Int. J. Environ. Res., 8(1):93-100,Winter 2014
ISSN: 1735-6865

Application of Biochemical Tests to Evaluate the Pollution of the Unislaw Basin soils with Heavy Metals

Bartkowiak, A.[1] and Lemanowicz, J.[2*]

[1] Department of Soil Science and Soil Protection, Faculty of Agriculture and Biotechnology, University of Technology and Life Sciences, Bernardyńska 6 St., 85-029 Bydgoszcz, Poland

[2] Sub-Department of Biochemistry, Faculty of Agriculture and Biotechnology, University of Technology and Life Sciences, Bernardyńska 6 St., 85-029 Bydgoszcz, Poland

Received 10 Feb. 2013; Revised 10 June 2013; Accepted 20 June 2013

ABSTRACT: To determine the extent and the size of the environment pollution with heavy metals, biological methods, investigating the enzymatic activity of soil can be applied. Most frequently, the enzymes investigated in the soils threatened with anthropogenic contamination are phosphatases, dehydrogenases as reacting fastest to the increase in the content of heavy metals in the environment. The aim of the paper was to apply the results of the research to the activity of selected oxydo-reducing and hydrolytic enzymes to define the pollution of soils with zinc, copper and nickel of the Unisław Basin, the Kujawy and Pomorze Province. The total contents of Cu, Zn and Ni in the soils of the region allow for classifying the soils as unpolluted with those metals, while the contents of the forms extracted with the DTPA solution point to a low mobility of the elements and to their availability to plants. In terms of the negative effect of those heavy metals on the activity of selected enzymes, they have been ordered as follows: dehydrogenases: Ni>Zn, alkaline phosphatase: Zn>Cu, acid phosphatase: Zn>Cu. The use of enzymatic tests to evaluate the ecochemical condition of soils even with a natural content of heavy metals facilitates the long-term monitoring and identifying the trends.

Key words: Soil, Heavy metals, Dehydrogenases, Catalase, Phosphatases

INTRODUCTION

The natural content of heavy metals in soils does not pose a threat to plants, animals or to the man. However, a greater and greater industrial and agricultural development, due to the application of mineral fertilisers, plant protection agents and sewage, has resulted in an accumulation of toxic compounds, including heavy metals, in the natural environment (in the soil, water and in the air) (Terelak *et al*., 2000). The agrotechnical practises modify the course of natural soil processes by intensifying the soil degradation by decreasing the content of humus or the accumulation of trace elements. Heavy metals are mostly accumulated in the surface horizons of soil pedons; they do not undergo the process of biodegradation and when penetrating into the environment, they affect all the links of the food chain, starting from soil microorganisms, through plants and animals, to end up in the man, triggering various mutagenic and cancerogenic processes in living organisms. The content of heavy metals in soils depends on the natural factors which affect the so-called biogeochemical background, while the others concern the soil pollution processes.

Heavy metals occur in soils in various compounds and forms, affecting the solubility and then the availability to plants. Some are referred to as the vital elements, however when found in higher amounts, they demonstrate a negative effect on living organisms. Most heavy metals are indispensable for the right operation of the enzymes in metabolic processes of the organism, however, once they cross a certain threshold amount, they contribute to the inhibition of the microbiological and enzymatic activity of soil. They are referred to as toxic and they pose the key threat to living organisms, even when found in trace quantities. The degree and the size of the environment pollution can be determined with biological methods by investigating the enzymatic activity of the soil. The most frequently investigated enzymes in the soils exposed to the threat of anthropogenic pollution are phosphatases, dehydrogenases, urease and protease since they react fastest to an increase in the content of heavy metals in the environment (Mocek-Płóciniak,

*Corresponding author E-mail:lemanowicz@utp.edu.pl

2010). Modulators (activators and inhibitors) present in the enzymatic reaction environment affect its rate by a change in the affinity between the enzyme and the substrate, which results in changes in the conformation of the enzyme protein and a decrease in the catalytic activity of the enzyme. The effect of heavy metals, which occur in excessively high amounts in soil, on the enzymes comes both from a direct effect by the denaturation of proteins and the destruction of the integrity of cell membranes, as well as indirectly, involving a change in the sate of soil acidification affecting the crop and decreasing the number of soil microorganisms, being one of the enzymes sources (Piotrowska and Wilczewski, 2012).

An important protective role in the soil is played by humus compounds which show a high sorption capacity. For that reason in the agricultural areas polluted with heavy metals the content of soil humus is of paramount importance. At present the man modifies and affects various soil parameters and, at the same time, can destroy them completely. With that in mind, the basic global task is the protection of soil reservoirs from a further degradation. Enzymatic tests facilitate the evaluation of both the effect of natural factors and the anthropogenic impact on the agroecosystems functioning.

The aim of the present paper was to use the results of research into the activity of selected redox and hydrolytic enzymes to determine the pollution of soils with zinc, copper and nickel in the region of the Unisław Basin, the Kujawy and Pomorze Province, Poland.

MATERIAL & METHODS

The research material was made up of the soil sampled in autumn (October) from the humus horizons of arable soils. The research involved 7 soil profiles from which mineral surface samples (Ap) were taken from the depth of 0-30 cm and below the surface (Aa) from the depth of 30-60 cm. The soils were classified as representing the following type: Fluvisols, subtype: Humic Fluvisols (WRB 2006), demonstrating a high fertility. It is the Unisław Basin which is the region the research material comes from; it is a part of the South Baltic Lakes, being part of the Lower Vistula Valley, stretching from Bydgoszcz all the way to Gniew; it covers the area of about 1 thousand km^2 (Kondracki, 2000) (Fig.1). That area remains under the agricultural use, especially under the field cultivation of carrot, onion, wheat and rapeseed.

The parent material here is mostly made up of various alluvial material deposited as a result of the alluvial process. The process is conditioned by the effect of the surface flowing water in river valleys, which is reflected in the layer-like nature of the material deposited.

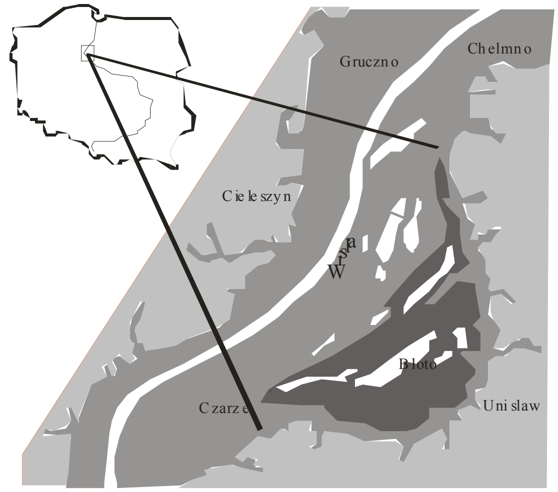

Fig. 1. Location of the study area (Kordowski, 2003)

For the samples taken, using the methods commonly applied in soil-science laboratories, the following were determined: grain size composition (fraction < 0.002), reaction, humus and total nitrogen. Similarly, the measurement was taken of the total content of Zn, Cu and Ni after mineralization in the mixture of acids HF+ $HClO_4$ with the Crock and Severson method (1980) and easily available forms, extracted with DTPA, according to Lindsay and Norvell (1978). The total content and mobile forms were assayed by applying the method of atomic absorption spectroscopy using the spectrometer PU 9100X (Philips). All the results were verified using certified reference material Loamy Clay1 (CRM 052-050). There was also determined the activity of selected enzymes representing the class of oxidoreductases: catalase activity (KAT) [E.C. 1.11.1.6] with Johnson's and Temple's (1964) method, dehydrogenases (DEH) [E.C. 1.1.1] with the method of Thalmann (1968) as well as the class of hydrolases: alkaline phosphatase [E.C. 3.1.3.1] (AlP) and acid phosphatase [E.C. 3.1.3.2] (AcP) with the method of Tabtabai and Bremner (1969). All the assays were made in three reps; the paper presents the arithmetic means of the results.

To identify any potential correlations between soil parameters, the statistical analysis of the results was made using the Statistica software. A correlation matrix studied properties was based on Pearson's correlation coefficients using $p < 0.05$ to indicate the 95% probability levels.

RESULTS & DISCUSSION

The soils were identified as clearly different from most of the soils in Poland. Besides calcium carbonate, it was organic matter which was their main component. In the arable soils of the region analysed there were reported high contents of humus, 8.65-13.84 % and 0.88-13.45%, respectively (Table 1). A high content of C- organic is one of the characteristics which differentiate those soils in the area; they are classified as Humic Fluvisols (Bartkowiak, 2010). In the soils analysed the amounts of $CaCO_3$ determined a neutral or slightly alkaline reaction, in the surface horizons there was recorded the value of exchangeable acidity ranging from pH $_{KCl}$ 7.03 to 7.41 and in sub-surface horizons pH $_{KCl}$ 6.96-7.30 (Table 1). The total nitrogen content ranged from 6.1g·kg^{-1} to 17.3 g/kg in Ap and from 2.1 to 8.0 g/kg in Aa horizons. A variable content of total nitrogen in the soils can result from both the content and the quality of humus compounds as well as the biological activity of those soils. Yet another author confirms that the nitrogen accumulation in organic matter in organic and mineral-and-organic soils is related to muck-formation processes. The soil samples from the Unisław Basin demonstrated a high variation in the content of the fraction with ø < 0.002 mm (Table 1) and they were classified to represent the following grain size composition groups: sandy loam, clay loam, silt loam, clay and silt loam /Annex 2 PTG 2008/ (PTG 2009).

The occurrence of trace elements in soils is mostly determined by the content in the parent rocks of soils and their richness in iron and clay minerals. The main factors, however, affecting the distribution of heavy metals in the soil profile are the content of soil humus, iron and manganese oxides, as well as the grain size composition and soil pH (Salama and Helmke, 1998). The content of total zinc in the surface horizons of the soils did not exceed 18.58 mg/kg, and in subsurface horizons – 18.76 mg/kg, namely the mean value for the soils of agricultural land of Poland (Terelak *et al.*, 2000). The total copper content ranges from 1.60 mg/kg in profile no 5 to 8.40 mg/kg in profile no 2, while the nickel content – from 3.38 mg/kg to 18.04 mg/kg (Fig. 2). The reports (Kabata-Pendias and Pendias, 2001) confirm a special capacity for bonding those metals by organic substance.

However, in none of the samples there were found interactions between the content of those metals and organic substance. The total content of elements in soil was similar to the content of the geochemical background (Kabata-Pendias and Pendias, 2001) and it did not exceed the admissible concentrations for the non-polluted soils (RME, 2002).

Determining the content of phytoavailable forms in soils is important due to its availability to the plants, determined by the concentration of mobile forms in soil. To determine the bioavailability of metal forms, there are applied various extraction solutions, however Komisarek (2008) show that the forms of metals extracted with the solution of diethylene triamine pentaacetic acid (DTPA) are considered available to plants and they accumulate mostly in the humus horizons of many soil types. The elements are considered to be mobile. Their availability and mobility

Table 1. Some physicochemical properties of soils

Profile	Horizon	pH KCl	Content			
			Nitrogen	Humus	Fraction <0.002mm	$CaCO_3$
			g/kg	%		
I	Apca	7.03	7.2	9.62	48.3	16.2
II	Apca	7.13	7.0	11.02	64.0	21.8
	Aaca	7.15	7.3	11.36	60.3	27.2
III	Apca	7.41	7.0	10.50	40.6	24.4
	Aaca	7.27	7.9	11.33	38.5	25.6
	Aaca	6.96	4.6	10.88	11.3	5.3
IV	Apca	7.30	7.4	10.24	51.0	26.5
	Aaca	7.23	2.4	3.40	18.3	6.7
V	Apca	7.33	8.3	13.84	60.0	23.6
	Aaca	7.26	8.0	13.45	64.5	24.9
VI	Apca	7.20	6.1	8.65	66.5	15.9
	Aaca	7.30	2.1	0.88	29.3	69.3
VII	Apca	7.20	17.3	11.19	35.6	25.0

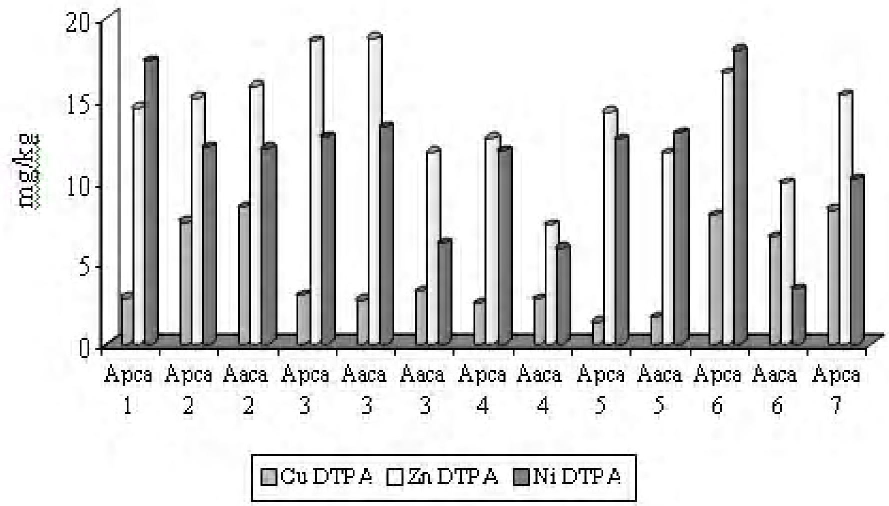

Fig. 2. Total content of copper, zinc and nickel [mg/kg] in the soil horizons

in soil are affected by very many factors, including the content of organic matter, the concentration of iron compounds as well as pH and the grain size composition of the soil itself (Rogóż and Grudnik, 2004; Rooney *et al.*, 2007). Organic matter limits the amount of forms of heavy metals available to plants and thus their toxicity decreases (Kwiatkowska, 2006; Skłodowski *et al.*, 2006). Similarly the reaction is considered one of the main factors affecting the availability of those metals (Kwiatkowska-Malina and Maciejewska, 2011). In the soil samples the content of DTPA-extractable copper ranges from 0.9 to 2.08 mg/kg in surface horizons and from 0.58 to 1.39 mg/kg in sub-surface horizons, the zinc content – from 1.28 to 2.6 mg/kg and from 0.54 to 2.4 mg/kg and the content of nickel from 0.56 to 1.72 mg/kg and from 0.46 to 1.59 mg/kg, respectively (Fig. 3). A change in the soil reaction affected the concentration and the mobility of heavy metals considerably. The increasing soil reaction is accompanied by a decrease in the concentration of zinc, which can be due to its increased bonding by iron and aluminium oxides, or they can be precipitated to less soluble forms. As for nickel, increased soil acidity enhances complex Ni bonds getting solved in the soil solution and increases its bioavailability (Szatanik-Kloc, 2004). In the soils with acid reaction its solubility increases considerably, however, the susceptibility to the formation of bonds with organic substance also triggers a high mobility of nickel when exposed to neutral or alkaline reaction (Kabata-Pendias and Pendia, 2001). Besides a lower content of organic matter plays a smaller role in limiting the bioavailability of nickel, as compared with acid soil (Domańska, 2009). Soil liming is, therefore, a factor limiting the phytoavailability of nickel and zinc. The alkaline reaction of the soils could have resulted in a low content of zinc and nickel, close to the bottom limit of its content in the soils showing the grain size composition of clays **(**Kabata-Pendias and Pendias, 2001). The comparison of the content of copper available to plants with the critical level of 0.2 mg/kg shows that the soils are rich in copper bonds easily available to crops (Kruger *et al.*, 1985).

The statistical calculations made for the purpose of this paper showed a positive correlation between the content of available forms of the elements analyzed and the content of colloidal clay (Table 3). Literature reports (Kabata-Pendias and Krakowiak, 1995; Czekała and Jakubus, 2000) confirm the positive relationships between the contents of those elements and the percentage content of the silt and clay fraction. The contents of Cu, Zn and Ni available to plants were similar to the values most often observed in the soils of the region (Kobierski and Dąbkowska-Naskręt, 2012).

Catalase is an enzyme participating in the defence of plants against the effects of the oxidation stress. The activity of catalase ranged from 0.017 to 0.030 mg H_2O_2/g/h and it was decreasing with the depth of the soil profiles (Table 2). The decreasing activity of the enzymes deep down the soil profile is connected with the spatial distribution of humus as well as soil microorganisms and a decreasing amount of carbon substrates available to microorganisms and enzymes (Bilińska and Mocek-Płóciniak, 2012). No significant relationship was found between the total content of

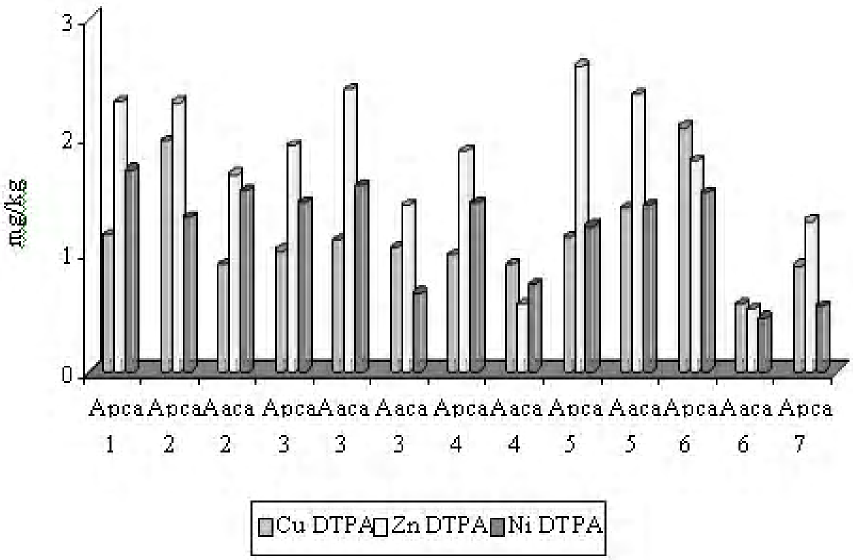

Fig. 3. Content of available forms of copper, zinc and nickel [mg/kg] in the soil horizons

Cu, Zn and Ni as well as their available forms in soil and the activity of catalase. According to Olko and Kujawska (2011), as a result of the effect of heavy metals representing the group of transition metals (e.g. Cu, Fe, Pb), in the presence of H_2O_2 there is an intensive production of ROS (*reactive oxygen species*) as a result of the Fenton and Haber-Weiss reaction, whereas heavy metals which do not show the activity in the cellular redox processes (e.g. Cd, Zn) can result in an increase in the ROS level by the activation of the NADPH oxidase. The deficit of some heavy metals can also trigger oxidation stress in plants. No dependence between the content of Cu, Zn, Ni and the catalase activity in the soil investigated can be due to the natural content of those elements, and thus they did not trigger any oxidation stress in the vegetable crops.

The activity of soil dehydrogenases ranged from 0.283 to 0.481 mg TPF/kg/h (Table 3). The highest activity of that enzyme (0.481 mg TPF/kg/h) was found in the Apca horizon, profile no 7, where rapeseed was grown and the lowest – in the Aaca horizon, profile 5, where onion was grown. Some authors (Gilewska and Płóciniczak, 2004) point to changes in the enzymatic activity depending on the species composition of the plant cover, which is connected with a varied species composition of soil microorganisms which infest the plant roots and their secretions, which affects the concentration of the soluble carbon in soil, thus determining changes in the enzymatic activity. Other authors (Wyszkowska *et al.*, 2008) observed that growing yellow lupin alleviated the unfavourable effect of nickel on dehydrogenases, as compared with the non-sown soil. There was reported a significant negative relationship between the activity of dehydrogenases and the content of available forms of zinc (r= -0.577) as well as nickel (r= -0.617) (Table 3). Similarly other reports (Wyszkowska and Zaborowska, 2002) confirm the inhibiting effect of zinc on the activity of soil dehydrogenases since it is the enzyme most sensitive to changes in the soil environment, reflecting the activity of the entire soil microorganism population.

However, interestingly, zinc as an element of metalloenzymes can both inhibit and stimulate the enzymatic activity.

The activity of alkaline phosphatase ranged from 1.50 to 1.77 mM pNP/kg/h, while the activity of acid phosphatase was lower and it ranged from 0.91 to 1.29 mM pNP/kg/h (Table 2), which is connected with the alkaline soil reaction which is optimal for alkaline phosphatase. Phosphomonoesterases are among the enzymes most sensitive to pH changes (Koper and Lemanowicz, 2008; Lemanowicz and Siwik-Ziomek, 2010; Nagórska-Socha *et al.*, 2006).

The content of heavy metals in the soils investigated had a negative effect on the activity of phosphomonoesterases (Table 3). There was found a significant negative value of the coefficient of correlation between the content of available copper forms in soil and the activity of alkaline (r = -0.621) and acid phosphatase (r = -0.664). The hydrolytic enzymes can also be a good marker of soil pollution with zinc. There were reported significant negative values of the coefficients of correlation between the content of Zn_{DTPA} and the activity of AlP (r = -0.635) and AcP (r =

Table 2. Activity of catalase [mg H_2O_2/g/h], dehydrogenases [mg TPF/kg/h], alkaline [mM pNP/kg/h] and acid phosphatases [mM pNP/kg/h] in soil

Profile	Horizon	Catalase	Dehydrogenases	Phosphatases	
				Alkaline	Acid
1	Apca	0.023	0.312	1.53	1.03
2	Apca	0.020	0.299	1.50	0.94
	Aaca	0.019	0.287	1.64	1.12
3	Apca	0.027	0.322	1.60	1.07
	Aaca	0.028	0.309	1.56	0.97
	Aaca	0.024	0.341	1.60	0.94
4	Apca	0.030	0.337	1.55	1.10
	Aaca	0.017	0.328	1.57	1.07
5	Apca	0.021	0.301	1.54	0.91
	Aaca	0.019	0.283	1.53	0.93
6	Apca	0.026	0.411	1.54	0.96
	Aaca	0.022	0.427	1.77	1.29
7	Apca	0.024	0.481	1.53	1.06

Table 3. Correlation coefficients significant at $p<0.05$

Variable	Cu_{DTPA}	Zn_{DTPA}	Ni_{DTPA}
Fraction<0.002	0.618	0.679	0.701
DEH	-	-0.577	-0.617
AcP	-0.664	-0.698	-
AlP	-0.621	-0.635	-

-0.698). Similar results were recorded by Nagórska-Socha *et al.* (2006) who stated that the enzymatic soil activity (especially dehydrogenase and phosphatases) can be considered an indicator of soil pollution with heavy metals. The highest activity of alkaline phosphatase (1.77 mM pNP/kg/h) and acid phosphatase (1.29 mM pNP/kg/h) were noted in the Aaca horizon, profile 6, (Table 2) in which there was recorded the lowest content of available Cu forms (0.58 mg/kg), Zn (0.54 mg/kg) and Ni (0.46 mg/kg) (Fig. 3). Some authors (Shindo and Huang, 2001), testing the effect of Cu, Zn and Cd on the activity of phosphatases, found that the capacity of Cu for inhibiting the activity of that enzyme was greater than that of Zn and Cd.

CONCLUSION

Total contents of Cu, Zn and Ni in the soils investigated in the region help us classify those soils as non-polluted with those metals, while the contents of the forms extracted with the DTPA solution point to a low mobility of those elements and the availability to plants.

As for the negative effect of the heavy metals on the activity of selected enzymes, they have been ordered as follows: dehydrogenases: Ni>Zn, alkaline phosphatase: Zn>Cu, acid phosphatase: Zn>Cu. The natural content of heavy metals in soils did not trigger any oxidation stress in the crops.

Determining the activity of selected redox and hydrolytic enzymes in the soils of the Unisław Basin can provide a method of long-term soil environment quality monitoring and define the trends found in it.

REFERENCES

Bartkowiak, A. (2010). Morphology and selected physicochemical properties of heterogeneous carbonate sediments in the Unislawski Basin. Soil Science Annual, **61,** 5-12. (in Polish)

Bielińska, E. J. and Mocek-Płóciniak, A. (2012). Impact of the tillage system on the soil enzymatic activity. Archives Environmental Protection, **38**, 75-82.

Crock, J. G. and Severson, R. (1980). Four reference soil and rock samples for measuring element availability in the western energy regions. Geochemical Survey Circular, **841**, 1-16.

Czekała, J. and Jakubus, M. (2000). Occurrence of copper, zinc and manganese in areable soils. Advances Agricultural Sciences Problem Issues, **471**, 219-228. (in Polish)

Domańska, J. (2009). The content and uptake of Ni by plants at differentiated pH of natural, and Cd or Pb contaminated soils. Environmental Protection and Natural Resources, **40,** 236-245. (in Polish)

Gilewska, M. and Płóciniczak, A. (2004). Enzymatic activity of soils originating from past mining soils. Soil Science Annual, **55, 2,** 123-129. (in Polish)

Johnson, J. I. and Temple, K. L. (1964). Some variables affecting the measurements of catalase activity in soil. Soil Science Society of America, **28**, 207-216.

Kabata-Pendias, A. and Krakowiak, A. (1995). Soil parameters as a base for the calculation of background heavy metal status. W: Wilker R., Förster U., Knöchel A. (red.) International Conference "Heavy Metals in the Environment". Hamburg **2**, 398-401.

Kabata-Pendias, A. and Pendias, P. (2001). Trace Elements in Soils and Plants. 3re.ed., CRC Press.

Kobierski, M. and Dąbkowska-Naskręt, H. (2012). Local background concentration of heavy metals in various soil types formed from glacial till of the Inowrocławska Plain. Journal of Elementology, **17, 4,** 559-585.

Komisarek, J. (2008). Spatial analysis of the copper and zinc content in forest haplic luvisols (arenic, spodic) of "Wigry" sample area of integrated monitoring. Science Nature Technologies, **2,** 3-22. (in Polish)

Kondracki, J. (2000). Polish regional Geography. Wyd. Nauk. PWN. (in Polish)

Koper, J. and Lemanowicz, J. (2008). Effect of varied mineral nitrogen fertilization on changes in the content of phosphorus in soil and in plant and the activity of soil phosphatases. Ecological Chemistry and Engineering S, **15,** 465-471.

Kordowski, J. (2003). Internal structures and granulometry of lower Vistula valley overbank deposits in the Toruń and Unisław Basins. Polish Geographical Review, **75,** 601-621. (in Polish)

Kruger, G. A., Karamanos, R. E. and Singh, J. P. (1985). The copper fertility of Saskatchewan soils. Canadian Journal of Soil Science, **65**, 89-99.

Kwiatkowska, J. (2006). The effect of organic amendments on the phytoavailability of heavy metals in polluted soil. Ecohydrology and Hydrobiology, **6,** 181-186.

Kwiatkowska-Malina, J. and Maciejewska, A. (2011). The uptake of heavy metals by plants at differentiated soil reaction and content organic matter. Environmental Protection and Natural Resources, **49,** 43-51. (in Polish)

Lemanowicz, J. and Siwik-Ziomek, A. (2010). Concentrations of available phosphorus and sulphur and activities of some hydrolytic enzymes in a Luvisol fertilized with farmyard manure and nitrogen. Polish Journal of Soil Science, **43,** 37-48.

Lindsay, W. L. and Norvell, W. A. (1978). Development of a DTPA soil test for zinc, iron, manganese, copper. Soil Science of Society America Journal, **43**, 421-428.

Mocek-Płóciniak, A. (2010). Utilisation of enzymatic activity for the evaluation of the impact of anthropogenic changes caused by heavy metals in soil environment. Science Nature Technologies, **4,** 86. (in Polish)

Nagórska-Socha, A., Łukasik, I., Ciepał, R. and Pomiarny, S. (2006). Activity of selected enzymes in soil loaded with varied Levels of heavy metals. Acta Agrophysica, **8,** 713-725.

Olko, A. and Kujawska, M. (2011). Double function of H_2O_2 in plant response to stress conditions. Kosmos, **60, 1-2,** 161-171. (in Polish)

Piotrowska, A. and Wilczewski, E. (2012). Effect of catch crop cultivated for green manure and mineral nitrogen fertilization on soil enzyme activities and chemical properties. Geoderma, **189-190**, 72-80.

PTG, (2009). Particle size distribution and textural classes of soils and mineral materiales-classification of Polish Society of Soil Sciences 2008. Soil Science Annual, **60,** 5-16. (in Polish)

RME, (2002). Regulation of Minister of the Environment on the soil and land quality standards. Dz.U. of 2002, No. **165,** item 1359 (in Polish)

Rogóż, A. and Grudnik, J. (2004). Assessment of trace element pollution of soil and root crops. Ecological Chemistry and Engineering, **11,** 775-785.

Rooney, P. C., Zhao, F. J. and Mc Grath, P. S. (2007). Phytotoxicity of nickel in a range of European soils: Influence of soil properties on Ni solubility and speciation. Environmental Pollution, **145,** 596-605.

Salama, K. and Helmke, P. A. (1998). The pH dependence of free ionic activities and total dissolved concentration of copper and cadmium in soil solution. Geoderma, **83**, 281-291.

Shindo, H. and Huang, Q. Y. (2001). Comparison of the influence of Cu, Zn, and Cd on the activity and kietics of free and immobilized acid phosphatase. Soil Science and Plant Nutrition, **47,** 767-772.

Skłodowski, P., Maciejewska, A. and Kwiatkowska, J. (2006). The effect of organic matter from brown coal on bioavailability of heavy metals in contaminated soils. Soil and Water Pollution Monitoring, Protection and

Remediation. NATO Science Series IV. Earth and Environmental Sciences, **69,** 299-307.

Szatanik-Kloc, A. (2004). Effect of ph and selected heavy metals in soil on their content in plants. Acta Agrophisica, **4,** 177-183.

Tabatabai, M. A. and Bremner, J. M. (1969). Use of p–nitrophenol phosphate for assay of soil phosphatase activity. Soil Biology and Biochemistry, **1**, 301-307.

Terelak, H., Motowicka-Terelak, T., Stuczyńsk, T. and Pietruch, C. (2000). Trace elements (Cd, Cu, Ni, Pb, Zn) in agricultural soils of Poland. IUNG, Warszawa, 1-69. (in Polish)

Thalmann, A. (1968). Zur methodic derestimung der Dehydrogenaseaktivität i Boden mittels Triphenyltetrazoliumchlorid (TTC). Landwirdschaftliche Forschung, **21**, 249-258.

WRB, (2006). IUSS Working Group WRB, World reference base for soil resources. World Soil Resources Reports No. 103. FAO, Rome. 132 pp.

Wyszkowska, J., Boros, E. and Kucharski, J. (2008). Enzymatic activity of nickel contaminated soil. Journal Elementology, **13,** 139-151.