DIAGNOSIS OF THE CONTENT OF SELECTED HEAVY METALS IN THE SOILS OF THE PAŁUKI REGION AGAINST THEIR ENZYMATIC ACTIVITY

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Abstract: The paper presents the research results for the soils sampled from the area located in the eastern part of the Chodzieskie Lakes, between the Middle Noteć River Valley and the Wełna River Valley, the right tributary of the Warta River. The research involved 7 soil samples from the surface horizons, allocated to the cultivation of various plant species (cereals and vegetable crops). The following were determined in the soil material: the content of phytoavailable forms of selected heavy metals Zn, Cu, Pb, Ni, Fe and Mn, active and available to plants phosphorus against the activity of selected oxydo-reduction and hydrolytic enzymes. The soil under the vegetable crops showed a very high richness in phosphorus available to plants, which must have been related to an intensive fertilisation. There were identified relatively low contents of the available forms of the heavy metals investigated, the fact that points to their natural content in soil, which triggered the inhibition of neither the oxydo-reduction nor hydrolytic enzymes.

INTRODUCTION

The contamination of soils with heavy metals is, next to industrial and municipal solid waste, also caused by agriculture since increased contents of such elements can occur in many mineral fertilisers [16, 11] and sewage sludge [9]. Their excessive doses application to enhance the yield can, at the same time, lead to soil contamination. The deteriorating soil properties caused by unbalanced fertilisation result in the changes in the availability of nutrients; their ions can migrate deep down the soil profile [34, 39] or they are accumulated in the topsoil layers and, when uptaken by plants, they reach successive links of the food chain, triggering mutagenic and cancerogenic changes in living organisms [26, 10]. With that in mind, it is recommended to maintain the soils in the optimal state of the equilibrium, which should be today’s objective of agrotechnical methods connected with the intensification of agriculture.

Some heavy metals, such as zinc, iron, nickel, copper, manganese, and cobalt, are considered to represent a group of trace elements participating in the biochemical cellular reactions. They are components of some enzymes, and they are also indispensable...
for the right pattern of biochemical processes. Heavy metals affect the metabolism of soil microorganisms triggering protein denaturation as well as the destruction of cell membranes [3], and through their presence in the environment of the enzymatic activity, they influence their rate by a change in the affinity of the enzyme to substrate, which results in the changes of the conformation of protein and a decrease in the catalytic activity of the enzyme. According to Zaborowska et al. [38], Hinojosa et al. [12] and Bielińska and Mocik-Połciniak [4], to evaluate the state of soil contamination, biological methods are used; hence the use of the measurements of the enzymatic activity, mostly dehydrogenases, phosphatases, ureases and proteases [20]. The key value of the biological diagnostic methods to evaluate the state of the environment, based on enzymatic analyses, is the summary capacity for expressing the impact of many anthropogenic and natural factors. The factors most decisive in terms of the microelements availability to plants are the chemical properties of each element and soil properties [1].

The aim of the paper was to evaluate the contents of selected heavy metals Zn, Cu, Pb, Ni, Fe and Mn in the Luvisol of the Pałuki Region against the activity of selected oxydo-reduction enzymes (catalase and dehydrogenases) as well as hydrolytic enzymes (alkaline and acid phosphatase).

**MATERIAL AND METHODS**

The area analysed is located in the eastern part of the Chodzieskie Lakes, between the Middle of the Noteć River Valley, and the Welna River Valley, the right tributary of the Warta River (Poland) [17]. The analysis involved 7 one-kilogramme soil samples taken in the autumn (the third decade of September) of 2011 from the surface horizons (0–30 cm), allocated to growing various plant species (cereals and vegetable crops). All the tillage and cultivation treatments were performed compliant with commonly applied guidelines of good agrotechnical practises for triticale. The vegetable crops (broccoli, rhubarb, carrot, onion, cauliflower) were exposed to intensive mineral fertilisation and irrigation.

In the air-dried soil samples of a disturbed structure, screened through the ∅ 2 mm sieve, the following physicochemical properties were determined:

- granulometric composition following the modified Cassagrande-Proszynski method
- pH in H₂O and pH in CaCl₂ at the concentration of 0.01 M·dm⁻³,
- carbon of organic compounds (TOC) was determined with the TOC analyser Primacs provided by Scalar. The results were converted into humus.
- content of available phosphorus (P₄₀₋₄₀) in soil by the Egner-Riehm method – DL [PN-R-04023, 1996],
- content of active phosphorus (P₄₀₋₄₀) in soil by the Houba method [13],
- the activity of catalase (CAT) [E.C. 1.11.1.6] in soil with the Johnson, Temple [14],
- the activity of dehydrogenases (DEH) [E.C. 1.1.1] in soil by the Thalmann method [37],
- the activity of alcaline (AcP) [E.C. 3.1.3.1] and acid phosphatases (AcP) [E.C. 3.1.3.2] in soil by the Tabatabai, Bremner method [36].

There were also measured the contents of easily available forms of heavy metals (Zn, Cu, Pb, Ni, Fe and Mn), DTPA-extracted (1 M diethylenetriaminepentaacetic acid),
The content of mobile forms was assayed applying the method of atomic absorption spectroscopy with the PU 9100X spectrometer (Philips).

The paper presents the arithmetic means of the results from three reps. Besides, the results of the analyses of the features investigated were exposed to the analysis of simple correlation (p<0.05) and (p<0.01) which determined the degree of dependence between respective features. The analysis of the correlation was made using ‘Statistica for Windows Pl’ software.

RESULTS AND DISCUSSION

The basic physicochemical properties of the soil samples are given in Table 1, showing that the soils showed the reaction from acid to neutral or alkaline; the values expressed in pH H₂O ranged from 5.8 to 8.5, while in 0.01 M CaCl₂ from 5.6 to 6.9. The amount of humus ranged from 0.72 to 2.5% (Table 1). The values were low, lower than the mean values for the Kujawy and Pomorze Province, reported by Mocek and Owczarzak [25]. The accumulation of organic substance is mostly connected with the types and the kinds of soils. However, the variation in the content of that parameter can be connected with a different soil use [29]. Humus affects the migration and detoxification of heavy metals as well as protects the activity of enzymes. Intensive agricultural production connected with simplified crop rotation or monoculture can limit the amount of organic residue which enters the humus transformation cycle and, as a result, it can lead to a decrease in its content in soils. The decomposition and biodegradation of humus can take place also due to the application of physiologically acid fertilisers and the activation of soil microorganisms under intensive mineral fertilisation.

The research of the samples grain size composition demonstrated the grain size composition of the sandy loam, and the content of clay fraction (⌀<0.002 mm) ranged from 3 to 9%, silt from 17 to 27%, and sand from 71 to 83% (Table 2). In the area investigated Albic Luvisol, formed from glacial tills of the Baltic glaciation of the grain size composition of those clays dominates.

Table 1. Some physicochemical properties of soils

<table>
<thead>
<tr>
<th>Sample no</th>
<th>Plant</th>
<th>$P_{ER}$ mgP·kg⁻¹</th>
<th>$P_{AC}$ mgP·kg⁻¹</th>
<th>Humus %</th>
<th>pH H₂O</th>
<th>pH CaCl₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Triticale</td>
<td>45.86</td>
<td>11.08</td>
<td>2.5</td>
<td>7.5</td>
<td>6.5</td>
</tr>
<tr>
<td>2</td>
<td>Broccoli 1</td>
<td>69.16</td>
<td>12.68</td>
<td>0.9</td>
<td>6.5</td>
<td>5.7</td>
</tr>
<tr>
<td>3</td>
<td>Broccoli 2</td>
<td>164</td>
<td>13.57</td>
<td>1.32</td>
<td>7.3</td>
<td>6.2</td>
</tr>
<tr>
<td>4</td>
<td>Rhubarb</td>
<td>88.05</td>
<td>13.47</td>
<td>0.72</td>
<td>8.5</td>
<td>6.9</td>
</tr>
<tr>
<td>5</td>
<td>Carrot</td>
<td>74.03</td>
<td>12.07</td>
<td>0.83</td>
<td>6.9</td>
<td>6.3</td>
</tr>
<tr>
<td>6</td>
<td>Onion</td>
<td>44.48</td>
<td>11.64</td>
<td>1.21</td>
<td>5.8</td>
<td>5.8</td>
</tr>
<tr>
<td>7</td>
<td>Cauliflower</td>
<td>167</td>
<td>18.89</td>
<td>0.92</td>
<td>6.3</td>
<td>5.6</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>93.28</td>
<td>13.34</td>
<td>0.92</td>
<td>6.3</td>
<td>5.6</td>
</tr>
<tr>
<td>SD*</td>
<td></td>
<td>51.806</td>
<td>2.613</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SD* – standard deviation
Trace elements occur in the soils of various forms and chemical compounds, affecting their solubility and availability to plants. The kind of agricultural use is connected with the application of various doses of mineral and organic fertilisers affecting the content of the elements in soil. In the arable soils analysed the content of available forms ranged for Zn 0.914–5.746 mg·kg⁻¹; Cu 0.540–2.234 mg·kg⁻¹; Pb 0.536–0.826 mg·kg⁻¹; Ni 0.121–0.242 mg·kg⁻¹; Fe 22.36–117.06 mg·kg⁻¹ and Mn 5.236–33.76 mg·kg⁻¹ (Table 3). When evaluating the contamination level of the soils with selected heavy metals, compliant with the Regulation of the Minister of the Environment of September 2, 2002, on the soil quality standards and the earth crust quality standards [Dz.U. No 165, item 1359], one shall observe that no admissible contents have been exceeded, which points to their natural content in soil. The availability of those elements and their mobility in soil are affected by very many factors; the content of organic matter, the concentration of iron compounds and pH as well as the grain size composition of the soil itself [31, 32]. The metal toxicity also decreases with an increase in organic substance which limits the amount of the forms of heavy metals available to plants [35]. The uptake of heavy metals can be limited or inhibited by some macro- and micronutrients. The presence of phosphorus in soil is an essential factor limiting the uptake of heavy metals by plants since together with a higher content of its easily soluble forms there can precipitate hard-soluble phosphates of zinc, cadmium, lead and copper. The availability of heavy metals and micronutrients uptaken by plants in a form of cations increases with soil acidification since, under those conditions, their solubility increases [8]. In the soil samples analysed there was found, however, no interaction between the soil parameters and the content of available forms of those elements.

The compounds of phosphorus which occur in nature are not harmful to living organisms. Nevertheless its excess in terrestrial ecosystems can lead to a decrease in the biodiversity, while in the aquatic ecosystems – to limiting the availability of oxygen which, in turn, leads to disappearance of life [35].

The content of available phosphorus ($P_{E-R}$) in the soil ranged from 44.48 mgP·kg⁻¹ to 167 mgP·kg⁻¹ (Table 1). According to the criteria provided for in PN-R-04023 [1996], the soil can be classified as class I of a very high content of $P_{E-R}$. It is assumed that 30 mgP·kg⁻¹ of soil is a critical phosphorus content for plants. However, the accumulation

<table>
<thead>
<tr>
<th>Sample no</th>
<th>Horizon</th>
<th>Content of fraction [%]</th>
<th>2–0.05 mm</th>
<th>0.05–0.002 mm</th>
<th>&lt;0.002 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ap</td>
<td>78</td>
<td>16</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Ap</td>
<td>77</td>
<td>17</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Ap</td>
<td>80</td>
<td>16</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Ap</td>
<td>83</td>
<td>14</td>
<td>3</td>
<td></td>
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<tr>
<td>5</td>
<td>Ap</td>
<td>71</td>
<td>20</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Ap</td>
<td>78</td>
<td>18</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Ap</td>
<td>70</td>
<td>27</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

Ap – humic horizon (surface horizon)
of that available form of phosphorus in soil varied depending on the plant species grown and on the intensity of fertilisation and irrigation. The highest $P_{E-R}$ content was identified in soil under cauliflower (167 mgP·kg$^{-1}$) and broccoli 2 (167 mgP·kg$^{-1}$), while the lowest – under traditional cultivation of triticale (45.86 mgP·kg$^{-1}$) and onion (44.48 mgP·kg$^{-1}$). An intensive irrigation of vegetable crops increased the soil moisture and thus decreased the share of soil pores filled with air, and so the conductivity of water and nutrients penetration were made easier. The decrease in the available form of phosphorus in soil under onion can be considerably affected by the acid reaction of soil (Table 1).

The content of active phosphorus ($P_{AC}$) determined by the method of Houby et al. [13] allows for defining the current availability of phosphorus found in the soil solution which, however, is present at very low amounts. The content of $P_{AC}$ fell within the range of 11.08–18.89 mgP·kg$^{-1}$ (Table 1) (mean of 13.34 mgP·kg$^{-1}$). The highest content of $P_{AC}$ was also identified under cauliflower (18.89 mgP·kg$^{-1}$) and broccoli 2 (13.57 mgP·kg$^{-1}$).

Table 3. Content of the available forms of Zn, Cu, Pb, Ni, Fe and Mn

<table>
<thead>
<tr>
<th>Sample no</th>
<th>Plant</th>
<th>Content of DTPA-extractable forms mg·kg$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Triticale</td>
<td>0.914 0.596 0.674 0.158 22.36 5.236</td>
</tr>
<tr>
<td>2</td>
<td>Broccoli 1</td>
<td>1.408 0.540 0.826 0.121 54.40 7.294</td>
</tr>
<tr>
<td>3</td>
<td>Broccoli 2</td>
<td>5.338 2.234 0.560 0.242 117.06 33.76</td>
</tr>
<tr>
<td>4</td>
<td>Rhubarb</td>
<td>1.700 1.270 0.536 0.158 25.90 6.640</td>
</tr>
<tr>
<td>5</td>
<td>Carrot</td>
<td>1.360 0.878 0.644 0.134 56.86 14.26</td>
</tr>
<tr>
<td>6</td>
<td>Onion</td>
<td>1.994 0.690 0.612 0.152 63.98 22.38</td>
</tr>
<tr>
<td>7</td>
<td>Cauliflower</td>
<td>5.746 0.974 0.602 0.142 93.98 21.42</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>2.637 0.997 0.636 0.158 62.791 15.855</td>
</tr>
<tr>
<td>SD*</td>
<td></td>
<td>2.015 0.579 0.095 0.039 34.062 0.158</td>
</tr>
</tbody>
</table>

SD* – standard deviation

Table 4. Person's correlation coefficients (n=7)

<table>
<thead>
<tr>
<th></th>
<th>$P_{AC}$</th>
<th>$P_{E-R}$</th>
<th>ZnDTPA</th>
<th>CuDTPA</th>
<th>FeDTPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAC</td>
<td></td>
<td>0.80*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AIP</td>
<td></td>
<td>0.83*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DEH</td>
<td>0.77*</td>
<td>0.85*</td>
<td>0.82*</td>
<td>0.78*</td>
<td></td>
</tr>
<tr>
<td>KAT</td>
<td></td>
<td></td>
<td>0.87**</td>
<td>0.79*</td>
<td>0.77*</td>
</tr>
</tbody>
</table>

*Significant at p<0.05; **Significant at p< 0.01

The content of active phosphorus ($P_{AC}$) determined in 0.01 M solution of CaCl$_2$ is about 86% lower than the content of mobile phosphorus ($P_{E-R}$) defined by the Egner-Riehm method. There was also noted a significant coefficient of correlation between the content of $P_{AC}$, a $P_{E-R}$ in soil ($r=0.80, p<0.05$) (Table 4). Active phosphorus uptaken by plants is
supplemented from the mobile pool of that macronutrient and so the content of those two forms is quite closely correlated.

All the phosphorus transformations which occur in soil are stimulated by phosphatases; the enzymes conditioning their transformation into forms available to plants. The response of the plants to phosphorus deficit in soil is the synthesis of phosphatases secreted by plant roots and microorganisms.

The activity of alkaline phosphatase ranged from 0.537 to 1.202 mM pNP·kg\(^{-1}\)·h\(^{-1}\), while in acid phosphatase – from 0.559 to 1.593 mM pNP·kg\(^{-1}\)·h\(^{-1}\) and it was 39% higher than the alkaline phosphatase, which was due to the acid soil reaction. A higher activity of acid phosphatase comes from the fact that phosphomonoesterases are enzymes most susceptible to changes in the soil reaction; the optimum pH of soil for the activity of alkaline phosphatase is 9.0–11.0, and for acid phosphatase – 4.0–6.5 [18, 23, 21].

The highest activity of alkaline phosphatase was noted in the soil under broccoli 2 (1.202 mM pNP·kg\(^{-1}\)·h\(^{-1}\)) and cauliflower (0.882 mM pNP·kg\(^{-1}\)·h\(^{-1}\)). Similar values were reported for the activity of acid phosphatase; the highest value was recorded in the soil under broccoli 1 (1.593 mM pNP·kg\(^{-1}\)·h\(^{-1}\)), broccoli 2 (1.554 mM pNP·kg\(^{-1}\)·h\(^{-1}\)) and cauliflower (1.539 mM pNP·kg\(^{-1}\)·h\(^{-1}\)) (Fig. 1A). In those soils there was found, at the same time, the highest content of available and active phosphorus (Table 1). There was reported a significant positive value of the coefficient of correlation between the activity of alkaline phosphatase and the content of available phosphorus in soil (r=0.83, p<0.05). According to Kieliszewska-Rokicka [15], on the other hand, an intensive supply of mineral fertilisers can lower the activity of some enzymes since e.g. an increased level of inorganic phosphorus in soil acts as a competition inhibitor decreasing the activity of phosphatases. At the same time mineral fertilisers cause the proliferation of soil microorganisms less considerably than organic fertilisers, at the same time its effect is lower than that of organic fertilisation [19].

With the values of the activity of alkaline and acid phosphatase reported, there was calculated the enzymatic index of the pH soil level [6]. The value of the AlP:AcP ratio during the research was 0.47–1.12. The value optimal for plant growth and development can be considered such a value of soil pH under the conditions of which the adequate ratio of the AlP:AcP activity is ensured, namely 0.50 [6]. The value of the AlP:AcP ratio lower than 0.50 points to an acid soil reaction and limiting is recommended. The enzymatic index of the pH level below 0.50 was noted in the soil under broccoli 1 (0.47), carrot (0.49), onion (0.47) (Fig. 1B), which is in those soils where the soil reaction was acid (Table 1). The enzymatic indicator of the pH level can be used as an alternative method to determine soil pH as well as the changes in it [21, 22].

The activity of catalase ranged from 0.006 to 0.062 H\(_2\)O\(_2\)·g\(^{-1}\)·h\(^{-1}\). Catalase is an enzyme taking part in the plant defence from the effects of oxidation stress. The activity of the oxydo-reduction enzyme in the soil under vegetable crops intensively fertilised and irrigated was much higher than in the soil under triticale traditionally cultivated (0.013 H\(_2\)O\(_2\)·g\(^{-1}\)·h\(^{-1}\)) (Ryc. 2A). According to Olko and Kujawska [27], due to the effect of heavy metals representing the group of transition metals (e.g. Cu, Fe, Pb), in the presence of H\(_2\)O\(_2\), an intensive production of ROS (reactive oxygen species) occurs. Heavy metals, on the other hand, which do not show any activity in the redox cell processes (e.g. Cd, Zn) can increase the ROS level through the activation of NADPH oxydase. A deficit of some
heavy metals can also trigger oxidation stress in plants. Significant positive values of the correlation between the activity of soil catalase and the content of available forms of zinc \((r=0.87, p<0.01)\) and copper \((r=0.79, p<0.05)\) as well as of iron \((r=0.77, p<0.05)\) in soil point to the defence of the plants from the effects of oxidation stress caused by a natural amount of Zn, Cu, and Fe in soil.

The activity of dehydrogenases is an intermediary indicator of the soil microorganism biomass and the level of their activity increases with the abundance of microorganisms and the rate of their metabolism, being the key source of many soil enzymes [5]. The activity of dehydrogenases ranged from 0.271 to 0.438 mg TPF·kg\(^{-1}\)·h\(^{-1}\). The lowest activity of dehydrogenases \((0.271 \text{ mg TPF·kg}^{-1}\cdot\text{h}^{-1})\) (Fig. 2B) was reported in the soil under wheat. A higher activity of that enzyme was found in the soil under the other plants which were additionally irrigated. According to Pascuala et al. [28], at a higher soil moisture there is observed an increase in the dehydrogenases activity, which is connected with an increased occurrence of anaerobic bacteria. The highest activity of dehydrogenases was reported in the soil sampled under broccoli 2 \((0.438)\). There were recorded significant positive values of the coefficients of correlation between the activity of dehydrogenases and the content of zinc \((r=0.82, p<0.05)\) and copper \((r=0.78, p<0.05)\) in soil. An increased activity of dehydrogenases can be due to the fact that heavy metals,
especially at low concentrations, show a stimulating effect on the rate of growth and development of soil microorganisms. Neutral-reaction soils do not trigger any DHA inhibition, as compared with the heavily acidified environment (pH 1.5–4.5). Besides, as a result of the environment acidification, one also observes an increase in the availability of heavy metals and a decrease in available P forms, which decreases the activity of soil dehydrogenases, which was not noted in the soil of the Pałuki Region under study. There was also noted a positive significant value of the coefficient of correlation between the activity of dehydrogenases and the content of available phosphorus (r=0.85, p<0.05) as well as active phosphorus (r=0.77, p<0.05) in soil.

The activity of the enzymes depended on the species of the crop being cultivated. The highest activity of dehydrogenases and phosphatases was reported in the soil where broccoli 2 and cauliflower were grown (Figs 1 A, 2 A and B). According to [15], the plant species has a significant effect on the concentration of soluble carbon in soil, affecting the changes in the activity of enzymes. The effect of plants on the enzymatic activity is also connected with the chemical composition of plants, root exudates as well as the species composition of microorganisms infesting roots [2].

CONCLUSIONS

As a result of the analyses made, there were reported relatively low contents of available forms of the elements which point to their low mobility. The soils can be classified as non-contaminated soils.

The natural richness of the Pałuki Region soils in heavy metals triggered the inhibition of neither the oxydo-reduction nor the hydrolytic enzymes.

The soil under vegetable crops showed a very high content of phosphorus available to plants, which must have been due to intensive fertilisation.

Determining the enzymatic activity of soils can be a long-term monitoring method for the quality of the environment and the environmental changes.

REFERENCES


**DIAGNOZA ZAWARTOŚCI WYBRANYCH METALI CIĘŻKICH GLEB PAŁUK NA TLE ICH AKTYWNOŚCI ENZYMATYCZNEJ**

W pracy przedstawiono wyniki badań gleb pobranych z terenu leżącego we wschodniej części Pojezierza Chodzieskiego, znajdującego się pomiędzy Doliną Środowej Noteci, a doliną Welny, prawego dopływu Warty. Badaniom poddano 7 próbek glebowych pobranych z poziomów powierzchniowych, przeznaczonych pod uprawę różnych gatunków roślin (zboża i warzywa). W materiale glebowym oznaczono zawartość fitodostępnych form wybranych metali ciężkich Zn, Cu, Pb, Ni, Fe i Mn, aktywnego oraz przyswajalnego dla roślin fosfora na tle aktywności wybranych enzymów oksydoredukcyjnych i hydrolitycznych. Badana gleba spod roślin warzywnych charakteryzowała się bardzo wysoką zasobnością w fosfor przyswajalny dla roślin, co miało związek najprawdopodobniej z intensywnym nawożeniem. Stwierdzono stosunkowo niskie zawartości form przyswajalnych badanych metali ciężkich, co świadczy o naturalnej ich zawartości w glebie, która nie spowodowała inhibicji badanych enzymów oksydoredukcyjnych jak i hydrolitycznych.