Chapter 11
Effects of Heavy Metals on Soil Enzyme Activities

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11.1 Introduction

Heavy metals are considered one of the major sources of soil pollution (Huang and Shindo 2000). Heavy metal pollution of the soil is caused by various metals, especially Cu, Ni, Cd, Zn, Cr, and Pb (Effron et al. 2004). Zeng et al. (2007) reported that Pb is one of the most abundant heavy metal soil pollutants (Eick et al. 1999). Many authors have reported that heavy metals cause long-term hazardous effects on soil ecosystems and negatively influence soil biological processes (Chen et al. 2005; D'Ascoli et al. 2006; de Mora et al. 2005; Effron et al. 2004; Kunito et al. 2001; Kuperman and Carreiro 1997; Lorenz et al. 2006; Malley et al. 2006; Shen et al. 2005; Speir et al. 1999). For this reason, heavy metals need to be monitored and their concentrations in soils regulated. For example, the Commission of the European Community (CEC) has established permissible heavy metal limits in agricultural soils; for Hg, Pb, and Zn these are 1–1.5, 50–300, and 150–300 mg kg^{-1} dry soil, respectively (CEC 1986). The heavy metal contamination of soils has become a serious environmental issue around the world for various reasons, including industrial activities, solid waste disposal, fertilizer and sludge application, irrigation with wastewater, and automobile exhausts (Karaca et al. 2002; Karaca 2004; Khan et al. 2007; Yang et al. 2006). Heavy metals affect many characteristics of soils, including their biological properties (Huang and Shindo 2000). Khan et al. (2007) concluded that heavy metals have an inhibitory influence on soil enzyme

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activities and as well as microbial community structure. The strong inhibition of enzyme activity exerted by heavy metals has been well documented by many researchers (Effron et al. 2004; Kahkonen et al. 2008; Kizilkaya 2008; Kunito et al. 2001; Malley et al. 2006; Oliviera and Pampulha 2006; Shen et al. 2005; Speir et al. 1999; Wang et al. 2008). Soil enzyme activities are considered to be good bioindicators, reflecting natural and anthropogenic disturbances, and evaluating soil enzyme activities is one of the cheapest and easiest techniques that can be used to evaluate soil pollution (Hinojosa et al. 2004; Khan et al. 2007). Some researchers describe the toxicity of metals to enzymes using the ED_{50} value, which is defined as the heavy metal concentration at which the enzyme activity is half of its uninhibited level (Huang and Shindo 2000). Soil enzymes are inhibited by heavy metals to different extents depending on the characteristics of the soil, such as its clay, silt and organic matter contents and its pH value (Doelman and Haanstra 1986; Effron et al. 2004; Geiger et al. 1998). Yang et al. (2007a,b) reported that the reduction in soil microbes and the inhibition of soil enzyme activities caused by metal contamination negatively affect soil fertility.

11.2 Inhibition of Soil Enzymes

An enzyme inhibitor is an agent that reduces enzyme activity, whereas an enzyme activator is an agent that stimulates enzyme activity (Voet and Voet 1995). The effects of inhibitors and activators on enzymes are shown in Fig. 11.1. Both of these types of agents affect the parameter K_m for the enzyme reaction of interest (K_m is the substrate concentration at which the reaction rate is half of the maximum rate; Stryer 1995). As seen in Fig. 11.1, K_m values increase in the presence of an inhibitor and decrease in the presence of an activator.

The inhibition of soil enzyme activities by heavy metals is a very complex issue, as there are many factors that affect this inhibition. These factors can be divided into four main classes: metal factors, enzyme factors, soil factors, and plant factors. Metal factors include the heavy metal element in question, the concentration of the heavy metal, the chemical form of the heavy metal, the availability of the heavy

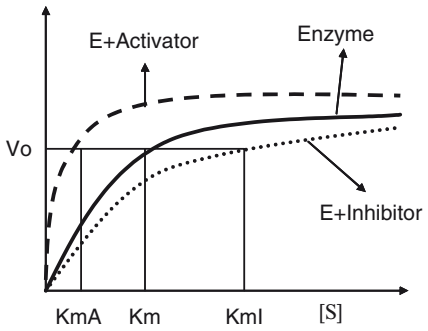

Fig. 11.1 The effects of inhibitors and activators on enzyme activity (Voet and Voet 1995)

metal, and indirect effects of the heavy metal. Enzyme factors include the enzyme sensitivity, the structural inhibition of the enzyme, and the major properties of the enzyme. Soil factors include pH, organic matter, and clay. Finally, plant factors include metal accumulation and plant community effects. We now take a closer look at these factors.

11.2.1 Metal Factors

11.2.1.1 Heavy Metal Element

Enzyme activities are influenced in different ways by different metals due to the different chemical affinities of the enzymes in the soil system. Khan et al. (2007) found that Cd was more toxic to enzymes than Pb because of its greater mobility and lower affinity for soil colloids. Shen et al. (2005) found a negative interaction between Zn and Cd resulting from competition between them for sorption sites. Zn concentrations are generally higher (by factors of 100–1,000) than Cd concentrations (Christensen 1987). Also, different metals affect soil enzymes in different ways. Geiger et al. (1998) found that copper inhibited β-glucosidase activity more than cellulase activity. Balyaeva et al. (2005) found that Pb decreased the activities of urease, catalase, invertase, and acid phosphatase significantly. Speir et al. (1999) found that phosphatase and sulfatase were inhibited by As(V) but that urease was unaffected. Lorenz et al. (2006) found that As contamination significantly affected arylsulfatase activity but not those of xylanase, invertase, protease and alkaline phosphatase; Cd contamination had a negative effect on the activities of protease, urease, alkaline phosphatase and arylsulfatase but no significant effect on that of invertase. Each soil enzyme exhibits a different sensitivity to heavy metals. Shen et al. (2005) reported that the order of inhibition of urease activity generally decreased according to the sequence Cr>Cd>Zn>Mn>Pb (Zheng et al. 1999). Effron et al. (2004) found that heavy metals inhibited the activities of arylsulfatase, acid phosphatase, protease and urease. The relative toxicities of the metals toward enzyme activity were found to be: Cd≈Cu>Pb. Acosta-Martinez and Tabatabai (2001) found that Ag(I), Hg(II) and Cd(II) were more effective inhibitors than the other 18 trace elements examined. Renella et al. (2005) found that Cd inhibited alkaline phosphatase, arylsulfatase and protease, but did not affect acid phosphatase, β-glucosidase and urease.

Vig et al. (2003) published a review of the bioavailability and toxicity of Cd towards soil microorganisms and their activities. The effects of Cd on soil enzymes are extensively summarized in their review. A summary of studies on the effects of Cd on soil enzyme activities is given in Table 11.1.

11.2.1.2 Metal Concentration

Actually, all metals, including heavy metals, are generally found in the soil at low concentrations and provide essential micronutrients for soil organisms; however,

Table 11.1 The effect of Cd on soil enzyme activity in different studies (adapted from Vig et al. 2003)

Soil type/treatment	Cd (mg kg^{-1} soil)	Inhibition (−), activation (+) or no effect (NE)	References
Field studies. Oak forest near abandoned zinc smelter: pH 5.0–6.2, 0.5–0.7% OC	Cd 26, Cu 15.0, Pb 21.6, Zn 478	+DHA 93% +UR 88%	Pancholy et al. (1975)
Lab amendments: pH 5.1–6.1, 1.5–2.9% OC, 10–21% clay	$CdCl_2$ 562	−ARA 55–82%	Acosta-Matinez and Tabatabai (2001)
Lab amendments. Soil 1: pH 6.2–7.6, 2.7–5.3% OC, 26–34% clay. Soil 2: pH 7.6, 3.2 %OC, 30% clay	2810281	−ASL 23–55% −ASL 7%	Al-Khafaji and Tabatabai (1979)
Sandy loam: pH 7.9, 0.47% OC. Loam: pH 8.1, 1.61% OC. Clay-loam: pH 7.7, 0.72% OC	$CdCl_2$ 50	−DEH, ALP	Dar (1996)
Sandy: pH 7.0, 1.6% OM. Sandy peat: pH 4.4, 12.8% OM	$CdCl_2$ 150 $CdCl_2$ 1980 $CdCl_2$ 40	−UR 10%, 6 weeks −UR 10%, 6 weeks −UR 10%, 1.5 years	Doelman and Haanstra (1986)
pH 5.6, 2.6% OC, 28% clay	562	−ADS 6%	Frankenberger and Tabatabai (1981)
Forest soil: pH 4.8, 2.3% OC, 87% sand, 8% silt, 5% clay	$CdSO_4$ 500 $CdSO_4$ 50	−DEH, ACP −ACP	Landi et al. (2000)
Montepaldi soil: pH 8.1, 1.7% TOC, 66% sand, 21% silt, 13% clay	$CdSO_4$ 3–400	−DEH, UR	Moreno et al. (2001)
Agricultural soil: 1.3% OC	$Cd(NO_3)_2$ 150	−DEH 48% −CL 29 % −AML 34%	Rogers and Li (1985)
Fir needle litter: 78% OM	$CdCl_2$ 1000	NE IN, XY, BD, PPO	Spalding (1979)
pH 4.6-7.0, 1.99–5.32% OC, 24–36% clay	$CdSO_4$ 2810	−PYP 19–50%	Stott et al. (1985)
Surface soils: pH 5.1–7.8, 2.6–5.5% OC, 17–42% clay	$CdSO_4$ 562	−UR	Tabatabai (1977).

OC organic carbon; *TOC* total organic carbon; *OM* organic matter; *ARA* arylamidase; *ASL* arylsulfatase; *DEH* dehydrogenase; *ALP* alkaline phosphatase; *ADS* amidase; *ACP* acid phosphatase; *CL* cellulase; *AML* amylase; *IN* invertase; *XY* xylanase; *BD* β-glucosidase; *PPO* polyphenoloxidase; *PYP* pyrophosphatase

their levels have increased drastically due to anthropogenic pollution (Carine et al. 2008). Zeng et al. (2007) observed a stimulating effect of Pb on soil enzyme activities at low concentrations of Pb. However, when the level of Pb was increased to

500 mg kg^{-1}, soil enzyme activities decreased. Similarly, Shah and Dubey (1998) reported that an enhancement in protease activity was observed at low Cd levels (50–100 μM); however, protease activity was inhibited above these levels. Fliessch et al. (1994) reported that sludge containing low levels of metals had a stimulating affect on soil microbial activity. Furthermore, Dar (1996) found that the addition of Cd aat 10 μg g^{-1} soil (in Sw) did not result in any significant changes in soil enzyme activity. However, the addition of Cd at 50 μg g^{-1} soil decreased the soil enzyme activity, and this effect was greater in sandy loam than in loam or clay loam soils.

Tejada et al. (2008) reported that soil enzyme activities decreased with increasing Ni concentration. Lorenz et al. (2006) found that increasing the level of Cd decreased enzyme activities. Zeng et al. (2007) stated that "it is well known that any element under specific environmental conditions would bring about the adverse effect to plants and microorganisms if its concentration is higher than a certain range." Cellulase and β-glucosidase activities were inhibited at copper concentrations above 200 μM (Geiger et al. 1998). However, it was observed that the enzyme activities were slightly reduced at 1 mM copper compared to 600 μM. Hemida et al. (1997) found that urease activity completely disappeared at 2,000 μg heavy metals (Cu^{2+} and Zn^{2+}) g^{-1} soil. Wyszkowska et al. (2006) concluded that concentration of 50 mg kg^{-1} of metals (Cu, Zn, Ni, Pb, Cd and Cr) inhibited soil enzyme activities (those of dehydrogenase, urease, acid phosphatase and alkaline phosphatase).

Mikanova (2006) studied the effects of heavy metals on the enzyme activities (arylsulfatase, invertase, urease and dehydrogenase) of heavy metal polluted alluvial soils. Increasing the heavy metal concentration inhibited all of the soil enzymes studied, but arylsulfatase and dehydrogenase were more sensitive to lower concentrations of metal than invertase and urease (Table 11.2). Hinojosa et al. (2004) conducted a study to determine enzyme sensitivity in order to find the magnitude of the heavy metal pollution (Cd, Pb, Cu and Zn) resulting from a mine spill.

Table 11.2 Effects of Cd, Pb, and Zn on soil enzyme activities in heavy metal polluted alluvial soils (adapted from Mikanova 2006)

	Heavy metal (mg kg^{-1} dry soil)			Inhibition				Activation			
Soil properties	Cd	Pb	Zn	ASL	IN	UR	DEH	ASL	IN	UR	DEH
Alluvium of Litavka River											
Unpolluted	1.9	106.0	202.5								
Low-level pollution	2.4	113.5	249.8	S		W	S		W		
Moderate	5.4	530.5	407.0	S	W	M	S				
Medium	59.0	3,450.7	6,230.8	S	M	M	S				
High	61.3	7,040.3	7,497.9	S	M	M	S				
High	113.8	6,335.9	12,557.4	S	S	S	S				
Czech standards	1	140	200								

ASL arylsulfatase; *IN* invertase; *UR* urease; *DEH* dehydrogenase; *S* strong; *M* moderate; *W* weak

Similarly, increasing the degree of pollution caused decreased soil enzyme activities. The highest enzyme activity was found in unpolluted soil and the lowest in the most polluted soil.

11.2.1.3 Chemical Form of the Heavy Metal

Different chemical forms of heavy metals can affect soil enzymes differently. Carine et al. (2008) found that phenoloxidase activity was inhibited Al chloride salt than Al sulfate salt, at higher rate and lower Al level. Yang et al. (2007a,b) found that mercury ($HgCl_2$) markedly inhibited soil urease activity, and that there was a logarithmic relationship ($P < 0.05$) between the concentration of Hg and the activity of the soil urease.

11.2.1.4 Availability of the Heavy Metal

Bioavailability is an important factor when evaluating metal toxicity. Bioavailability can be defined as "the fraction of all contaminants in the soil particles that is available to receptor organisms" (Vig et al. 2003). Bioavailability is particularly important for soil microorganisms and plants, since they are the main sources of enzymes. The bioavailability of Cd (one of the most toxic heavy metals) depends on several factors, such as soil type, Cd speciation, aging, nature of Cd applied, and the nature of the microorganisms (Vig et al. 2003). Vig et al. (2003) reported that the availability of Cd in a soil–plant system increased in the order: mineral lattices > Fe and Mn oxides > organics > metal-organic complexes > carbonates > exchangeable (Krishnamurti 2000). They also reported that the bioavailability of a heavy metal declines with the time it is in contact with the soil (Naidu et al. 2003).

The available forms of a metal are significant when attempting to understand metal toxicity, and its available forms are related to its chemical forms in the soil (Wang et al., 2007a,b). Water and NH_4NO_3 extractions can be used as methods to define the solubilities of metals, by either releasing heavy metals in a soil solution (water extraction) or by extracting soluble and exchangeable metals (NH_4NO_3 extraction). Generally, heavy metal concentrations in soil solutions decrease at neutral or alkaline pH (Munoz-Melendez et al. 2000). Soluble forms of heavy metals are considered to be most available to microorganisms and enzymes (Huang and Shindo 2000). Bhattacharyya et al. (2008) reported that water-soluble and exchangeable forms of metals showed strong inhibitory effects on soil enzyme activities. Chaperon and Sauve (2007) concluded that, since higher dissolved metal concentrations were found in agricultural soil, metals were more toxic for the studied enzymes. The metal fractions (total, soluble, or extractable) present are an important aspect of the availability of metals. Wang et al. (2007a,b) found that soil phosphatase activity was significantly negatively correlated with Cu and Zn (soil solution, NH_4NO_3-extractable, and total fractions).

11.2.2 Enzyme Factors

11.2.2.1 Enzyme Sensitivity

Shen et al. (2005) investigated the interactions of polycyclic aromatic hydrocarbons (phenanthrene, fluoranthene, benzo[*a*]pyrene) and heavy metals (cadmium, zinc and lead) with soil enzymes (urease and dehydrogenase). The results showed that dehydrogenase was more sensitive to the combined pollution than urease. Similarly, Maliszewska-Kordybach and Smreczak (2003) demonstrated that dehydrogenase activity is most sensitive to the combined effects of pollutants (heavy metals and PAHs). Shen et al. (2005) reported that urease and dehydrogenase could be suitable indicators of combined pollution (heavy metals and PAHs), particularly at the early stages of pollution (Baath 1989; Yang and Liu 2000). Renella et al. (2003) reported that alkaline phosphatase was more susceptible in acid soil, whereas acid phosphatase was more susceptible in alkaline soil. Wyszkowska et al. (2006) found that the metal sensitivities of enzymes followed the order: dehydrogenase > urease > alkaline phosphatase > acid phosphatase. The metal sensitivities of soil enzymes that have been reported in the literature are given in Table 11.3.

Table 11.3 Metal sensitivities of soil enzymes, as reported in the literature

Heavy metal	Treatment	Metal sensitivity			References
		High	Moderate	Low	
		DEH			Maliszewska-Kordybach and Smreczak (2003), Hinojosa et al. (2004) cit. Khan et al. (2007)
	Long-term pollution	UR, ACP, DEH			Aoyama and Naguma (1996) cit. Zeng et al. (2007)
Cu	Vermicomposting	DEH		PR	Malley et al. (2006)
Cu	Long-term pollution	PH			Wang et al. (2008)
Cd	Phosphate fertilizer and sewage sludge	PME	βG, ASL	UR	Karaca et al. (2002)
CdCu Pb	Incubation experiment	ASL, PR PH, PR PR			Effron et al. (2004)
Cd, Zn, Pb	Combined pollution (heavy metals and PAHs)	DEH	UR		Shen et al. (2005)
As[V]	Experiment	PH	SL, UR		Speir et al. (1999)
Hg, As	Long-term pollution	DEH			Oliveira and Pampulha (2006)
Zn	Long-term sludge-amended soil	DEH, UR, IN			Kunito et al. (2001)
Zn	Organic wastes and Zn	DEH			Kizilkaya (2008)

UR urease; *ACP* acid phosphatase; *DEH* dehydrogenase; *PH* phosphatase; *PR* protease; *SL* sulfatase; *PME* phosphomonoesterase; *ASL* arylsulfatase; *IN* invertase; *βG* β-galactosidase

11.2.2.2 Structural Inhibition of the Enzyme

Enzyme reactions are inhibited by heavy metals in three different ways: (1) complexation of the substrate; (2) combination with protein-active groups on the enzyme, and; (3) reaction with the enzyme–substrate complex (Tejada et al. 2008; Megharaj et al. 2003). D'Ascoli et al. (2006) reported that heavy metals inhibited enzyme activity in several ways: (1) by masking catalytically active groups; (2) denaturing the protein conformation, or; (3) competing with metal ions that are needed to form enzyme–substrate complexes (Gianfreda and Bollag 1996).

Khan et al. (2007) reported that extracellular enzymes were inactivated by heavy metals. Mechanisms involved the metals binding to some of the amino acids in the enzymes and indirectly reducing the number of microorganisms responsible for producing the enzymes (Doelman and Haanstra, 1986; Kuperman and Carreiro 1997; Bandick and Dick 1999; Kunito et al. 2001).

Geiger et al. (1998) reported that the interaction of a metal cation with an enzyme is largely dependent on the amino acid composition of the protein. It is assumed that the catalytic reactions of cellulases involve a hydrolysis reaction that proceeds via an acid–base mechanism involving aspartic and glutamic acid. There are two components to this mechanism: (1) acting as a catalyst (aspartic acid), (2) acting as a nucleophile (glutamic acid). Cellulose binds to cellulase in the region of the cellulose-binding domain (Esterbauer et al. 1991). Cellulose-binding domains contain plenty of glycine and cysteine, which are stabilized by two or three disulfide bridges (Wood and Garcia-Campayo 1990). In other words, the shape of the active site of cellulase is mainly provided by amino acids (glycine and cysteine) and bonds between them (disulfide bridges). The cellulose-binding domain also contains tryptophan residues (Teeri et al. 1995). Copper can form complexes with tryptophan residues in the cellulose-binding domain, resulting in the inhibition of cellulase.

Khan et al. (2007) stated that "it is well documented that heavy metals react with sulfhydryl groups of enzymes and inhibit and/or inactivate the enzymatic activities." Lorenz et al. (2006) reported that enzyme activities decreased due to the binding of Cd^{2+} to sulfhydryl groups (Sanadi 1982). Hemida et al. (1997) reported that Tabatabai (1977) stated that "there was a marked decrease in urease activity with increasing trace element ion concentrations due to the reaction of –SH groups on urease (which are involved in urease activity) with the trace element ions." Bhattacharyya et al. (2007) specified that As ions inactivate enzymes by reacting with sulfhydryl groups resulting from the formation of arsenic sulfide. They also reported that As decreases enzyme activity in three ways: (1) by interacting with the enzyme–substrate complex; (2) by denaturing the enzyme protein, or; (3) interacting with the active protein groups (Dick 1997).

Hemida et al. (1997) indicated that the amidase activity in soil to which Cu^{2+} and Zn^{2+} had been added was not strongly inhibited compared to the activities of urease and nitrate reductase, and explained this by citing the different functional groups at the active sites of amidase. Wood and Oris (1974) stated that thiol groups had no direct effect on the catalytic activity of amidase, but they were necessary to stabilize the active amidase conformation. Frankenberger and Tabatabai (1980) suggested

that α-amino groups may be effective at catalyzing amidase function, and that these groups do not react with metal ions.

Bhattacharyya et al. (2007) reported that phosphatase activity was negatively influenced by a high phosphorus content in the soil because of the structural similarity of phosphate and arsenate (Juma and Tabatabai 1977; Speir et al. 1999). Arsenic is a highly inhibitory heavy metal, even at low concentrations, due to its chemical properties (uncharged at neutral pH, can diffuse across the cell membrane). When arsenic reaches the inside of the cytoplasm, it crosslinks with sulfhydryl groups and permanently inactivates the enzyme (Dick 1997).

11.2.2.3 Seasonal Effects of Enzymes

Soil enzymes are season-dependent macromolecules because they derive from living organisms. Microorganisms, plants and animals show seasonal fluctuations in activity. Zhang et al. (2008) found that there was a seasonal difference in the effect of heavy metals on soil enzymes – the effect of the heavy metals was more obvious in spring and summer than in autumn.

11.2.3 Soil Factors

11.2.3.1 pH

Effron et al. (2004) reported that enzyme activity was sensitive to changes in pH. When a metal enters the soil, it can alter the soil pH, and usually results in acidification. Increasing the pH influences Cd sorption, reducing the concentration of Cd in the soil solution and making less Cd available in soil (Vig et al. 2003). Geiger et al. (1998) found that the effect of copper on the enzymatic decomposition of cellulose by cellulase and β-glucosidase in suspensions of montmorillonite and aluminum-treated montmorillonite was strongest in the pH range 5.0–5.5. Copper lowered the pH values corresponding to the optimal activities of cellulase and β-glucosidase. Generally, amino acids of enzymes are deprotonated at high pH involved in metal interaction. Geiger et al. (1998) reported that, in the presence of kaolinite, the optimal pH for clay-absorbed enzyme activity was shifted by one or two pH units toward alkaline values (Pflug 1982). Campbell (1988) suggested that almond β-glucosidase had a catalytic function involving two key groups, aspartic and glutamic carboxyl groups at the enzyme's active site, when they were in the appropriate protonation state. Campbell's model assumes that enzyme activity can be lost in two ways: (1) deprotonation of the aspartic carboxyl group; (2) protonation of the glutamic carboxyl group. Geiger et al. (1998) found that the effect of copper was strongest in the pH range 5.0–5.5, in which case 200 μM Cu reduced enzyme activities (of cellulase and β-glucosidase) by 25% or more. However, when the pH was close to 4, the enzyme activities were reduced by only 5% by the same level of copper.

Different enzymes can respond differently at the same pH values and metal levels. Under conditions of pH 5.5 and 600 μM copper, β-glucosidase activity was reduced by 90% whereas cellulase activity dropped by 60%.

11.2.3.2 Soil Organic Matter

D'Ascoli et al. (2006) investigated the effects of heavy metal contamination on the biological and biochemical properties (FDA hydrolase, dehydrogenase, β-glucosidase, urease, arylsulfatase, and acid phosphatase) of a soil onto which a river contaminated with Cr(III) and Cu overflowed. The results showed negative correlations between the activities of dehydrogenase, arylsulfatase, and acid phosphatase and Cr fractions (soluble, exchangeable, and carbonate-bound). Although Cu pollution negatively influenced soil biological and biochemical properties, the soil organic matter was able to mask these negative impacts of Cu on the microbial community.

Similarly, many other studies have shown that organic amendments (with municipal waste, compost, biosolid compost, leonardite, gyttja, and litter) reduce the toxicities of heavy metals to soil enzymes (de Mora et al. 2005; Karaca et al. 2006).

Karaca et al. (2002) indicated that many of the effects of Cd were reduced by sewage sludge and phosphate fertilizer amendments. Therefore, reducing the amount of fertilizer added to a contaminated agricultural site will result in an increase in the availability of Cd at that site. A positive way of reducing the impact of Cd contamination is therefore to continue phosphate and sewage sludge/organic matter amendments, which are low in pollutants, on a limited basis. For example, if 80% of the Cd added to the soil remains in the topsoil each year (Taylor 1997), the addition of phosphate or organic matter resulting in a <20% increase in the soil Cd content will eventually result in a reduction of Cd in the soil. This will also reduce the availability of Cd, resulting in less toxic soil and less Cd being sequestered by crop biomass.

Tejada et al. (2008) found that increasing Ni levels reduced soil enzyme activities, and that soil amendment with organic wastes (crushed cotton gin compost, poultry manure) reduced the toxicity of nickel to soil enzyme activities (urease, BBA-protease, alkaline phosphatase, β-glucosidase and arylsulfatase). Organic amendments enhance soil enzyme activity for the following reasons: (1) intra- and extracellular enzymes stimulate microbial activity in the added materials, (2) carboxyl, phenolic, alcohol, and carbonyl functional groups in the humic substances react with toxic ions, forming metal–humate complexes (metal chelation) and stabilizing them (Nannipieri 1994; Dick 1997; Pascual et al. 1998).

Tejada et al. (2008) summarized the following results from different studies. Carboxyl groups play an important role stabilizing toxic ions in the humic acids (McKnight et al. 2001). Although fulvic acids contain more carboxyl groups than humic acids (Stevenson 1994), studies show that metal chelation by humic acids is more effective than metal chelation by fulvic acids since humic acids provide more binding sites due to their larger molecules and more complex nature (Lobartini et al. 1994). Also, humic substances have more strongly acidic groups than fulvic

acids (Hayes 1991). Tejada et al. (2008) concluded that soil microbial biomass and soil enzyme activities are greater in humic acid (crushed cotton gin compost) than in fulvic acid-amended (poultry manure) soil, that the addition of these organic materials may be considered a good strategy for heavy metal polluted soil remediation, and also that the addition of organic materials with a higher humic acid than fulvic acid concentration is more advisable.

11.2.3.3 Clay Minerals

Zeng et al. (2007) studied the effect of lead treatment on the soil enzyme activities in a soil–lead–rice system in a greenhouse pot experiment. High inhibition was observed in sandy soil with a low organic matter content. Similarly, Renella et al. (2003) found that enzyme inhibition was greater in sandy than in fine-textured soils because the clay fraction protects soil enzyme activity.

Geiger et al. (1998) investigated the effect of copper on the enzymatic decomposition of cellulose by cellulase and β-glucosidase in suspensions of montmorillonite and aluminum-treated montmorillonite. The results showed that montmorillonite and Al-montmorillonite reduced the activities of cellulase and β-glucosidase. Also, the use of montmorillonite resulted in the largest reduction in enzyme activity due to its larger specific surface and higher surface area. Gianfreda et al. (1991) indicated that the specific surface areas of montmorillonite and Al-montmorillonite when fully dispersed were approximately 700 and 450 $m^2 g^{-1}$, respectively. There are various reasons for the different specific surface areas of these clay minerals: (1) the adsorption of enzyme molecules on both external and internal surfaces by montmorillonite (Fusi et al. 1989), and; (2) the larger net negative charge of montmorillonite (87 meq $100g^{-1}$) compared to Al-montmorillonite (15 meq $100g^{-1}$) (Lothenbach et al. 1997).

Montmorillonite and Al-montmorillonite did not reduce the toxic effect of the metal. To explain this, Geiger et al. (1998) cited the higher affinity of copper for cellulase and β-glucosidase than for montmorillonite or Al-montmorillonite, and the synergetic effects of clay minerals and copper on the inhibition of enzyme activity. Geiger et al. (1998) proposed that clay surfaces interact with both enzymes and metals and ultimately reduce the toxicity of metals.

Clay minerals can strongly affect extracellular enzyme activity in soil (Geiger et al. 1998). The adsorption of enzymes at clay surfaces caused two different responses: (1) the inactivation of enzymes due to conformational changes (Burns 1978; Boyd and Mortland 1990; Geiger et al. 1998), or; (2) enzyme activity enhancement caused by increased concentrations of enzyme and substrate at the solid–water interface (Burns 1978).

Tietjen and Wetzel (2003) investigated the effect of clay adsorption on enzyme activities (alkaline phosphatase, glucosidase, protease, and xylosidase). Montmorillonite clay (M) and clay extracted from Elledge Lake (EL) were used in enzyme–clay solutions in an adsorption experiment. While adsorption onto the EL clay decreased alkaline phosphatase activity, adsorption onto the M clay decreased the activities of

all of the studied enzymes. They also found that the adsorption of enzyme onto clay protects the enzyme from photodegradation.

Wyszkowska et al. (2006) investigated the effects of copper on soil enzymes (dehydrogenase, urease, acid phosphatase, and alkaline phosphatase) and its interactions with other heavy metals (Zn, Ni, Pb, Cd, Cr). They found that the activity of dehydrogenase was greater in heavy loamy sand, while the activities of other enzymes were higher in light silty clay. In another words, enzyme inhibition due to heavy metals was greater in heavy loamy sand than in light silty clay (except in the case of dehydrogenase).

11.2.4 Plant Factors

11.2.4.1 Metal Accumulator Plants

Wang et al. (2008) defined metal accumulator plants as those that can grow in heavy metal contaminated soils, and have evolved mechanisms to tolerate high levels of heavy metal from the soil inside their cells (Tang et al. 1999; Song et al. 2004). Mining sites, in particular, contain high heavy metal concentrations in soil and metal-tolerant plants. *Elsholtzia splendens* is a Cu-tolerant plant that is widely found at Cu mining sites and is used as a Cu-mine indicator (Wang et al. 2008). Such plants can be used in the phytoremediation of heavy metal soils because they accumulate the metals and thus reduce metal levels in the soil. Wang et al. (2008) investigated the acid phosphatase activity in the rhizospheres of a copper accumulator (*Elsholtzia splendens*) and a nonaccumulator plant (*Trifolium repens*) upon different Cu treatments (0, 200, 500, 1,000 mg kg^{-1}). The results showed that enzyme inhibition was strong in the unplanted and nonaccumulator plant rhizospheres and weak in the rhizosphere of the Cu-accumulator plant. Wang et al. (2007a,b) studied the effect of heavy metal pollution on enzyme activity near a copper smelter. They found a strong inhibition of alkaline phosphatase activity near the copper smelter (<200 m).

11.2.4.2 Plant Community Effect

Yang et al. (2007a,b) investigated the effects of coexisting plant species on soil microbes and soil enzymes in lead-contaminated soils. In a mesocosm experiment carried out in greenhouse, four different plant species (*Festuca arundinacea*: FA, *Kummerowia striata*: KS, *Echinochloa crusgalli*: EC, and *Solidago canadensis*: SC), three different species mixtures (one: FA, two: FA + KS, four: FA + KS + EC + SC), and three different lead application rates (0, 300, and 600 mg kg^{-1}) were used. Urease activity was significantly affected by plant species and Pb concentration. It was significantly greater for the four-species mixture than for the one- or two-species mixtures. Alkaline phosphatase activity was not significantly impacted

by plant species but was affected by Pb concentration. Acid phosphatase and dehydrogenase were not significantly influenced by either species mixture or Pb concentration.

11.2.5 Special Inhibition Parameters

11.2.5.1 Ecological Dose

The effect of the heavy metal on soil enzyme activity can be quantified by determining the ED_{50} (ecological dose) parameter, which is the concentration of heavy metal at which the enzyme activity, or some other biological activity, is reduced to 50% of its uninhibited value (Tejada et al. 2008). Tejada et al. (2008) reported that ED_{50} values may be more suitable indicators of the sensitivity of an ecosystem to stress, because a 50% reduction in the basic ecological process may be too extreme for its continued functioning (Babich et al. 1983). Many researchers have used this inhibition parameter to evaluate soil enzyme inhibition by heavy metals, and their results are summarized in Table 11.4.

11.2.6 Understanding the Inhibition of Soil Enzymes by Heavy Metals

11.2.6.1 Combined Effects

Heavy metals exert inhibitory effects on soil enzymes, but these effects depend on many factors in the soil.

Combined Effects of Two Metals

Khan et al. (2007) investigated soil enzyme activities (catalase, alkaline phosphatase, and dehydrogenase) when various levels of Cd and/or Pb were applied to the soil. This work thus provides a good example of the combined effects of heavy metals on soil enzyme activities (see Table 11.5). Strong inhibition was observed at high heavy metal concentrations in both the single-metal and dual-metal systems; however, the inhibition was greater in the dual-metal system than the single-metal systems; in other words, a "synergistic effect" was observed. However, some combinations of metals exhibit this synergism while others do not. Wyszkowska et al. (2006) concluded that treatment with copper alone was more inhibitory towards soil enzyme activity than copper applied in conjunction with other heavy metals (Cu with Zn, Ni, Pb, Cd, and Cr).

Table 11.4 ED_{50} values for evaluating soil enzyme inhibition by heavy metals

Soil type												
pH	OM[a]	CEC[b]	Sand[c]	Clay[c]	Treatment	ED_{50}						Reference
5.94	26.4	13.30	54	16	Pesticides + Hg	UR						Yang et al. (2007a,b)
					Hg: 0.92, 1.85, 3.69, 7.39, 14.77, 29.54	88						
6.19	20.7	15.35	62	18		5.5						
6.26	31.6	28.05	29.8	32		24						
6.71	29.4	23.0	22	36		20						
								ACP		ALP		
5.1	3.7[c]	–	77.8	2.2		Cd		558.2		15.1		Renella et al. (2003)
						Cd + Cu		46.1		9.7		
						Cd + Zn		260.7		16.1		
						Cd + Cu + Zn		159.9		7.9		
7.2	0.7[c]	–	81.9	11.4	Cd	14.3				4.1		
					Cd + Cu	3.9				2.9		
					Cd + Zn	3.5				3.1		
					Cd + Cu + Zn	3.1				2.6		
8.1	2.2[c]	–	20.5	42.2	Cd	30.0				25.5		
					Cd + Cu	15.1				15.9		
					Cd + Zn	28.1				17.1		
					Cd + Cu + Zn	10.7				14.4		
7.68	1.31[c]	6.9[d]	78.2	7.1	Pyrite sludge amended	ASL	ALP	ACP	BG	DEH	UR	Hinojosa et al. (2008)
					As 2.8, Cd 0.1, Zn 38.8, Cu 9.5,	0.61	0.55	1.89	0.68	1.71	2.74	
7.66	2.02[c]	12.3[d]	14.8	36.2	Cr 0.01, Fe 234.1, Mn 0.3,	1.36	2.69	3.65	3.38	2.12	6.27	
7.72	1.58[c]	11.3[d]	41.5	11.6	Hg 0.1, Ni 0.003, Pb 39.9	n.s.	3.15	0.88	0.49	2.71	14.59	
7.37	17.1[c]	-	4	41	Enzyme	Cr[VI]0.4		Cr[III]0.2				Samborska et al. (2004)
					Suspension soil + enzyme	0.5		0.4				
					Suspension soil + irrigated, purified wastewater	0.5		0.6				

[a]mg kg^{-1}; [b]cmol kg^{-1}; [c]%; [d]meq 100 g^{-1}; *UR*, urease; *ACP*, acid phosphatase; *ALP* alkaline phosphatase; *DEH* dehydrogenase; *ASL* arylsulfatase; *BG* β-glucosidase

Table 11.5 Combined effects of Cd and Pb on enzyme activities in soil[a] in a pot experiment performed in a greenhouse (Khan et al. 2007)

Enzyme[b]	Cd and Pb application rates[c]	Incubation time (weeks)	Inhibition of activity (%)
CATCAT	Cd1Cd3	22	5.9639.3
CAT	Cd3 + Pb3	2	39.9
CAT	Cd1 + Pb1	2	8.8
ALP	Cd1	2	7.8
ALP	Cd3	2	41.5
ALP	Pb1	2	7.8–19.3
ALP	Pb2	2	11.9–20.9
ALP	Pb3	2	13.1–24.3
ALP	Cd1 + Pb1	2	25.5
ALP	Cd2 + Pb2	2	40.5
ALP	Cd3 + Pb3	2	43.5
DEH	Cd1	2	19.3
DEH	Cd2	2	25.9
DEH	Cd3	2	32.4
DEH	Pb1	2	2.9–15.8
DEH	Pb2	2	7.2–23.7
DEH	Pb3	2	12.1–18.2
DEH	Cd1 + Pb1	2	8.9–24.1
DEH	Cd2 + Pb2	2	11.9–32.5
DEH	Cd3 + Pb3	2	15.5–41.6

[a]Soil properties: pH 8.0; OM:17.9 g kg^{-1}; 42.5% sand; 10.4% clay; total Cd: 0.14 mg kg^{-1}; total Pb: 2.57 mg kg^{-1}

[b]*CAT* catalase; *ALP* alkaline phosphatase; *DEH* dehydrogenase

[c]Cd added as $CdSO_4$, Pb as $Pb(NO_3)_2$; application rates (in mg kg^{-1}) were: *Cd1*, 1.5; *Cd2*, 3; *Cd3*, 5; *Pb1*, 150; *Pb2*, 300; *Pb3*, 500

Combined Effects of Three Metals

Yang et al. (2006) investigated the combined effects of Cd, Zn, and Pb on catalase, urease, invertase, and alkaline phosphatase in soil. The results showed that Cd significantly inhibited the activities of all of the enzymes studied, Zn only inhibited those of urease and catalase, while Pb was not significantly inhibitory compared to the other heavy metals towards the studied enzymes, and actually had a protective influence on catalase activity when all of the metals were present (Cd, Zn and Pb). Cd was the most effective enzyme inhibitor, followed by Zn. The order of the effect of Cd, Zn and Pb was Cd > Zn > Pb. There was a negative synergistic inhibitory effect of Cd and Zn on urease and catalase activity in the presence of Cd, Zn, and Pb, which can be explained by the similar ionic properties of Zn and Cd. Urease activity was enhanced by Cd and Pb at low concentration; however, it was inhibited at higher concentrations of Cd and Pb. Urease activity was reduced by 20–40% in the Cd–Zn–Pb combined metal system. Therefore, three-metal treatments had a greater inhibitory effect than the single heavy metal treatments because of a synergistic effect of the metals on enzyme activity. In this study, the enzymes showed different sensitivities to the single- and three-metal treatments. Urease was the most

sensitive of the enzymes to combined pollution (Cd, Zn and Pb). Yang et al. (2006) reported that the magnitude of enzyme inhibition or activation depends on (1) the heavy metal ion, its concentration, and the type of enzyme assayed, (2) the interaction between the heavy metals, (3) the reactions between the heavy metals in solution and the functional groups of the enzymes, (4) the chemical and physical properties of the soil (pH, organic matter content, and type and amount of clay).

Combined Effects of pH, Organic Matter (OM), Clay, and Four Metals

Irha et al. (2003) studied the effect of heavy metals and PAHs on dehydrogenase in soil. Decreasing the organic matter, clay and pH slightly inhibited the dehydrogenase (Table 11.6). Rendzina alvar and Brown pseudopodzolic soils differ only in their organic matter and amorphous mineral phase contents; their clay contents are the same. The dehydrogenase was more inhibited at lower organic matter and higher amorphous mineral phase contents (i.e, in Brown pseudopodzolic soil). Organic matter and the amorphous mineral phase may therefore mask dehydrogenase inhibition by heavy metals.

Combined Effects of pH, OM, Clay, Cation Exchange Capacity (CEC), and Chemical Form of Metal

Carine et al. (2008) evaluated the effects of different metals in different chemical forms (chloride, sulfate and acetate salt) on soil phenoloxidase activity. This study is a very good example of an investigation of soil enzyme inhibition by heavy metals because the researchers considered many factors and examined many heavy metals. The influential factors are obvious from Table 11.7. The study results lead us to conclude that soil enzyme inhibition by heavy metals depends on: (1) the heavy metal its concentration; (2) soil texture (clay content); (3) the chemical form of the heavy metal (Karaca et al. 2000).

Table 11.6 Effects of heavy metals and PAHs on soil dehydrogenase activity (adapted from Irha et al. 2003). Soils were artificially contaminated with heavy metals (as their chloride salts) at the following levels: Cr (Cr^{+3}) 3 mg L^{-1}; Pb 6 mg L^{-1}; Cu 20 mg L^{-1}; Cd 60 mg L^{-1}

Soil type	Inhibition
Rendzina alvar: pH 7.0, 22.94% OM, 30% clay, 2% amorphous phase	Weak DEH
Brown pseudopodzolic: pH 7.2, 6.64% OM, 30% clay, 1% amorphous phase	Moderate DEH
Sod podzolic: pH 6.2, 4.88% OM, 15% clay, 31% amorphous phase	Strong (no activity) DEH

Table 11.7 Combined effects of heavy metals on the enzyme activity of phenoloxidase

	Soil types					
	Sandy loam 1: pH 6.9, 11.8 g kg^{-1} OM, 7.5 cmol kg^{-1} CEC, 67% sand, 13% clay		Loam: pH 8.5, 8.8 g kg^{-1} OM, 9 cmol kg^{-1} CEC, 36% sand, 23% clay		Sandy loam 2: pH 8.2, 9.9 g kg^{-1} OM, 4 cmol kg^{-1} CEC, 67% sand, 12% clay	
	Phenoloxidase activity					
Treatments	Inhibition (−) degree	100% (−) level or activation (+) range (nM)	Inhibition (−) degree	100% (−) level or activation (+) range (nM)	Inhibition (−) degree	100% (−) level or activation (+) range (nM)
BaCl salt	Strong	150	Strong	150	Strong	50400
$CdSO_4$ salt	Weak	150	No effect	400	Weak	400
CoCl salt	Strong	150	Strong	400	Strong	200
$CoSO_4$ salt	Strong	10	Strong	10	Strong	10
CuCl salt	Moderate	5	Weak	0.5–200	Strong	50–400
$CuSO_4$ salt	Moderate		Weak	5	Moderate	5–400
$FeSO_4$ salt	Strong		Strong	150	Strong	400
MgCl salt	No effect		No effect	400	No effect	10
MnCl salt	No effect		No effect		(+) Strong	150
$MnSO_4$ salt	No effect		(+) Weak		(+) Strong	200
NiCl salt	No effect		No effect		Weak	
Pb-acetate salt	Moderate		Moderate		Strong	
SnCl salt	Strong		Strong		Strong	
ZnCl salt	(+) Weak		(+) Weak		(+) Weak	
$ZnSO_4$ salt	(+) Weak		(+) Weak		(+) Weak	
AlCl salt	Moderate		Strong		Strong	
$AlSO_4$ salt	Weak		Strong		Strong	
(0, 0.5, 5, 10, 50, 100, 150, 200, 400, 800 nM)						

Combined Effects of Metal, Metal Oxidation State, and Organic Matter

Senwo and Tabatabai (1999) conducted a study on the effects of heavy metals on aspartase activity in soils. They concluded that: (1) the most effective inhibitors of aspartase activity were Ag(I) and Hg(I); (2) aspartase activity was significantly correlated with organic carbon, total nitrogen, and clay content; (3) activity inhibition was higher in air-dried soils than in field-moist soils because the air-dried soils provided more exposure of the enzyme to heavy metals. The results of this study are shown in Table 11.8, and can be summarized as follows. (1) Higher organic matter and clay contents along with a higher soil pH results in less inhibition of aspartase activity. (2) Higher oxidation states of heavy metals are less inhibitory than lower oxidation states. (3) Ag and Hg are highly toxic elements.

11.3 Conclusion

As a result of increasing metal concentrations in the soil due to either natural or anthropogenic contamination, it has been found that soil enzyme activities are influenced by different metals in different ways, depending on the type of metal and the metal salt. However, soil characteristics such as pH, clay content, and soil organic matter, can modify the negative or positive impacts of heavy metals on soil enzymes. Therefore, in addition to monitoring changes in soil metal content, an assessment of changes in soil enzyme activities would be a useful tool for monitoring soil quality and fertility under heavy metal pollution. This definitely depends on the enzyme, the metal, and its concentration. Based on the

Table 11.8 Effects of heavy metal species on aspartase activity in soils

	Inhibition of aspartase activity (%) in following soil types:		
Heavy metal species (5 μmol g^{-1} soil)	Weller soil: pH 6.0, 12.2% OC, 235 g kg^{-1} clay, 46 g kg^{-1} sand	Webster soil: pH 6.9, 32.45 OC, 264 g kg^{-1} clay, 250 g kg^{-1} sand	Harps soil: pH 7.9, 44.0% OC, 356 g kg^{-1} clay, 188 g kg^{-1} sand
Ag(I)Cu(I)	9856	9134	8731
Cd(II)	63	54	49
Fe(II)	41	26	35
Hg(II)	97	95	87
Sn(II)	53	32	31
Fe(III)	53	32	28
Ti(IV)	48	31	25
As(V)	32	45	25
Mo(VI)	28	45	27

research findings discussed in this chapter, it can be concluded that intracellular oxidoreductases (i.e., dehydrogenase) that take part in microbial processes are more vulnerable to metal-related short-term changes than extracellular ones. Without a doubt, this is due to the linkage of the extracellular enzymes to the colloidal soil fractions, especially clay and organic matter, through adsorption and crosslinking, microencapsulation, copolymer formation, entrapment, ion exchange, and covalent attachment, and hence them becoming resistant to environmental factors. However, again, the research findings presented in this chapter reveal that different salts of a particular metal affect enzyme activities differently, and that metal solutions prepared from various metal salts cause different degrees of enzyme inhibition (Karaca et al. 2000). This fact has generally been neglected in most incubation studies that have examined the effects of heavy metals on soil enzyme activities, but it should be taken into consideration in future experimental studies.

In many laboratory studies, the application of increasing concentrations of metal nitrate or sulfate salts also results in the addition of large amounts of nitrogen and sulfur, which are nutrients for soil microflora that synthesize soil enzymes. Following the application of these metal solutions to the soil, the heavy metals would probably inhibit enzyme activity while the nutrients would support the enzyme production system. This balance between the inhibitory effects of the metals and the stimulatory effects of the nutrients in the solution may blur the actual influence of the metal on soil enzyme activities. Similarly, solutions of metal salts that do not contain microflora-activating ions (i.e., chlorides) could also result in complex effects. Therefore control treatments where only the nutrients or salt constituents are applied to the soil should also be included in laboratory incubation experiments.

This is also necessary in laboratory studies evaluating the effects of multiple heavy metals on soil enzyme activity.

On the other hand, the increase in enzyme activity resulting from the application of various metal solutions to the soil at low concentrations may be related to either the metal itself or other anions in the metal salt solution, and we need to clarify which one of these options is correct. Also, thus far, low concentrations of some heavy metals like Zn and Cu have been shown to have nutritional value, while this is not the case for other metals like Cd. The reason for the increase in enzyme activity at lower Cd concentrations, as reported in numerous research papers, needs to be clarified.

The main soil characteristics that control the influence of heavy metals on soil enzyme activities are the clay and organic matter contents and the soil pH. Since these are the primary factors that affect the binding of metals to soil colloids and their uptake by biological systems, any changes to these soil characteristics will affect the interactions between heavy metals and soil enzymes. However, most works have shown that although different soils have different physicochemical features, increasing the heavy metal concentration largely inhibits the biological activity of the soil, and so soil enzymes are highly sensitive indicators of soil degradation due to heavy metal accumulation.

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