Chapter 8
Enzyme Activities in Soils Contaminated with Heavy Metals in Varying Degrees

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8.1 Introduction: Soil Enzymes and Their Role in Soil

The biological activity of the soil, which is determined, among other things, by estimating the enzyme activity, is an important component indicating the regularity of soil processes. Soil enzymes catalyze processes related to the breakdown of organic matter released into the soil during plant growth. They also stimulate the processes connected with the formation, decomposition of soil humus, and a distribution and release of mineral substances. Moreover, enzymes are responsible for making those substances available to plants and for binding of molecular nitrogen, detoxification of xenobiotics, as well as nitrification and denitrification processes (Das and Varma 2011; Friedlova 2010; Hang et al. 2013; Khan et al. 2007; Pang and Yu 2011). Soil enzyme activity results from the activity of the enzymes accumulated in soil (extracellular enzymes) and from the enzyme activity of reproducing microorganisms (intracellular enzymes). Cells of microorganisms, as well as remains of plants and animals, are sources of enzymes accumulated in the soil (Mukhopadhyay and Maiti 2010). The enzyme activity of the soil is most often assessed on the basis of the effect of five enzymes: dehydrogenase, phosphatase, urease, invertase, and protease. The activity of soil enzymes is also known as a sensitive indicator of natural and anthropogenic changes in ecosystems, which is used to assess the impact of various pollutants including heavy metals in the soil, both in a long and short period of time (Gulser and Erdogan 2008; Januszek 1999; Kuziemska 2012; Yang et al. 2007). Moreover, the activity of enzymes can be used to indicate the effectiveness of rehabilitation processes of ecosystems or to reflect the quality of the soil following the restoration

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of soil environment which has been destroyed by industrial processes (Ciarkowska et al. 2014; Finkenbein et al. 2013; Mukhopadhyay and Maiti 2010).

8.2 Characterization of Selected Soil Enzymes

Dehydrogenases catalyze redox processes. Under aerobic conditions, they are transferred by a series of intermediaries onto the components of the respiratory chain, and ultimately onto oxygen, while in anaerobic conditions, oxidized inorganic forms such as NO^{3-}, Mn (IV), Fe (III), SO_4^{2-}, and CO_2 serve as acceptors of organic compounds, in the fermentation process. Thus, dehydrogenases are indicators of the respiratory metabolism of soil microorganisms—mainly of bacteria and actinomycetes (Burns et al. 2013; Hang et al. 2013; Kumar et al. 2013; Kuziemska 2012; Wolińska and Stępniewska 2012). They constitute an integral part of living cells and do not accumulate extracellularly in the soil. They belong to constitutive enzymes, that is, those which are present in almost constant amounts in the cell, independently of the amount of substrate (Das and Varma 2011; Friedlova 2010; Stępniewska and Wolińska 2005). It has been stated that the optimal soil pH value for the activity of dehydrogenases is 6.6–7.2. These enzymes are located in the cytoplasm or in specific structures developed from the cytoplasmic membrane. Dehydrogenase activity depends on the total population of microorganisms living in the soil, and that is why they are considered to be an indicator of overall soil microbial activity (Mukhopadhyay and Maiti 2010).

Phosphatases belong to a large group of enzymes that catalyze the hydrolysis of organic phosphorus compounds and are used to assess a potential rate of mineralization of these compounds in the soil (Gulser and Erdogan 2008; Hang et al. 2013; Yang et al. 2007). They play an essential role in the phosphorus circulation and are responsible for the management of phosphorus in the plant. They belong to periplasmic enzymes which means that they are deposited on the membrane or between the cytoplasmic membrane and the cell wall. These are the following types of phosphatases: acidic, neutral, and alkaline ones. Acid reaction, which corresponds to pH value ranging from 4 to 6, is optimal for acid phosphatase, whereas alkaline pH (pH 8–10) is accurate for alkaline phosphatase. The neutral phosphatase shows optimal activity at a pH value of 6.5–7.0. Microorganisms are the main source of phosphatases in the soil, but they are also produced by roots of plants and soil fauna. Phosphatase activity in soil reflects the activity of enzymes associated with soil colloids and humic substances (Das and Varma 2011; Śliwińska-Wyrzychowska and Nadgórska-Socha 2011).

Urease catalyzes the hydrolysis of urea in the soil to carbon dioxide and ammonia, which is one of the sources of nitrogen for plants. The activity of this enzyme reflects the ability to transform organic nitrogen to effective nitrogen and the ability to provide inorganic nitrogen (Ciarkowska et al. 2014; Gulser and Erdogan 2008; Friedlova 2010; Ianelli et al. 2012; Li et al. 2012; Yang et al. 2007). The rate of decomposition of urea depends on the soil reaction. The

optimal value of soil pH for urease activity is between 6 and 7. This enzyme is present in the cells of plants and many microorganisms, especially bacteria as both intra- and extracellular enzyme. Urease is bound in the soil mainly by humic substances but also by the clay minerals on a smaller scale (Das and Varma 2011).

Proteases catalyze the hydrolysis of proteins in the soil environment, breaking down the peptide bonds (CO-NH) to amino acids. They are produced mainly by aerobic and anaerobic bacteria, fungi, and actinomycetes. Most proteases produced by bacteria are most active at pH = 7–8. Proteases produced by the fungi are less sensitive to acid reaction because they are active even at pH = 4. Extracellular proteases are enzymes whose activity indicates not only the biological activity of the soil in the enzymatic transformations of substrates, which is independent of the activity of microorganisms, but also play a role in microbial ecology of ecosystems. These enzymes are bound by the mineral and organic colloids (Das and Varma 2011; Mocek-Płóciniak 2011).

Invertase is an enzyme which is widely distributed in soils. It plays a significant role in increasing the content of soluble nutrients in the soil and carbon transformations. The activity of this enzyme reflects the ability of the soil to break down sucrose and release simple sugars, which are the main energy sources of soil microorganisms (Ciarkowska et al. 2014; Li et al. 2012; Yang et al. 2007).

8.3 Natural Environmental Factors Influencing the Activity of Soil Enzymes

Research findings on the variability of enzyme activity in soils indicate the impact of many environmental factors such as soil type, soil profile depth, composition and diversity of soil microorganisms, soil pH, organic matter content, and temperature on the enzyme activity. Many studies have shown that the activity of enzymes, such as dehydrogenases, urease, protease, phosphatase, and invertase, is proportional to the content of organic matter (organic content of C and total N) and is much higher in the rhizosphere than in the deeper horizons of the soil profile (Chodak and Niklińska 2010; Januszek 1999; Mocek-Płóciniak 2011). According to many authors (Friedlova 2010; Mukhopadhyay and Maiti 2010; Wolińska and Stępniewska 2012), the factors which have a significant positive effect on the activity of dehydrogenases are the water content and cations, Ca, Mg, K, and Fe, whereas a negative impact is exerted by the oxidizing-reducing potential and aerating of the soil. Under conditions of the humid temperate climate of Poland, it is observed that in most cases, enzymes are most active in summer. Nevertheless, high levels of activity have also been noted in spring or even in winter, which shows the large impact of freezing and thawing effects on the soil enzyme activity (Januszek 1999). The rate of enzymatic reaction is also conditioned by the concentration of the enzyme involved in the reaction, the substrate concentration, as well as the presence of activators and inhibitors (Kuziemska 2012).

The enzymes released from litter, living and dead microorganisms, as well as plant roots, are washed down to lower horizons. Their further fate, stability, and activity in the soil depend on the soil texture, mineral composition, as well as physical and chemical properties of soils. Extracellular enzymes secreted by living or dying cells can be present on the surface of cell walls and cell membranes, as well as fragments of cell organelles or plasma. They can also be accumulated in the soil, where they form labile enzyme-substrate compounds, are adsorbed onto the surface of mineral particles, or enter into complex compounds with colloids of humic substances. They can even remain partially, and on a short-term basis, in the soil solution (Burns et al. 2013). Free enzymes are very active but short lived, and they quickly undergo proteolysis. There are not only enzymes bound to organic colloids as a result of copolymerization during the process of the formation of humus and accumulated in humic complexes which may survive for long periods in the soil but also, to a smaller extent, enzymes bound to mineral colloids (Januszek 1999). In comparison with free enzymes, bound enzymes usually display lower levels of activities. It is because the complexation blocks the access to the substrate by occluding the active centers. Therefore, bound enzymes constitute a potential reservoir of enzymes. They can also be the source of enzymes in case of substrate deficiency or stressful conditions. One theory states that the enzyme spread increases as the enzyme-substrate availability decreases, which allows the producer of enzyme to access more distant substrate (Burns et al. 2013). The level of enzyme activity in the soil can also depend on vegetation and its succession, as extracellular enzymes are often released by plant roots. Research findings of Yang et al. (2007) indicate that the urease activity varies depending on the species of a plant or a combination of plants.

8.4 Mechanisms Connected with the Influence of Heavy Metals on Soil Properties

The increasing interest in the environmental pollution caused by heavy metals results from their toxic effect on plants, animals, and humans. They suppress the activity of enzymes, damage nucleic acid chains, and tend to bioaccumulate in tissues of living organisms. The consequences of their occurrence in the environment are mutagenic and carcinogenic changes in the metabolism or limitation of photosynthesis (Lock et al. 2003; Mocek-Płóciniak 2011; de Mora et al. 2005).

The effect of heavy metals on soil enzyme activity may be direct or indirect. The direct impact refers to the extracellular free enzymes, while the indirect one is manifested through the influence on the biosynthesis of enzymes performed by microorganisms. The indirect impact is also manifested by the effect of heavy metals on the composition of the population of microorganisms in the soil, as well as root exudates production and the release of enzymes from dead cells (Wyszkowska et al. 2006). The toxicity of discussed elements results from their

possible influence on the growth and metabolism of soil microorganisms by disturbing the integrity of cell membranes or adversely affecting their functioning. Heavy metals also reduce soil enzyme activities by interacting with the complex enzyme-substrate and masking catalytically active groups, which produces the effect of denaturation toward active proteins of enzymes (Gianfreda et al. 2005; Zaborowska et al. 2006). Heavy metals affect the number, diversity, and activity of microorganisms. In the soil polluted with heavy metals, there prevail less diverse, slowly growing microorganisms which are more resistant to metals, but are characterized by a lower biological activity (Friedlova 2010). Lock and Janssen (2005) claim that the zinc contamination of soil causes microorganisms sensitive to this metal to die out. This can increase the number of resistant microorganisms.

Many authors think that as a result of the heavy metal contamination of the soil, the activity of intracellular soil enzymes produced by soil microorganisms is inhibited to a greater extent than the extracellular soil enzyme activity (Ciarkowska et al. 2014; Gulser and Erdogan 2008; Khan et al. 2007; Pang and Yu 2011). The influence of the heavy metal pollution of the soil on the activity of enzymes depends on the degree of contamination, the type of metal, time of exposure, and environmental factors, including mainly the soil reaction, which plays the key role in the solubility and the activity of metals in the soil (Januszek 1999; Oliveira and Pampulha 2006; Wyszkowska et al. 2006). Neutral or alkaline soil reaction contributes to the formation of metal compounds which are hardly soluble and therefore unavailable to plants (Ciarkowska and Gambuś 2004). The enzyme activity inhibited by heavy metals can also be associated with decreased contents of C, N, and P, which results in a lack of balance in the nutrient content and the soil degradation (Zhang et al. 2010). However, heavy metals which are present in small quantities in soils may stimulate the activity of enzymes. Not until they exceed the threshold content do they play a role in the reduction of microorganism activity and the production of extracellular enzymes (Wyszkowska et al. 2006).

8.5 Effects of the Short-Term Influence of Heavy Metals on the Activity of Soil Enzymes Observed During the Incubation and Pot Experiments

Incubation laboratory experiments enable us to trace the relationship between the content of heavy metals in soil and the level of activity of soil enzymes. The results of several studies show that as more time passes since the start of incubation and as the concentration of metals in the soil grows bigger, the enzyme activity gets lower. The extent of inhibition and the time after which the greatest reduction of enzyme activity is observed depend on the enzyme, the type of metal, their combination, and doses.

8.5.1 Cadmium and Lead

Khan et al. (2007) and Pang and Yu (2011) incubated the soil with the additional amounts of lead and cadmium salts. The researchers were progressively increasing amounts of those metals treating them both separately and as a combination. They observed that while they were increasing the doses of metals, the activity of urease, acid phosphatase, and dehydrogenase was getting lower proportionally to the dose of introduced metal. The enzyme activity was at its lowest after the scientists applied a combination of salts and the highest dose of metals. In the Pang and Yu (2011) experiment, the addition of 100 mg of Cd + 500 mg Pb kg^{-1} of soil resulted, after 10 weeks, in 50 % reduction of urease and dehydrogenase activity, whereas the acid phosphatase activity was reduced by 30 % after 2 weeks. In their incubation experiment, Khan et al. (2007) observed that the strongest inhibition of the activity of dehydrogenases and acid phosphatase occurred after 2 weeks and was followed by an increase in those enzyme activities. The authors explained that the decline in the enzyme activity which occurred over incubation time had been caused by the lack of nutrients which would be easily available for microorganisms. They also claim that the collapse of activity immediately after the application of metals and the increase in activity over time were both results of the adaptation of microorganisms to the conditions of contamination. Many authors (Khan et al. 2007; Mukhopadhyay and Maiti 2010; Pang and Yu 2011) unanimously state that a particularly negative effect on the activity of enzymes is exerted by cadmium and a combination of cadmium and lead. The impact of Cd on the enzyme activity is more toxic than Pb due to the greater mobility of the former element and a lower affinity with soil colloids. Mukhopadhyay and Maiti (2010) report that a significant inhibition of dehydrogenase activity occurs after applying very high doses of lead (5,000 mg of metal kg^{-1} of soil), while lower doses of the metal do not always reduce, but sometimes even slightly stimulate dehydrogenase activity. The effects lead has on the activity of enzymes can also be modified by the type of the plant cover. It was indicated by the result of the pot experiment in which lead had been introduced into the soil at a dose of 600 mg kg^{-1}. Such an amount of Pb led to the decline in the activity of urease activity in both cases: the soil covered with a combination of several plants and monoculture. However, the activity of this enzyme was greater in soils covered with a combination of several plants than in those with the monoculture plant cover. This may indicate that the presence of several plant species can reduce the negative influence of lead (Yang et al. 2007). The implemented dose of lead did not alter the activity of dehydrogenases and acid phosphatase and even resulted in an increase in the activity of alkaline phosphatase. The increased activity of alkaline phosphatase was explained by the increase in the soil pH value which had been caused by the introduction of alkalizing lead compounds (Yang et al. 2007). In her incubation experiment, Ciarkowska (2010) observed the varied impact of lead and cadmium on the activity of dehydrogenases. She compared the influence of low and high doses of lead and cadmium on the soils of sandy and silty textures, which were adjusted to the value of pH equal to 4.5, 5.5,

and 7.0. An increased lead content in the soil, which resulted from low and high doses of this metal introduced into both acid soils, slightly inhibited the activity of dehydrogenases. Artificial lead contamination at a low and medium level of a sandy soil with a pH of 5.5 led to the inhibition of dehydrogenase activity in the range of 7.2–11.7 %, while adding a small amount of lead to a silty soil of the same pH value increased the enzyme activity by more than 26 %. Under the same conditions, contamination with lead at a medium level slightly reduced the dehydrogenase activity. Increasing the concentration of lead in both soils with a neutral soil reaction, regardless of the amount of the metal added, generally stimulated the activity of dehydrogenases to a limited extent. A small amount of cadmium (2.25 mg kg^{-1}) incorporated into the silty soil stimulated enzyme activity in the range of 2.1–23.4 %. Such an impact of a small cadmium dose (1.5 mg kg^{-1}) on a sandy soil was observed only in the soil with pH = 7. Cadmium contamination of soil at a medium level (addition of 4.5 mg Cd to the light soil and 6.75 mg Cd kg^{-1} to the medium soil) had generally a clearly toxic effect in both soils and at each level of pH value, except for the soil with silty texture and pH = 5.5, in which a slight increase in the activity of dehydrogenases was recorded.

8.5.2 Zinc

The results of incubation experiments, in which different doses of zinc had been introduced into the soil, showed that this metal was a stronger inhibitor of the activity of enzymes than lead and cadmium. In the experiment of Ciarkowska (2010), the introduction of an increased amount of zinc into the soil strongly suppressed the activity of dehydrogenases from the very beginning. In the sandy soil, a negative effect of additional amounts of zinc was clearly seen since the first day after the introduction of the metal. In the silty soil, which has a greater sorption capacity than the sandy soil, the toxic effect of small additional amounts of zinc was observed only after 7–14 days since the start of the experiment. The introduction of zinc played a role in reducing the dehydrogenase activity in the range of 5.8–46.1 % in relation to the control soil. The amounts added were the following: 200 mg Zn kg^{-1} into the light soil and 300 mg Zn kg^{-1} into the medium soil. The enzyme activity decreased to the range of 34.5–74.5 % when 600 mg Zn kg^{-1} was introduced into the light soil and 750 mg Zn kg^{-1} was added into the medium soil.

Wyszkowska et al. (2006) showed a similar inhibiting effect of zinc on enzyme activity in the incubation experiment, in which they added increasing doses of zinc into the soil to test their effect on the activity of dehydrogenases, urease, as well as acid and alkaline phosphatases. The dose of 5 g Zn kg^{-1} decreased dehydrogenase by 3.5 %, and a dose of Zn, which was 400 times higher, brought the dehydrogenase activity down by 89 %. Excessive doses of zinc led to the decline of urease activity, but the influence was much weaker than in the case of dehydrogenases. The highest dose of zinc made the activity of the discussed enzyme 4.3 times smaller. Phosphatase activities were even less affected by the increased zinc content. The highest

dose of zinc used in this experiment (2,000 g Zn kg^{-1}) resulted in a more than twofold decrease in alkaline phosphatase activity and only a 1.5-fold decrease in acid phosphatase activity.

8.5.3 Combination of Zinc, Lead, and Cadmium

Yang et al. (2006) studied the effect that the increasing amounts of Zn, Pb, and Cd, individually or as a whole, had on catalase, urease, alkaline phosphatase, and invertase activities. They found out that, in case of all objects of experiment, lead, either as a single agent or in combination with the other two metals, stimulated the activity of catalase. The authors explain the fact by a reaction of Pb with the functional groups of catalase. The influence of heavy metal contamination of soil on the activity of urease was always inhibitory and proportional to the dose of introduced metals. It was stronger when they were applying the combination of metals rather than each one individually. A particularly strong synergistic interaction was observed for Zn and Cd. The activity of alkaline phosphatase was inhibited the most by the addition of Cd, while the effect of lead was not significant. Invertase was most strongly inhibited by a combination of three metals. Each of the enzymes responded differently to the single metal contamination and a combination of three metals. Various effects of individual metals on the enzyme activity resulted from different reactions into which enzyme functional groups and metals entered. In the experiment of Yang et al. (2006), cadmium was a strong inhibitor of all analyzed enzyme activity. Whereas zinc inhibited urease and catalase activities, lead proved to have the least inhibitory effect on enzymes and even a stimulating effect on catalase activity. The inhibitory effect of Cd on catalase and urease was strongly intensified by the addition of Zn. As far as the studied enzymes are concerned, urease has proven to be the most sensitive to the combinations of heavy metals. According to the authors, this enzyme can be a good biochemical indicator for the evaluation of Zn, Pb, and Cd contamination of soils.

8.5.4 Chromium

It was also observed that chromium (Cr (III) and Cr (VI)) was another element which had a negative influence on the activity of dehydrogenases in various types of soils. The results of the incubation experiment showed a rapid and significant decrease in the activity of dehydrogenases in each of the soils after the addition of higher doses of chromium, whereas small doses of chromium (2 mg kg^{-1}) increased the enzyme activity. The authors explained that the small additional amounts of Cr compensated for deficiencies of this element in the soils. The inhibitory effect of chromium on the activity of dehydrogenases depends on the type of soil, especially on the content of the organic substance. The activity of

dehydrogenases was more sensitive to the effects of chromium in *Haplic Luvisol* and *Eutric Cambisol* than in *Mollic Gleysol* (Stępniewska and Wolińska 2005).

8.5.5 Nickel

The purpose of the 4-year pot experiment of Kuziemska (2012) was to determine the effect which the increased amounts of nickel introduced into the soil after bean cultivation had on the dehydrogenase activity. The results showed a significant change in the enzyme activity in each year of the experiment. After nickel was introduced at the lowest dose of 50 mg Ni kg^{-1}, the scientists observed, during the three following years a significantly increased activity of dehydrogenases, whereas higher doses of the metal (100 and 150 mg Ni kg^{-1}) strongly decreased their activity. In the fourth year of the experiment, only the highest dose of nickel (150 mg kg^{-1}) lowered the activity of these enzymes. Liming of the acid soils reduced the negative impact of nickel.

8.5.6 Summary

The results of research connected with the impact of heavy metals on the enzyme activity in the soils contaminated with heavy metals for a short period of time are very ambiguous. This fact implies that various soil microbial populations have different sensitivities to heavy metals, even if the soils have similar physical and chemical properties. The differences result from the diverse structures of the microbial population, as well as from the various interactions of metals and their forms with the soil. They are also caused by the chemical and exchangeable sorption of heavy metals by the organic substance, clay minerals, metal oxides and hydroxides, as well as free amorphous aluminosilicates (Yang et al. 2007).

The abovementioned results of experiments can be summarized by stating that:

- The heavy metals discussed above, such as zinc, lead, cadmium, nickel, and chromium, tend to have a negative effect on enzyme activities in the soils which have not been contaminated for a long time.
- The inhibitory effect of metals on the activity of enzyme depends on the type of enzyme, soil properties, and the dose of metal.
- The inhibitory effect of zinc, lead, and cadmium can be arranged in the following order: Cd > Zn > Pb or Zn > Cd > Pb. Lead has a greater affinity for soil organic matter than zinc and is less mobile than cadmium. That is why it is complexed with soil organic matter to a greater degree and has a smaller inhibitory impact on the activity of enzymes.
- Combination of metals has a stronger negative impact on the activity of enzymes than a single metal because of a synergistic interaction of metal combinations.

– The toxic effect of metal interaction decreases over time.
– The results of studies do not unanimously point to one of the enzymes (dehydrogenase, urease, phosphatase, invertase) as an indicator of biochemical assessment of heavy metal contamination of soil.

8.6 Effects of the Long-Term Influence of Heavy Metals on the Activity of Soil Enzymes

Effects of long-term heavy metal pollution on the soil enzyme activity were studied in soil samples taken from the mining areas and/or metallurgy areas connected with nonferrous metals (copper, zinc, lead). Most authors observed a decrease in the activity of enzymes such as alkaline and acid phosphatases, urease, and dehydrogenase in soils which were in the direct vicinity of the emitter (Bielińska and Mocek-Płóciniak 2010; Castaldi et al. 2004; Januszek 1999). There was a close connection between the distance from metal smelters and enzyme activities, which indicates a significant negative effect of heavy metals on the activity of soil enzymes. The authors emphasize the particularly close relationship between the activity of enzymes and the content of bioavailable forms of zinc and cadmium (extracted by 0.01 M $CaCl_2$ or 1 M NH_4NO_3) (Śliwińska-Wyrzychowska and Nadgórska-Socha 2011). The negative effect of heavy metals on enzyme activity is also confirmed by the results of the research analyzing the activity of urease and alkaline phosphatase in soils which are located at different distances from roads with heavy traffic. The research indicated that the enzyme activity was higher as the distance from a road was growing. It also showed a significant negative correlation between the activity of enzymes (urease and alkaline phosphatase) and the content of Mn, Cr, and Pb. This suggests that heavy metal contamination of the soils located along roads inhibited the activity of the enzymes discussed above (Gulser and Erdogan 2008).

Friedlova (2010) showed a high inactivation of dehydrogenases in soils under conditions of long-term industrial emissions when she carried out the study of the enzyme activity of soils contaminated with emissions of lead industry, which had been functioning since the eighteenth century. The activity of dehydrogenases significantly decreased when the pollution increased, whereas changes in the urease activity were rather ambiguous and correlated with the content of nitrogen. However, one can also cite results of research on dehydrogenase activity in soils strongly contaminated with heavy metals over centuries. These soils are characterized by the significant activity of dehydrogenases. This can be explained by the fact that microbial populations which are exposed to long-term impact of toxic metals are increasingly tolerant to metals (Ciarkowska and Gambuś 2004; Januszek 1999; Zaborowska et al. 2006).

According to some researchers, extracellular enzyme activity, as compared with the activity of dehydrogenases, can be more useful to evaluate changes in the soil

environment. Bielińska and Mocek-Płóciniak (2010) believe that the phosphatase and urease activities are sensitive indicators of changes in the soil environment exposed to a long-term impact of metals. Mocek-Płóciniak (2011) also described the activity of phosphatase as a good indicator of heavy metal pollution of the soil, but the results of her study did not demonstrate the negative impact of a high content of heavy metals (Cu and Pb) on urease activity. Studies of Castaldi et al. (2004) confirm that the urease activity is not significantly correlated with the content of heavy metals in the soil. Resistance of this enzyme to heavy metal pollution of the soil apparently results from the specific properties of urease, which is resistant to external factors, and can even increase its activity in extreme conditions. The only limiting factor is the availability of urea because urease, as an extracellular enzyme, is synthesized only in its presence. Results of analyses of urease and invertase activities which were conducted by Ciarkowska et al. (2014) on the land that had been subjected to the long-term effects of strong pollution by zinc, lead, and cadmium showed that long-term and significant accumulation of metals in the soils had no reducing effect on the activity of enzymes and the invertase activity was even higher in polluted soils than in unpolluted forest soil. The authors explained that fact by arguing that the growth of metal-resistant microbial population and/or organic matter accumulation, as well as neutral reaction of the soil, caused heavy metals to occur mainly in biologically inactive forms. Also, according to Ciarkowska et al. (2014) and Finkenbein et al. (2013), the increased activity of enzymes in soils suffering from long-term stress, which resulted from heavy metal contamination, was the effect of changes in the composition of the microbial population. However, the decrease in the enzymatic activity may be the result of the consumption of energy in the process of the physiological adaptation of the microorganisms by which they develop the tolerance of heavy metals. This adaptation consists of the following processes: synthesis of intracellular and extracellular proteins which sequester metals as well as biochemical reactions of metal precipitation or capture which occur on the surfaces of microbial cells (Zhang et al. 2010). Śliwińska-Wyrzychowska and Nadgórska-Socha (2011) explain also that the lack of relationship between the enzymatic activity of certain enzymes (dehydrogenases, urease, and phosphatases) and the content of heavy metals in soils characterized by various degrees of heavy metal contamination, including those located near metallurgical plants of nonferrous metals, results from a high content of organic matter and a low concentration of soluble forms of heavy metals.

Drawing conclusions from the above-presented research results, we can observe that a variety of enzyme reactions to long periods of heavy metal pollution may result from the various levels of sensitivity of microorganisms to metal toxicity, as well as from the development of mechanisms of resistance to metals and changes in the structure of microbial population, which can compensate for losses in more sensitive populations. Therefore, it seems that a reliable assessment of the quality of the soil environment can be achieved only by studying a series of soil enzymes.

8.6.1 *Determining the Level of Degradation and the Progress of Ecosystem Restoration*

The determination of enzyme activity is used as an indicator of both the rate of degradation of ecosystems and the improvement in the quality of mining areas as a result of rehabilitation procedures (Ciarkowska et al. 2014; Schimann et al. 2012). The reclaimed soil has to be microbiologically active, so it is very important to study the activity of microorganisms by, for example, testing the enzyme activity (Finkenbein et al. 2013). Habitats created after the opencast mining of nonferrous metals, apart from strong contamination by heavy metals, are often characterized by very unfavorable soil conditions such as extreme pH values, as well as scarcity of organic matter and nutrients for plants. The activity of many enzymes, both intra- and extracellular, is determined by the organic matter content or composition of the vegetation growing on degraded lands. Soil texture and pH value are other important properties, which determine the enzyme activity, of the soil forming on degraded areas. They often are characteristics affecting the enzyme activity even more strongly than the vegetation or organic matter content. In strongly acid soils, the presence of organic carbon and total nitrogen may not stimulate microbial activity (Chodak and Niklińska 2010; Finkenbein et al. 2013).

An important and difficult issue is to determine the point at which the soil can be considered completely reclaimed. For this reason, indicators which reflect the state of the soil and its functions, such as the activity of enzymes which provide information about the soil microbiology as well as its physical and chemical parameters, are very useful (Antunes et al. 2011; Zhang et al. 2010). According to Ciarkowska et al. (2014), the activity of urease and invertase can be such an indicator. It has been noted that the activity of these enzymes in the soils of zinc and lead ores mining areas which have been restored in recent years is significantly lower than in the soils of the same area but reclaimed decades ago. This suggests that the newly reclaimed soils undergo a slow rehabilitation process. This process, however, is not yet completed, and microbial communities are still under the influence of mining activities (Ciarkowska et al. 2014). Similarly, Mukhopadhyay and Maiti (2010), who were examining the activity of dehydrogenases in reclaimed soils which were degraded after opencast mining of metal ores, found out that the activity of these enzymes in reclaimed soils was lower than in referential soils. The lowest activity was found in the soils where the period of time since the completion of the rehabilitation was the shortest. The activity of dehydrogenase in 3-year heaps was several times lower than in the soils of 20-year heaps, the latter level of activity being similar to the one noted in nondegraded soils of this area.

The results support the opinion that enzymatic activity is reduced immediately after the introduction of metals into the soil, but usually, as time passes, it returns to its original level (Ciarkowska and Gambuś 2004). The use of a biological parameter allows us to specify the ecologically negative effects of mining and metal ore processing as well as destabilization of the soil ecosystem.

8.7 Conclusions

Despite the fact that the results of various authors' research on the relationship between the content of heavy metals in soil and the level of enzyme activity are not fully unequivocal, one can draw some general conclusions, according to which the soil enzyme activity:

- Reflects the degree and size of heavy metal pollution soil
- Is a good indicator of both short- and long-term heavy metal contamination of the environment
- Allows to estimate the effects of reclamation of degraded ecosystems

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