The effect of organic fertilizers on the biochemical properties of soil contaminated with zinc

J. Wyszkowska, A. Borowik, J. Kucharski, M. Baćmaga, M. Tomkiel, E. Boros-Lajszner

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

ABSTRACT

This study evaluates the effectiveness of organic fertilizers in restoring the homeostasis of soils contaminated with zinc. The activity of selected enzymes participating in the transformation of carbon, nitrogen, phosphorus and sulfur and the sensitivity of white mustard plants to zinc were analyzed. A greenhouse pot experiment was carried out. Uncontaminated soil served as control. Six organic substances which potentially neutralize the adverse effects of zinc were used: tree bark, finely ground barley straw, pine sawdust, cattle manure, compost and cellulose. It was found that in less contaminated soil (300 mg Zn^{2+}/kg), all of the analyzed organic substances minimized zinc adverse effects on the biochemical properties of soil, including the activity of dehydrogenases, catalase, urease, acid phosphatase, alkaline phosphatase, β-glucosidase and arylsulfatase. In more contaminated soil (600 mg Zn^{2+}/kg), the negative consequences of zinc pollution were effectively mitigated only by cellulose, barley straw and manure. Cellulose had the highest soil restoration potential, as demonstrated by resistance indicator values for different enzymes. Cellulose, compost, manure and straw increased the resistance of white mustard plants to zinc, but only in treatments contaminated with 300 mg Zn^{2+}/kg. Bark and sawdust potentiated zinc toxic effects on mustard plants.

Keywords: soil pollution; enzyme activity; resistance index; organic fertilization

Technological, industrial and economic growth leads to heavy metal emissions to the natural environment (Mikanova et al. 2001, Bartoli et al. 2012). Heavy metals are among the most toxic environmental pollutants, and they pose a particular threat for soils which are the main reservoirs for contamination (Boros et al. 2011, Kucharski et al. 2011, Trevisan et al. 2012). Heavy metal pollution contributes to gradual degradation of the soil environment in many parts of the world (Bartoli et al. 2012); it may lead to permanent soil damage, loss of soil fertility and depletion of plant cover (Wyszkowska et al. 2009, Boros et al. 2011, Bartoli et al. 2012). Heavy metals have adverse effects on the biological properties of soil, such as enzymatic activity and microbial counts (Mikanova et al. 2001, Mertens et al. 2007, Wyszkowska et al. 2008), and plants (Ciećko et al. 2001, Wyszkowska et al. 2007, Bartoli et al. 2012, Hejcman et al. 2012).

Biological activity levels are determined by various factors, including type of pollutant (Wyszkowska et al. 2010, Pérez-Leblic et al. 2012, Wyszkowski and Ziółkowska 2013), exposure to pollution (Mertens et al. 2007), soil pH (Kucharski et al. 2011) and organic carbon content (Barančíková and Makovníková 2003, Ros et al. 2003, Borůvka and Drábek 2004). Organic carbon concentrations are a particularly important indicator because organic matter is a key determinant of soil quality which affects the physical, chemical and biological properties of soil (Ciećko et al. 2001, Wyszkowska et al. 2013). In view of the above, the objective of this study was to evaluate the effectiveness of organic fertilizers (tree bark, straw, sawdust, manure, compost and cellulose) in restoring the homeostasis of soils contaminated with zinc. The evaluation was based on the activities of selected enzymes participating in the transformation of

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carbon, nitrogen, phosphorus and sulfur, and the sensitivity of white mustard plants to zinc.

MATERIAL AND METHODS

Soil. Samples of Eutric Cambisol soil were collected from the organic and humus horizons (O-horizon and A-horizon) at the Educational and Experimental Center in Tomaszkowo (NE Poland). The collected soil samples had the granulometric composition of sandy loam based on the USDA soil classification system. The studied soil had the following properties: pH in 1 mol KCl/dm^3 – 7.0; $C_{organic}$ – 7.05 g/kg; N_{total} – 0.74 g/kg; Zn_{total} – 16.60 mg/kg; 16.60 hydrolytic acidity – 8.00 mmol$_+$/kg; sum of exchangeable bases Ca^{2+}, Mg^{2+}, K^+, and Na^+ – 111.00 mmol$_+$/kg; cation exchange capacity – 119.00 mmol$_+$/kg; base saturation – 93.28%.

Experimental design. The experiment was carried out in the greenhouse of the University of Warmia and Mazury in Olsztyn (NE Poland), in five replications, using 3.5 dm^3 polyethylene pots. The experimental variables were the level of soil contamination with zinc in mg Zn^{2+}/kg soil (0, 300, 600) and the type of organic substance (fermented bark, straw, sawdust, manure, compost and cellulose). Each pot was filled with 3 kg of soil. Prior to filling, soil was mixed with macronutrients in polyethylene pots and, subject to treatment, with zinc chloride and organic substances. Macronutrients and micronutrients were supplied at a constant rate in all treatments: N – 100 mg [$CO(NH_2)_2$], P – 44 mg [KH_2PO_4], K – 83 mg [KH_2PO_4 and KCl], Mg – 25 mg [$MgSO_4 \cdot 7\ H_2O$], Mn – 5 mg [$MnCl_2 \cdot 4\ H_2O$], Cu – 5 mg [$CuSO_4 \cdot 5\ H_2O$], Mo – 5 mg [$(NH_4)_6Mo_7O_{24} \cdot 4\ H_2O$] and B – 0.33 mg/kg [$H_3BO_3$]. The moisture content of potted soil was brought to 60% of capillary water capacity. White mustard plants (*Sinapis alba* L.) cv. Rota were sown at 8 plants per pot. Soil moisture content was maintained at 60% of maximum capillary water capacity throughout the experiment. White mustard was harvested at full flowering.

Organic substances. The following organic substances were tested for their ability to restore zinc-contaminated soil: fermented bark of coniferous trees (Athena Bio-Produkty Sp. z o.o., Szczecin, Poland), finely ground barley straw, pine sawdust, cattle manure, compost (Kommunal Service Vornkahl Polska Sp. z o.o., Tczew, Poland) and microcrystalline cellulose (Alfa Aesar GmbH&Co., Karlsruhe, Germany). All substances were applied in the amount of 0 and 9 g/kg dry mater (DM) soil.

Determination of soil enzyme activity. The activity of soil enzymes (methods in Table 1) was determined twice during the experiment (days 25 and 50) in soil samples from each replication in three successive replications. Enzyme activity levels, excluding catalase, were determined using the Perkin-Elmer Lambda 25 spectrophotometer (Massachusets, USA). The activity levels of soil enzymes were presented as mean values from two measurements.

Calculations. The indicators of enzyme resistance were determined based on the activity levels of soil enzymes and the yield of white mustard plants with the use of the formula proposed by Orwin and Wardle (2004).

Statistical analysis. The homogeneity of variance between groups at $P = 0.01$ was determined with the use of Tukey's test. The effect of zinc and organic substances on the activities of soil enzymes was

Table 1. Determined soil enzymes (DM – dry matter)

No.	Enzyme	Substrate	Unit	References
1	dehydrogenases (EC 1.1)	2,3,5-triphenyl tetrazolium chloride	triphenyl fomazan (μmol/kg DM of soil/h)	Öhlinger (1996)
2	catalase (EC 1.11.1.6)	H_2O_2 – aqueous solution	O_2 (mol/kg DM of soil/h)	Alef and Nannpieri (1998)
3	urease (EC 3.5.1.5)	urea – aqueous solution	N-NH_4 (mmol/kg DM of soil/h)	
4	β-glucosidase (EC 3.2.1.21)	*p*-nitrophenyl-β-D-gluco-pyranoside	*p*-nitrophenol (mmol/kg DM of soil/h)	
5	acid phosphatase (EC 3.1.3.2) and alkaline phosphatase (EC 3.1.3.1)	disodium 4-nitrophenyl phosphate hexahydrate	*p*-nitrophenol (mmol/kg DM of soil/h)	
6	arylsulfatase (EC 3.1.6.1)	potassium-4-nitrophenyl-sulfate	*p*-nitrophenol (mmol/kg DM of soil/h)	

evaluated by principal component analysis (PCA). Statistical analyses were performed with the use of Statistica 10.0 software (StatSoft Inc. 2012).

RESULTS AND DISCUSSION

Changes in the biochemical properties of soil induced by zinc contamination were confirmed by the values of enzyme resistance indicators (RS) (Table 2). Soil resistance to zinc was very rarely investigated based on the enzyme activity levels (Kucharski et al. 2011), and the correlations between the indicators of enzyme resistance are complex and influenced by specific environmental conditions (Orwin and Wardle 2004).

In nearly all cases, the lowest RS values were reported in treatments contaminated with 600 mg Zn^{2+}/kg soil, regardless of the applied organic substance (Table 2). The above could be attributed to microbial succession (Kucharski and Wyszkowska 2004) resulting from the elimination of sensitive microorganisms and the proliferation of resistant microbes (Mertens et al. 2007). Although enzyme resistance decreased with increasing levels of soil contamination, irrespective of the applied zinc dose, cellulose had a stimulating effect on the activity of 4 enzymes: catalase, urease, acid phosphatase and alkaline phosphatase, compost – on 3 enzymes: dehydrogenases, acid phosphatase and alkaline phosphatase, straw – 3 enzymes: urease, acid phosphatase and alkaline phosphatase, bark – on 3 enzymes: dehydrogenases, urease and alkaline phosphatase, manure – on 2 enzymes: dehydrogenases and alkaline phosphatase, and sawdust – on 1 enzyme: β-glucosidase. The varied effect of the analyzed organic fertilizers on enzyme activity can probably be attributed to differences in their chemical properties and susceptibility to decomposition (Boros et al. 2011). The applied substances, regardless of type, had the most stimulating effect on the RS values of alkaline phosphatase, followed by acid phosphatase and dehydrogenases.

The distribution of vectors around the axis representing the first principal component indicates that the activity of all analyzed enzymes, excluding

Table 2. Index of soil enzymes resistance depending on zinc pollution

Zn dose (mg/kg of soil)	Application of organic substances						
	control	cellulose	compost	cattle manure	barley straw	tree bark	pine sawdust
Dehydrogenases							
300	0.190^{f}	0.371^{a}	0.325^{b}	0.209^{e}	0.223^{d}	0.231^{c}	0.183^{g}
600	0.080^{k}	0.065^{m}	0.087^{j}	0.113^{h}	0.066^{m}	0.095^{i}	0.077^{l}
Catalase							
300	0.749^{cde}	0.702^{f}	0.617^{g}	0.744^{de}	0.709^{f}	0.589^{h}	0.574^{ij}
600	0.566^{ij}	0.878^{b}	0.445^{l}	0.754^{cd}	0.454^{jk}	0.992^{a}	0.406^{m}
Urease							
300	0.468^{e}	0.868^{a}	0.203^{m}	0.246^{jk}	0.484^{d}	0.640^{c}	0.414^{f}
600	0.259^{ij}	0.759^{b}	0.140^{n}	0.229^{kl}	0.393^{g}	0.373^{h}	0.301^{i}
β-glucosidase							
300	0.663^{fgh}	0.425^{j}	0.697^{efg}	0.924^{a}	0.809^{b}	0.723^{def}	0.781^{bcd}
600	0.627^{ghi}	0.397^{j}	0.420^{j}	0.685^{efg}	0.596^{hi}	0.689^{efg}	0.745^{j}
Acid phosphatase							
300	0.565^{ef}	0.788^{c}	0.771^{c}	0.515^{fgh}	0.923^{a}	0.851^{b}	0.723^{d}
600	0.325^{j}	0.485^{gh}	0.555^{ef}	0.398^{i}	0.533^{efg}	0.316^{j}	0.293^{j}
Alkaline phosphatase							
300	0.291^{i}	0.600^{de}	0.418^{fg}	0.629^{cd}	0.898^{a}	0.658^{c}	0.368^{gh}
600	0.194^{kl}	0.399^{fgh}	0.239^{jk}	0.465^{e}	0.707^{b}	0.369^{gh}	0.237^{jk}
Arylsulfatase							
300	0.424^{d}	0.770^{a}	0.603^{b}	0.542^{c}	0.431^{d}	0.615^{b}	0.377^{e}
600	0.446^{d}	0.305^{g}	0.302^{g}	0.391^{e}	0.252^{h}	0.421^{d}	0.346^{f}

The same letters within a given enzyme, both in columns and in rows, are assigned to homogenous groups

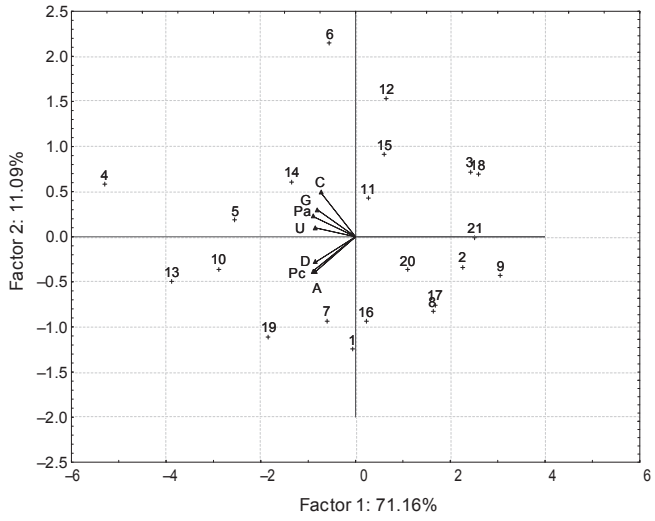

Figure 1. Enzyme activity in loamy sand contaminated with zinc – PCA method. Vectors represent the analyzed variables: D – dehydrogenases; C – catalase; U – urease; Pc – acid phosphatase; Pa – alkaline phosphatase; G – β-glucosidase; A – arylsulfatase; 1 – 0 mg Zn^{2+}; 2 – 300 mg Zn^{2+}; 3 – 600 mg Zn^{2+}; 4 – 0 mg Zn^{2+} with cellulose; 5 – 300 mg Zn^{2+} with cellulose; 6 – 600 mg Zn^{2+} with cellulose; 7 – 0 mg Zn^{2+} with compost; 8 – 300 mg Zn^{2+} with compost; 9 – 600 mg Zn^{2+} with compost; 10 – 0 mg Zn^{2+} with manure; 11 – 300 mg Zn^{2+} with manure; 12 – 600 mg Zn^{2+} with manure; 13 – 0 mg Zn^{2+} with straw; 14 – 300 mg Zn^{2+} with straw; 15 – 600 mg Zn^{2+} with straw; 16 – 0 mg Zn^{2+} with bark; 17 – 300 mg Zn^{2+} with bark; 18 – 600 mg Zn^{2+} with bark; 19 – 0 mg Zn^{2+} with sawdust; 20 – 300 mg Zn^{2+} with sawdust; 21 – 600 mg Zn^{2+} with sawdust

catalase, was significantly correlated with this variable (Figure 1). The first principal component gave rise to two homogeneous groups with negative values of vectors representing primary variables. The first group comprised acid phosphatase, dehydrogenases and arylsulfatase, and the second group – urease, alkaline phosphatase and β-glucosidase. The projection of data onto component space indicates that in treatments without zinc, enzyme activity was most significantly stimulated by cellulose, followed by straw, manure and sawdust. In zinc-polluted treatments, the distance between data points increased with the zinc dose, and enzyme activity was most profoundly stimulated by cellulose, straw and manure, whereas bark, sawdust and compost had the least stimulating effects. According to Ciećko et al. (2001) and Ros et al. (2003), indigenous organic substances as well as organic and natural fertilizers inhibit zinc's toxic effects on soil to a varying degree.

Zinc contamination also had a negative effect on white mustard plants. The analyzed plants responded differently to the applied organic fertilizers. The indicators of plant resistance (RS) proved to be a more reliable measure of the protective effects of organic matter than plant yield (Table 3). The RS values indicate that white mustard is highly sensitive to soil contamination with zinc. White mustard plants grown in contaminated soil were characterized by very low RS values. Fertilization with cellulose, compost, manure and straw increased the resistance of white mustard plants to zinc.

Bark and sawdust decompose less readily than the remaining organic substances. They lead a smaller decrease in the yield of white mustard plants (Table 3) in the control treatment than cellulose and straw which were characterized by wide C:N ratios. More nitrogen was probably immobilized in treatments fertilized with cellulose and straw, and it inhibited the growth and development of white mustard plants. Organic matter fractions are characterized by varied susceptibility to the formation of complexes with heavy metals, which explains differences in their bioavailability (Ciećko et al. 2001, Barančíková and Makovníková 2003, Borůvka and Drábek 2004).

It can be concluded that the ability of organic materials to restore the homeostasis of zinc-contaminated soil was determined by the type of organic substance and the level of soil contamination.

Table 3. Index of white mustard resilience depending on zinc pollution

Zn dose (mg/kg of soil)	Application of organic substances						
	control	cellulose	compost	cattle manure	barley straw	tree bark	pine sawdust
300	0.207^{d}	0.248^{b}	0.372^{a}	0.228^{c}	0.254^{b}	0.036^{f}	0.105^{e}
600	0.008^{ijk}	0.022^{ghi}	0.020^{ghij}	0.018^{ghij}	0.016^{ghij}	0.004^{ijk}	0.013^{ghijk}

The same letters within a given enzyme, both in columns and in rows, are assigned to homogenous groups

In less contaminated soil (300 mg Zn^{2+}/kg), all of the analyzed organic substances minimized zinc's adverse effects on the biochemical properties of soil. In more contaminated soil (600 mg Zn^{2+}/kg), the negative consequences of zinc pollution were effectively mitigated only by cellulose, straw and manure. Cellulose had the highest soil restoration potential. Cellulose, compost, manure and straw increased the resistance of white mustard plants to zinc, but only in treatments contaminated with 300 mg Zn^{2+}/kg. Bark and sawdust potentiated zinc's toxic effects on mustard plants.

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Corresponding author:

Prof. dr. hab. Jadwiga Wyszkowska, University of Warmia and Mazury, Department of Microbiology, Plac Łódzki 3, 10-727 Olsztyn, Poland
phone/fax: + 48 895 233 998, e-mail: jadwiga.wyszkowska@uwm.edu.pl