymes or the number of microorganisms in soil without an alleviating substance

Table 1. Some physicochemical properties of soil used in the experiment (kind of soil – loamy sand)

Granulometric composition of soil (% of fractions (*d*, mm))			pH_{KCl}	C_{org} (g/kg of soil)	HAC	TEB	CEC	BS (%)
Sand 2.0 ≥ *d* ≥ 0.05	silt 0.05 ≥ *d* > 0.002	clay *d* ≤ 0.002			mmol(+)/kg of soil			
75	20	5	5.8	6.4	14.75	48.67	63.42	76.75

C_{org} – organic carbon content per 1 kg of soil DM; pH_{KCl} – soil reaction; HAC – hydrolytic acidity; TEB – sum of exchangeable bases; CEC – cation exchange capacity; BS – base cation

doi: 10.17221/281/2014-SWR

Spring barley of the Rabel cultivar was sown at certain sites. After sprouting, plants were segregated and 15 items were left per pot. The plant vegetation period was 50 days. After harvesting the spring barley (BBCH 52, 20% of inflorescence emerged), dry matter yield was determined.

Microbiological and biochemical analysis. The soil samples collected on days 25 and 50 of the experiment were tested for the number of: ammonification bacteria, nitrogen-immobilizing bacteria, and *Arthrobacter* sp. and *Azotobacter* sp. on the substrate described by Wyszkowska *et al.* (2007). The number of microorganisms was determined with a colony counter. The same samples of soil were tested for urease activity (EC 3.5.1.5) with the method described by Alef and Nannipieri (1998). Urea ($(NH_2)_2CO$) was the substrate used for measurements of this enzyme. Absorbance of produced N-NH_4 was measured on a Perkin-Elmer Lambda 25 spectrophotometer (Perkin-Elmer, Waltham, USA) at the 410 nm wavelength. The results of biological measurements were presented as the activity of urease expressed in mmol of N-NH_4/kg of soil DM. The results were given as the rhizosphere effect R:S, i.e. the ratio of the number of microorganisms and urease activity in soil sown with spring barley (R) to the same parameters in unsown soil (S). Spring barley yielding was also determined. The activity of urease and the spring barley yield were used to evaluate the resistance of soil (RS) to contamination with cadmium. Calculations were made with a formula proposed by Orwin and Wardle (2004).

Calculations and statistical analysis. The results were statistically processed in STATISTICA 10.0 software (StatSoft Inc. 2012). Homogeneous groups were compared using Tukey's test at $P = 0.01$. Further, Pearson's simple correlation coefficients between incremental doses of cadmium and urease activity as well as microbiological properties were determined. The reaction of microorganisms to soil contamination with cadmium was analyzed with data clustering and a dendrogram with Ward's method. The impact of an alleviating substance on the number of microorganisms was depicted with the principal component analysis (PCA). The percentage of variation for all analyzed variables (η^2) was determined with the analysis of variance (ANOVA).

As the analysis of η^2 coefficient demonstrated that the duration of the experiment did not exert any significant impact on the urease activity and microbiological properties of soil, the obtained data is presented as the means for specific dates.

RESULTS AND DISCUSSION

Microbial activity. Classic indicators of a degree of soil degradation after cadmium application indicate that this metal shows a strong inhibitory potential on both urease activity and the number of microorganisms. The sensitivity of the examined groups of microorganisms to incremental pressure of the tested metal may be arranged in the following order: ammonification bacteria = nitrogen-immobilizing bacteria > *Arthrobacter* sp. > *Azotobacter* sp. (Figure 1). Having regard to the impact of the cultivated plant on the microbiological activity, according to the R:S values the same relationships between the studied groups of microorganisms were observed.

Bacteria representing the *Azotobacter* genus are one of the microorganisms that are most sensitive to heavy metals (Borowik *et al.* 2014). Ruyters *et al.* (2010) emphasize that cadmium exerts a negative impact on autochthonous soil microorganisms and, in the case of ammonification, it is 6- to 8-times a more powerful inhibitor of this process than zinc (Singha *et al.* 1998). The tested metal also negatively influences ammonification bacteria and it takes the following place in the toxicity order: Ni > Pb > Cr (III) > Cd > Zn > Hg (Wyszkowska *et al.* 2013b). Plants, to oppose the invasion of cadmium, activate mechanisms that give them a chance to detect cadmium as early as in root hairs (Irfan

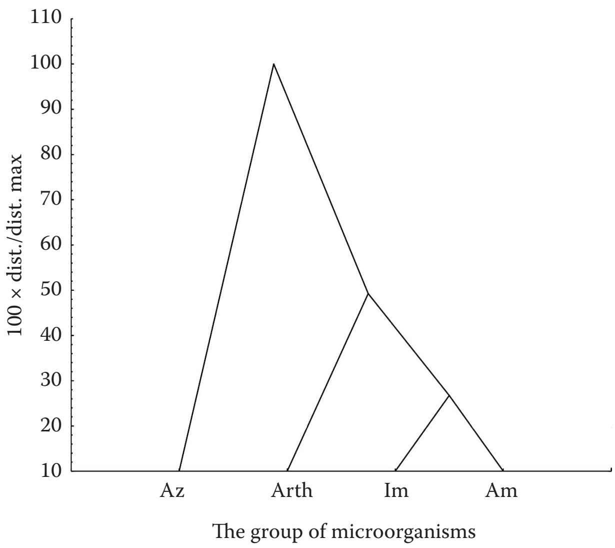

Figure 1. Similarity of microbial reaction to contamination of soil with Cd^{2+}
Am – ammonifying bacteria, Im – nitrogen immobilizing bacteria, Arth – *Arthrobacter* sp., Az – *Azotobacter* sp.

doi: 10.17221/281/2014-SWR

et al. 2013). On the other hand, they do stay neutral for the diversity and microbiological activity of root secretions that accumulate in the rhizosphere: glucose, glutamate acid, citric acid, and oxalic acid (Renella *et al.* 2006).

The effects of four potentially alleviating substances with the use of EF factor were analyzed with the PCA method (Figure 2). The distribution of vectors around the axis representing the first factor that described 58.40% (Figure 2a) and 42.14% (Figure 2b) of the total variance of data indicates that, regardless of the way soil is used, the number of tested microbial groups was positively correlated with this variable. In unsown soil, the number of *Azotobacter* sp. was of the highest importance for the second factor describing 27.90% (Figure 2a), whereas on the objects sown with spring barley (29.56%) (Figure 2b), these were *Azotobacter* sp. and ammonification bacteria. The PCA analysis demonstrated that, regardless of the way soil was used, the classic materials (straw and compost) were the main alleviators of the negative impact of cadmium on the microbiological properties of soil. Interestingly, increased EF values were recorded for ammonification bacteria on the objects supplemented with brown algae extract. In the case of *Azotobacter* sp. it was only straw that stimulated its numbers, although only to a minor degree in the samples contaminated with cadmium (up to 40 mg Cd^{2+}/kg of soil DM) (Figure 2). This is confirmed by the distances between the cases and the values of their coordinates.

According to Romera *et al.* (2006), brown algae and red algae are capable, when compared to other algae, of adsorbing heavy metals. However, Yoshida *et al.* (2006) emphasize that the growth of algae should be expected only in an environment with pH > 8 or pH < 4. Basalt flour did not have any significant, positive impact on the biochemical properties of soil. Under natural conditions, basalt dissolves slowly and thus its positive effects are delayed in time (Shammshuddin *et al.* 2011). Moreover, basalt flour, due to hydrolysis of silicic acid (H_4SiO_4), may deteriorate the conditions in which microorganisms thrive by lowering the pH of the soil environment (Anda *et al.* 2009).

Biochemical activity. Personal studies clearly indicate that urease is an enzyme that is very sensitive to shock caused by contamination of soil with cadmium (Table 2). In the samples without a fertilizing substance, the resistance of urease was reduced after the application of the highest cadmium dose (200 mg Cd^{2+}/kg of soil DM) by 55% in unsown soil and by 37% on the objects sown with spring barley.

The results of the experiment investigating the impact of Cd^{2+} on the biochemical resistance of soil correspond to the findings reported by researchers. Pan and Yu (2011) reported an inhibition of urease activity after the application of 10 mg Cd^{2+} by 17.5% and by 25% after the dose of 50 mg Cd^{2+} was applied.

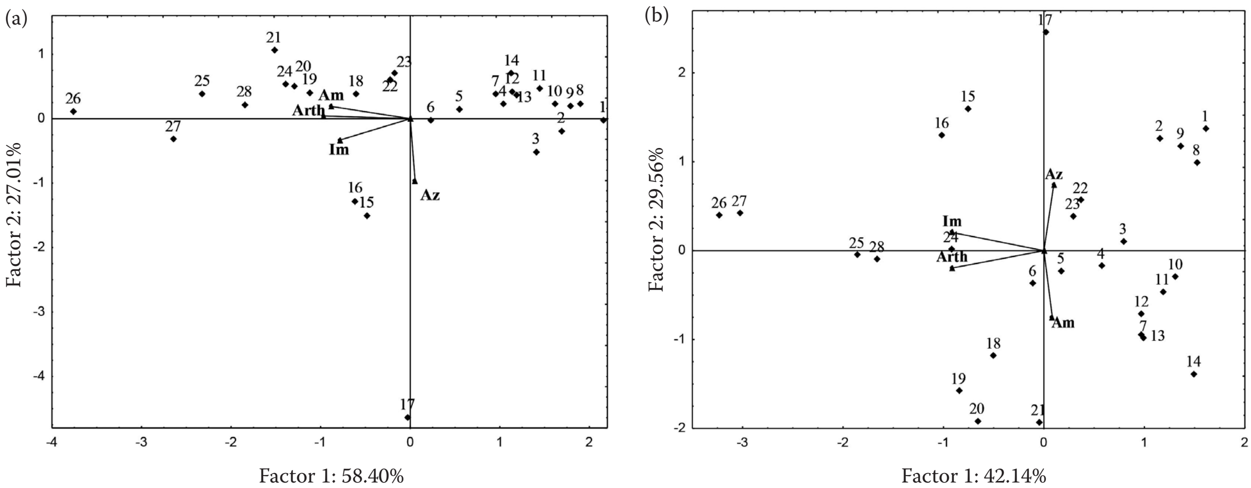

Figure 2. Fertilization effect of alleviating substance (EF) for number of microorganisms – PCA method: objects from the soil unsown (a) and with the soil sown (b)
Vectors represent the analyzed variables: Am – ammonifying bacteria, Im – nitrogen immobilizing bacteria, Arth – *Arthrobacter* sp., Az – *Azotobacter* sp.; dose Cd^{2+} (mg/kg DM soil): 0 (cases: 1, 8, 15, 22), 4 (2, 9, 16, 23), 40 (3, 10, 17, 24), 80 (4, 11, 18, 25), 120 (5, 12, 19, 26), 200 (7, 14, 21, 28), cases: 1–7 with basalt flour, 8–14 with algae, 15–21 barley straw, 22–28 with compost

doi: 10.17221/281/2014-SWR

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

Dose Cd^{2+} (mg/kg)	Control	Basalt flour	Labimar 10S	Barley straw	Compost
Unsown					
4	0.911[a]	0.843[b]	0.591[d]	0.571[c]	0.340[g]
40	0.832[a]	0.728[e]	0.559[f]	0.376[g]	0.318[h]
80	0.775[b]	0.753[d]	0.573[e]	0.376[g]	0.275[j]
120	0.518[cd]	0.751[d]	0.568[e]	0.413[e]	0.293[i]
160	0.423[ef]	0.817[c]	0.607[c]	0.393[f]	0.284[ij]
200	0.408[g]	0.685[f]	0.591[d]	0.353[h]	0.325[h]
Average	0.645[w]	0.763[u]	0.582[x]	0.414[y]	0.306[z]
r	–0.892*	–0.159	–0.011	0.061	0.013
Sown					
4	0.725[b]	0.947[a]	0.841[a]	0.907[a]	0.817[a]
40	0.575[c]	0.673[f]	0.648[b]	0.764[b]	0.792[b]
80	0.556[c]	0.630[g]	0.441[g]	0.475[d]	0.779[c]
120	0.518[cd]	0.463[h]	0.412[h]	0.342[i]	0.708[d]
160	0.499[cde]	0.352[i]	0.385[i]	0.255[j]	0.659[e]
200	0.454[efg]	0.325[j]	0.374[j]	0.214[k]	0.519[f]
Average	0.555[x]	0.565[w]	0.517[y]	0.493[z]	0.712[u]
r	–0.903*	–0.945*	–0.853*	–0.961*	–0.910*

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

According to Speir *et al.* (1999), the power of Cd^{2+} inhibition over urease is comparable to nickel and greater than that of copper, zinc, and chromium(III). In an experiment conducted by Wang *et al.* (2007), cadmium at 10 mg Cd^{2+}/kg of soil DM did not inhibit urease.

The use of fertilizing substance did not produce the expected results on the unsown objects (Table 2). These were only basalt flour and brown algae extract that – at high cadmium doses (120–200 mg Cd^{2+}/kg of soil DM) – generated increased RS values for urease in relation to the control samples. In the case of sown soil, compost most beneficially impacted the resistance of urease to contamination with cadmium. The average RS value increased by 22% in comparison with the control. The resistance of this enzyme also increased when all alleviating substances were present in the samples of soil where the lowest Cd^{2+} doses were applied (4 and 40 mg Cd^{2+}/kg of soil DM). Their efficacy on these objects can be arranged in the following order: straw > basalt flour > compost > brown algae extract.

A stimulating impact of compost on the activity of urease is also emphasized by Tejada (2009). Wyszkowska *et al.* (2013a) are of the opinion that straw generates much higher resistance of individual enzymes. It cannot be disputed that the stimulating effects of classic fertilizing agents were demonstrated by analyzing the tested soil samples with the EF coefficient. Both in unsown and sown soil, finely ground straw was the best at improving the fertility of soil (Figures 3 and 4) and together with compost, it increased the resistance of spring barley (Figure 5).

Wyszkowski and Wyszkowska (2009) also observed that straw effectively eliminates the inhibitory effect of the tested metal, predominantly at its

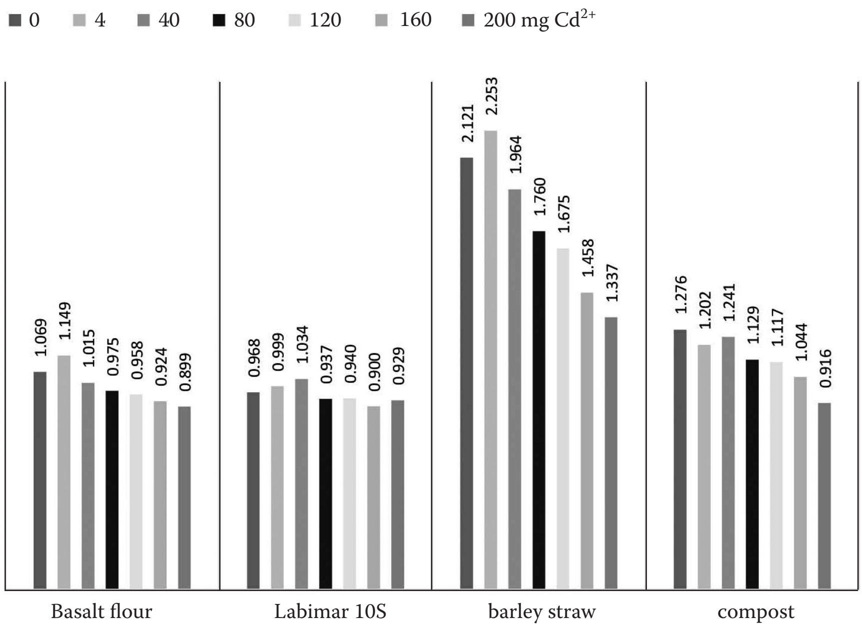

Figure 3. Fertilization effect of alleviating substance (EF) – for urease (objects from the soil unsown), in soil contaminated with Cd^{2+}

doi: 10.17221/281/2014-SWR

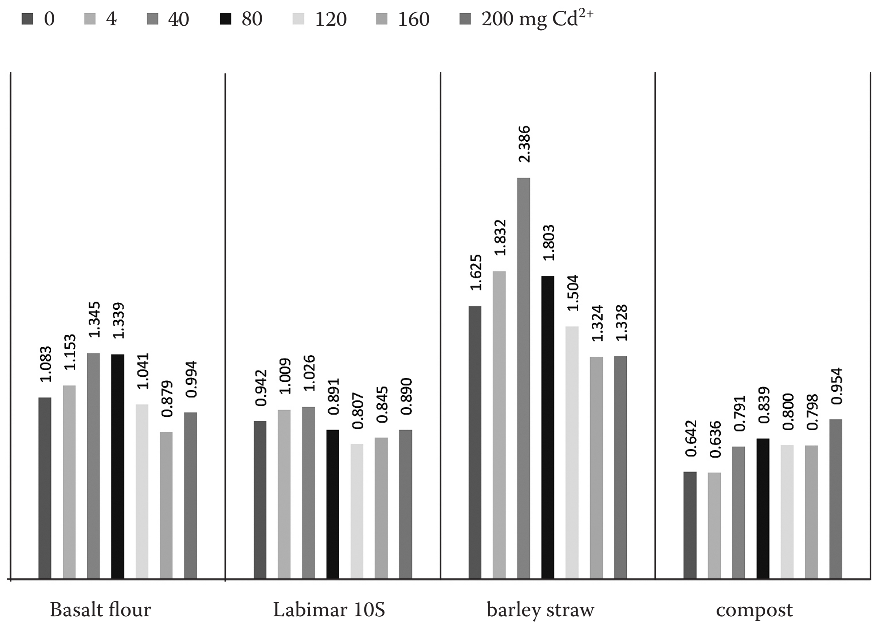

Figure 4. Fertilization effect of alleviating substance (EF) – for urease (objects from the soil sown), in soil contaminated with Cd^{2+}

lower doses, on the development of spring barley. Straw in soil as fertilizer acts beneficially by generating an accumulation of organic carbon in the soil ecosystem. However, Badía *et al.* (2013) indicated increased emissions of CO_2 in such case. Composts in turn complex heavy metals: lead in a larger extent and cadmium to a smaller degree (Tejada 2009).

The particularly positive effect of spring barley cultivation was reflected in the R:S values for urease (Table 3). The classic methods of soil fertilization generated an increase that was highest in the case of straw, namely, by approximately 20% in the samples of soil without cadmium application. According to Wenhao *et al.* (2013), a higher enzymatic activity in the rhizosphere prompts using plants as a phytoremediating factor.

CONCLUSION

Cadmium disrupts the soil balance and urease is a good indicator of soil pollution with this metal. Straw generated improving of biological activity, mainly

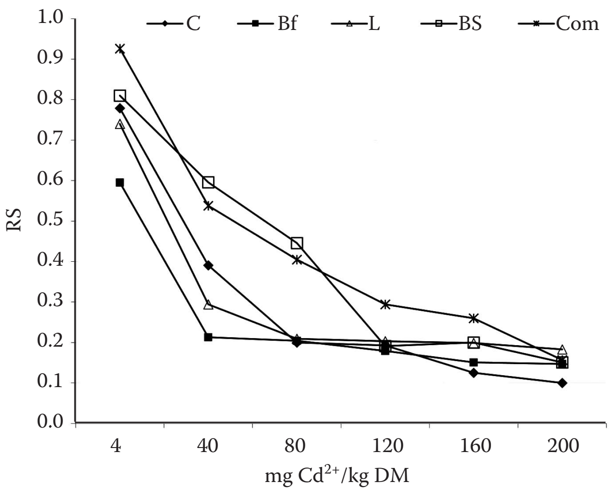

Figure 5. Index of resistance (RS) of spring barley depending on cadmium pollution
C – control; Bf - basalt flour; L – Labimar 10S; Bs – barley straw; Com – compost

Table 3. Effect of cultivation of spring barley on the activity of urease in soil contaminated with cadmium (rhizosphere effect, R:S ratio)

Dose Cd^{2+} (mg/kg)	Kind of neutralizing substances				
	control	basalt flour	Labimar 10S	barley straw	compost
0	1.520[a]	1.431[b]	1.566[a]	1.871[b]	1.206[d]
4	1.290[b]	1.465[a]	1.467[b]	1.832[c]	1.524[b]
40	1.085[c]	1.374[c]	1.176[c]	2.110[a]	1.536[b]
80	1.042[d]	1.318[d]	0.900[d]	1.509[d]	1.582[a]
120	0.910[e]	1.071[e]	0.866[e]	1.075[e]	1.424[c]
160	0.796[f]	0.795[f]	0.793[f]	0.890[f]	1.396[c]
200	0.728[g]	0.737[g]	0.779[g]	0.810[g]	1.098[e]
Average	1.053[z]	1.170[x]	1.078[y]	1.442[u]	1.395[w]
r	–0.941*	–0.972*	–0.917*	–0.933*	–0.413*

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

doi: 10.17221/281/2014-SWR

at lower doses of cadmium. Compost increased the values of resistance of spring barley. Basalt flour and brown algae extract were not effective soil improvers. *Azotobacter* sp. was the most sensitive to stress associated with the deposition of cadmium in soil.

References

Abalos D., Sanz-Cobena A., Misselbrook T., Vallejo A. (2012): Effectiveness of urease inhibition on the abatement of ammonia, nitrous oxide and nitric oxide emissions in a non-irrigated Mediterranean barley field. Chemosphere, 89: 310–318.

Alef K., Nannipieri P. (1998): Methods in Applied Soil Microbiology and Biochemistry. London, Academic Press, Harcourt Brace & Company.

Anda M., Shamshuddin J., Fauziah C.I., Omar S.R. (2009): Dissolution of ground basalt and its effect on oxisol chemical properties and cocoa growth. Soil Science, 174: 264–271.

Badía D., Marti C., Aguirre J.A. (2013): Straw management effects on CO_2 efflux and C storage in different Mediterranean agricultural soils. Science of the Total Environment, 465: 233–239.

Borowik A., Wyszkowska J., Kucharski J., Baćmaga M., Tomkiel M. (2014): Pressure exerted by zinc on the nitrification process. Journal of Elementology, 19: 327–338.

Čechmánková J., Vácha R., Skála J., Havelková M. (2011): Heavy metals phytoextraction from heavily and moderately contaminated soil by field crops grown in monoculture and crop rotation. Soil and Water Research, 6: 120–130.

Cordero B., Lodeiro P., Herrero R., Sastre de Vicente E.M. (2004): Biosorption of cadmium by *Fucus spiralis*. Environmental Chemistry, 1: 180–187.

EMEP/EEA (2013): EMEP/EEA Air Pollutant Emission Inventory Guidebook. Luxembourg, Publications Office of the European Union. Available at http://www.eea.europa.eu/publications/ emep-eea-guidebook-2013 (accessed Feb 5, 2014).

Griffiths B.S., Phillipot L. (2013): Insights into the resistance and resilience of the soil microbial community. FEMS Microbiology Reviews, 37: 112–129.

Irfan M., Hayat S., Ahmad H.A., Alyemeni M.N. (2013): Soil cadmium enrichment: Allocation and plant physiological manifestations. Saudi Journal of Biological Sciences, 20: 1–10.

Kucharski J., Wieczorek K., Wyszkowska J. (2011): Changes in the enzymatic activity in sandy loam soil exposed to zinc pressure. Journal of Elementology, 16: 577–589.

MacFarlane G.R., Burchett M.D. (2001): Photosynthetic pigments and peroxidase activity as indicators of heavy metal stress in the grey mangrove, *Avicennia marina* (Forsk.) Vierh. Marine Pollution Bulletin, 42: 233–240.

Maksymiec W., Wójcik M., Krupa Z. (2007): Variation in oxidative stress and photochemical activity in *Arabidopsis thaliana* leaves subjected to cadmium and excess copper in the presence or absence of jasmonate and ascorbate. Chemosphere, 66: 421–427.

Orwin K.H., Wardle D.A. (2004): New indices for quantifying the resistance and resilience of soil biota to exogenous disturbances. Soil Biology & Biochemistry, 36: 1907–1912.

Pan J., Yu L. (2011): Effects of Cd or/and Pb on soil enzyme activities and microbial community structure. Ecological Engineering, 37: 1889–1894.

Renella G., Egamberiyeva D., Landi L., Mench M., Nanipieri P. (2006): Microbial activity and hydrolase activities during decomposition of root exudates released by an artificial root surface in Cd-contaminated soils. Soil Biology & Biochemistry, 38: 702–708.

Romera E., Gonzalez F., Ballester A., Blazquez M.L., Munoz J.A. (2006): Biosorption with algae: a statistical review. Critical Reviews in Biotechnology, 26: 223–35

Ruyters S., Mertens J., Springael D., Smolders E. (2010): Stimulated activity of the soil nitrifying community accelerates community adaptation to Zn stress. Soil Biology & Biochemistry, 42: 766–772.

Shammshuddin J., Anda N., Fauziah C.I., Omar Syed S.R. (2011): Growth of cocoa planted on highly weathered soil as affected by application of basalt and/or compost. Communications in Soil Science and Plant Analysis, 42: 2751–2766.

Singha D.D., Anoop S., Gupta A.P. (1998): Nitrogen transformation in sewage – sludge amended soil as influenced by addition of zinc, cadmium, and nickel. The Indian Journal of Agricultural Sciences, 68: 96–100.

Speir T. W., Kettles H.A., Percival H.J., Parshotam A. (1999): Is soil acidification the cause of biochemical responses when soils are amended with soil heavy metal salts? Soil Biology & Biochemistry, 31: 1953–1961.

StatSoft Inc. (2012): Statistica. Version 10.0. Available at www.statsoft.com

Tejada M. (2009): Application of different organic wastes in a soil polluted by cadmium: Effects on soil biological properties. Geoderma, 153: 254–268.

Wang J., Lu Y, Shen G. (2007): Combined effects of cadmium and butachlor on soil enzyme activities and microbial community structure. Environmental Geology, 51: 1221–1228.

Wenhao Y., Hong H., Mei R., Wuzhong N. (2013): Changes of microbial properties in (near) rhizosphere soils after phytoextraction by *Sedum alfredii* H: A rhizobox ap-

doi: 10.17221/281/2014-SWR

proach with an artificial Cd-contaminated soil. Applied Soil Ecology, 72: 14–21.
Wyszkowski M., Wyszkowska J. (2009): The effect of soil contamination with cadmium on the growth and chemical composition of spring barley (*Hordeum vulgare* L.) and its relationship with the enzymatic activity of soil. Fresenius Environmental Bulletin, 18: 1046–1053.
Wyszkowska J., Boros E., Kucharski J. (2007): Effect of interactions between nickel and other heavy metals on the soil microbiological properties. Plant, Soil and Environment, 53: 544–552.
Wyszkowska J., Borowik A., Kucharski J., Baćmaga M., Tomkiel M., Boros-Lajszner E. (2013a): The effect of organic fertilizers on the biochemical properties of soil contaminated with zinc. Plant, Soil and Environment, 59: 500–504.
Wyszkowska J., Borowik A., Kucharski M., Kucharski J. (2013b): Effect of cadmium, copper and zinc on plants, soil microorganisms and soil enzymes. Journal of Elementology, 18: 769–796.
Yoshida N., Kieda R., Okuno T. (2006): Identification and characterization of heavy metal-resistant unicellular alga isolated from soil and its potential for phytoremediation. Bioresource Technology, 97: 1843–1849.

Received for publication December 22, 2014
Accepted after corrections March 2, 2015

Corresponding Author:

Prof. Dr. hab. Jadwiga Wyszkowska, University of Warmia and Mazury in Olsztyn, Department of Microbiology, Plac Łódzki 3, 10-727 Olsztyn, Poland; e-mail: jadwiga.wyszkowska@uwm.edu