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SOIL ENZYME ACTIVITY AND ITS RELATIONSHIP WITH HEAVY METALS

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Abstract-
Soil enzymes are vital indicators of soil quality, fertility, and microbial activity, as they mediate the decomposition of organic matter and nutrient cycling. Their activity reflects the functional capacity of soil ecosystems and is highly sensitive to environmental stressors, particularly heavy metals. Heavy metals such as cadmium (Cd), lead (Pb), zinc (Zn), copper (Cu), and arsenic (As) have been widely reported to inhibit soil enzyme activities, with the extent of inhibition varying according to metal type, concentration, chemical form, and bioavailability. Factors such as soil pH, organic matter, and clay content further influence enzyme response to heavy metal stress. Enzyme activities including urease, phosphatase, dehydrogenase, arylsulfatase, and β-glucosidase are among the most commonly studied as biomarkers of soil contamination. Evidence suggests that heavy metals may reduce microbial biomass and alter nutrient cycling, thereby threatening soil productivity and ecological stability. Hence, monitoring soil enzyme activity in relation to heavy metal contamination provides an effective tool for evaluating soil health, pollution impact, and sustainable land management.

Keywords: *Soil enzymes, Heavy metals, Enzyme inhibition, Bioavailability, Soil quality, Nutrient cycling, Soil contamination.*

1. INTRODUCTION-

The soil is a biologically active, exhaustible resource that can be used for the sustainable production of agricultural crops. Food production, environmental efficiency, and worldwide ecological stability are all affected by soil quality **(Binkley and Fischer, 2012)**. The quality of soil is a key factor determining the yield of crops, and can be sustained through using tools that are appropriate for predicting and evaluating changes in soil quality **(Almeida *et al.*, 2015)**. The soil's quality depends on several factors, including its composition and modifications due to human activity **(Abbott and Murphy, 2003)**. The soil enzymatic activity assay is one of the ways to measure soil ecosystem position. Soil enzymes play a crucial role in the biochemical process of reabsorbing organic matter from soil organic matter (SOM), soil physical properties, microbial activity and microbial biomass. As a result, they improve soil health and fertility management in ecosystems **(Makoi and Ndakidemi, 2008)**. A soil enzyme increases the rate of reaction at which plant residues decompose and release nutrients for plants **(Balezentiene, 2012)**. In conclusion, the rate of decomposition is associated with enzymes that act primarily on structural parts of plant material, thereby providing valuable information about the microbial community and succession.

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(Fioretto *et al.*, 2000). There are many different sources of soil enzymes, such as living or dead microbes, plant residues, and soil animals. Some enzymes help breakdown organic matter, such as hydrolase and glucosidase, whereas others help mineralize nutrients, such as amidase, *urease*, *phosphatase* and sulfates.

Classification-

Soil enzyme having various category in soil. An enzyme can be either Constitutive or Inducible depending on its quantity in a cell **(Figure 1)**. The location of enzymes further divides them into two types: endo or intracellular enzymes and exo- or extracellular enzymes **(Figure 2)**. It is also possible to categorize soil enzymes based on their function **(Figure 3)**.

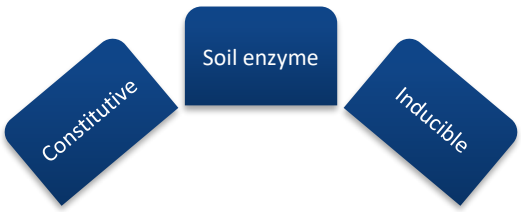

Figure 1: Based on quantity in a cell

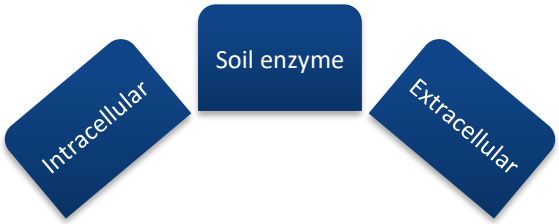

Figure 2: Based on location

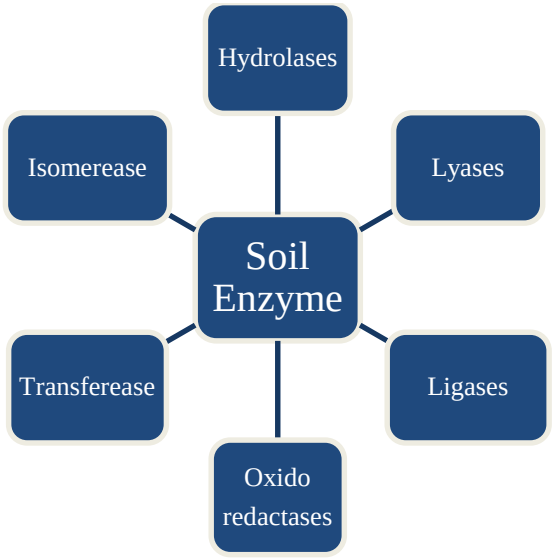

Figure 3: Based on their function

Mechanism of soil enzyme-

A soil enzyme is a protein-structured molecule that accelerates reaction rates by catalyzing them without permanently transformation the reaction **(Kandeler and Dick, 2006)**. The substance acted upon by a soil enzyme is called a substrate **(Figure 4)**

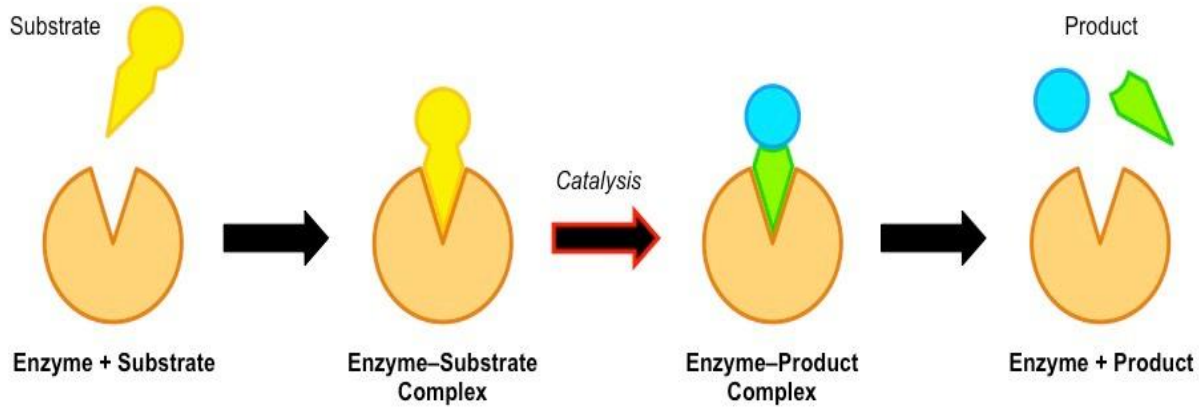

Figure 4: Lock and Key model of enzyme action given by **Koshland, (1958)**.

During the enzymatic reaction, the substrate is cleaved and a product is released, which may be a nutrient. A nutrient's availability affects enzyme production in part, which depends on microbial activity **Sinsabaugh, (1994)**, when nutrients are limited, microbes produce enzymes to mobilize resources from compound sources **(Harder and Dijikhuizen, 1983)**. As a result of their activities, soil enzymes can be used to evaluate the rate of important soil processes, soil productivity, microbial activity, and the inhibition of pollutants **(Naré *et al.*, 2014)** Major soil enzymes and their role are presented in **Table 1 (Srinivasrao *et al.*, 2017).**

Table 1: Major Soil Enzyme and their role

Enzyme	Source	Reaction catalyzed	End product	Soil function indicated
α-Amylase	Plants, animals, and microorganisms	Starch hydrolysis	Glucose and/or oligosaccharides	C-cycling
β-Amylase	Mainly plants	Starch hydrolysis	Maltose	β-Amylase
Dehydrogenase	Microorganisms	Oxidation of organic compounds	Transfer of H to NAD to NADP (electron transport system)	C-cycling,
Endo-1, 4-β-glucanase	Microorganisms, protozoa, and termites	Cellulose endohydrolysis	Oligosaccharides	C-cycling
Exo-1, 4-β-glucanase		Cellulose cleavage at ends	Glucose and cellobiose	
β-glucosidase		Cellobiose hydrolysis	Glucose (sugar)	
Phenol oxidase	Plants and microorganism	Lignin hydrolysis	C compounds (humic substances)	C-cycling
Urease	Microorganisms, plants, and some invertebrates	Urea hydrolysis	Ammonia (NH3) and CO2	N-cycling
Alkaline phosphatase	Mainly bacteria	Hydrolysis of esters and anhydrides of phosphoric acid	Phosphate (PO4)	P-cycling
Acid phosphatase	Plants, fungi, and bacteria			
Arylsulfatase	Microorganism plants and animals	Hydrolysis of sulfate esters	Sulfate (SO−2)	S-cycling

Enzyme	Source	Reaction catalyzed	End product	Soil function indicated
Protease	Microorganisms and plants	N mineralization	Plant available N N-cycling	N-cycling
Chitinase	Plants and microorganisms	Degradation and hydrolysis of chitin	Carbohydrates and inorganic nitrogen	C- and N-cycling

2. The inhibition of Soil Enzymes-

The enzyme inhibitor reduces enzyme activity, whereas the enzyme activator stimulates enzyme activity **(Voet and Voet, 1995)**. Enzyme inhibition and activation are show in **Figure 5**. This type of agent affects the Km parameter of the enzyme reaction of interest (Km is the substrate concentration at which the reaction rate is half of the maximum rate) **(Tabatabai, 1977)**. **Figure 5** shows that Km values increase in the presence of inhibitors and decrease in the presence of activators.

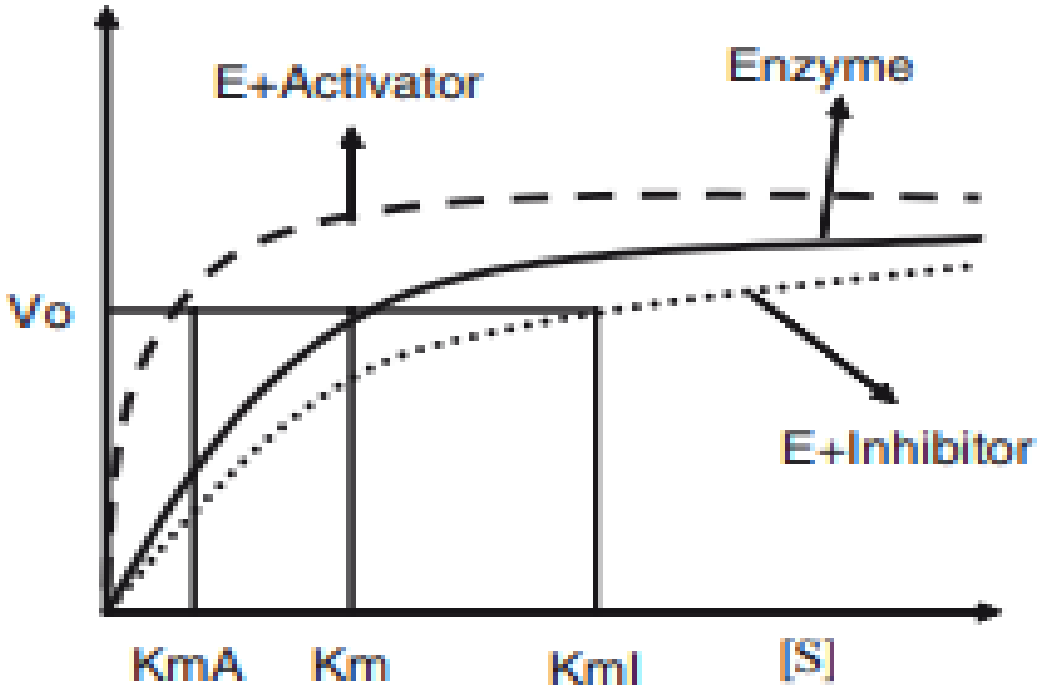

Figure 5: Effects of inhibitors and activators on enzyme activity

Heavy metals can inhibit soil enzyme activities as there are many factors that contribute to this inhibition. In general, these factors can be classified into many categories in these category metallic factors are most important factor which one inhibited enzyme activity in many ways.

3. Metal Factor-

This includes the heavy metal element in question, concentration of heavy metals, chemical form of heavy metals, and availability of heavy metals.

A. Heavy metal elements

Depending on the chemical affinity of each enzyme in the soil system, different metals influence enzyme activities in different ways. According to **Khan *et al.*, (2007)** due to its greater mobility and lower affinity for soil colloids, cadmium (Cd) was more toxic to enzymes than lead (Pb). **Shen *et al.*, (2005)** studied showed there is a negative interaction between zinc (Zn) and cadmium (Cd) resulting from competition for sorption sites. The effects of different metals on soil enzymes are different **Renella *et al.*, (2005)** investigation showed that cadmium (Cd) inhibited alkaline *phosphatase, arylsulfatase*, and protease, but not acid *phosphatase, b*-glucosidase, or *urease*. **Wyszkowska *et al.*, (2013)** found that cadmium (cd) inhibits *dehydrogenase, catalase* and *urease*, whereas zinc (zn) only inhibits catalase and urease, and lead only inhibits catalase and urease to a lesser extent than cadmium (Cd) and zinc (Zn). **Geiger *et al.*, (1998)** has been discovered that copper inhibits *b-glucosidase* activity more than cellulase activity. According to **Balyaeva *et al.*, (2005)** lead (Pb) significantly reduced *urease, catalase, invertase*, and *acid phosphatase* activities. Some study shown that total arsenic (As) and water-soluble As are the most toxic to soil enzymes, and that the activity of *urease, acid* and *alkaline phosphatases, arylsulfatases*, and *dehydrogenases* is negatively correlated with water-soluble arsenic **Koo *et al.*, (2012)**. Similarly in West Bengal, India, **Bhattacharyya *et al.*, (2008)** studied long-term As contamination of rice soils and found that soil enzyme activity was significantly negative correlated with the amount of water-soluble arsenic. It was also found that as contamination significantly affected *arylsulfatase* activity, but not *xylanase, invertase, protease*, or *alkaline phosphatase* activity **Lorenz *et al.*, (2006)**. In a study by **Speir *et al.*, (1999)**, *phosphatas*e and *sulfatase* were inhibited by As(V), but *urease* was not. **Shen *et al.*, (2005)** reported that Cr, Cd, Zn, Mn, and Pb generally inhibited urease activity in the order Cr>Cd>Zn>Mn>Pb. *Arylsulfatase, acid phosphatase, protease*, and *urease* were inhibited by heavy metals, according to **Effron *et al.*, (2004)**.

Cadmium (Cd) bioavailability and toxicity towards soil microorganism and their activities have been extensively summarized in **Vig *et al.*, (2003)** review. Detailed information on how Cd affects soil enzyme activity can be found in **Table 2**.

Table 2: Cd effect 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	Cu 15, Cd 26, , Zn 478, Pb 21.6	+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	2810 281	−ASL 23–55% −ASL 7%	**Al-Khafaji and Tabatabai (1979)**

Soil type/treatment	Cd (mg kg^{-1} soil)	Inhibition (−), activation (+) or no effect (NE)	References
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)**
Silty: pH 5.6, 2.6% OC, 28% clay	562	−ADS 6%	**Frankenberger and Tabatabai (1981)**
5 different soils—River sand, Gley, Gray lowland, Andosol and Humic Andosol (pH 5.7–8.5) +2% sludge	$CdCl_2$ 1124–3372	+ b-glucosidase	**Hattori (1989)**
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- BD	**Spalding (1979)**
Surface soils: pH 5.1–7.8, 2.6–5.5% OC, 17–42% clay	$CdSO_4$ 562	−UR	**Tabatabai (1977)**

Organic Carbon (OC); Total Organic Carbon (TOC); Organic Matter (OM); *Arylamides (ARA); Arylsulfatase (ASL); Dehydrogenase (DHE); Amidase (ADS); Acid Phosphatase (AC); Cellulase (CL); Amylase (AML); β-glucosidase (BD)*

B. Heavy Metal Concentration

It is generally known that all metals, including heavy metals, are found in soil at low concentrations and provide essential micronutrients for soil organisms however Anthropogenic pollution has dramatically increased their levels **(Carine *et al.*, 2008**). There was a very strong negative correlation between the heavy metal concentrations and the number of cultivable bacteria and fungi, soil microbial biomass carbon, and bacteria activity, as well as the activities of enzymes *dehydrogenases, urease, acid phosphatase,* and *arylsulfatase* **Chowdhury and Rasid, (2021)**. At low concentrations of Pb, **Zeng *et al.*, (2007)** observed a stimulating effect on soil enzyme activity. As the level of lead (Pb) was increased to 500 mg kg^{-1}, soil enzyme activities decreased. Likewise, **Dar, (1996)** found no significant changes in soil enzyme activity with the addition of 10μg g^{-1} of

cadmium (Cd) per gram of soil. But cadmium (Cd) addition at 50 μg g^{-1} soil decreased enzyme activity, with the impact being greater in sandy loam than in loam or clay loam.

According to **Tejada *et al.*, (2008)** soil enzyme activities decrease as Ni concentration increases. In their study, **Lorenz *et al.*, (2006)** found that enzyme activity decreased as cadmium (Cd) levels increased. It is well known that any element under specific environmental conditions will negatively affect plants and microorganisms if its concentration exceeds a certain threshold **(Zeng *et al.*, (2007).** The activities of cellulase and *b-glucosidase* were inhibited at copper concentrations above 200 mM **(Geiger *et al.*, 1998).** At 2,000 mg heavy metals (Cu^{+2} and Zn^{+2}) per gram of soil, **Hemida *et al.*, (1997)** found that *urease* activity completely disappeared. According to **Wyszkowska *et al.*, (2006)** metal concentrations of 50 mg kg^{-1} and higher inhibit the activities of soil enzymes (soil *dehydrogenase*, *urease*, acid and alkaline *phosphatase*s). Alluvial soils polluted with heavy metals were studied by **Mikanova, (2006)** for enzyme effects (*arylsulfatase*, *invertase*, *urease* and *dehydrogenase*). As the metal concentration increased, all soil enzymes were inhibited, but *arylsulfatase* and *dehydrogenase* were more sensitive than invertase and *urease* to lower metal concentrations **Table 3**. Soil enzyme activities decreased with increasing pollution levels. Unpolluted soil had the highest enzyme activity, while highly polluted soil had the lowest.

Table 3: Heavy metal pollution of alluvial soils: Effects of copper, lead, and zinc on enzyme activities (adapted from **Mikanova, 2006**)

S.No	Alluvium Soil property of Litavka River	Heavy metal (mg kg^{-1} dry soil)			Inhibition			
		Cd	Pb	Zn	DEH	ASL	UR	IN
1.	Unpolluted	1.9	106.0	202.5				
2.	Low-level pollution	2.4	113.5	249.8	S	S	W	
3.	Moderate	5.4	530.5	407.0	S	S	M	W
4.	Medium	59.0	3450.7	6230.8	S	S	M	M
5.	High	61.3	7040.3	7497.9	S	S	M	M
6.	High	113.8	6335.9	12557.4	S	S	S	S

Dehydrogenase (DEH); Arylsulfatase (ASL); Urease (UR); Invertase (IN); Strong (S); Moderate (M); Weak (W)

C. Chemical Form of the Heavy Metal

Heavy metals can affect soil enzymes in different ways depending on their chemical form. The activity of *phenoloxidase* was inhibited at a higher rate and at a lower level by Al chloride salt than Al sulfate salt, according to **Carine *et al.*, (2008)**. The study by **Yang *et al.*, (2007)** found that mercury ($HgCl_2$) inhibited soil *urease* activity, and that there was a logarithmic relationship ($P<0.05$) between Hg concentrations and the activity of soil *urease*

D. Availability of the Heavy Metal

The bioavailability of metals plays an important role in assessing their toxicity. The bioavailability of contaminants in soil particles is the fraction of contaminants that are available to receptor organisms, as a result, bioavailability is especially important for soil microbes and plants. Several factors determine cadmium (Cd) bioavailability, including soil type, cadmium (Cd) speciation,

aging, the type of Cd applied, and the type of microbes **Vig *et al.*, (2003)**. In soil plant systems **Vig *et al.*, (2003)** also found that cadmium (Cd) availability increased as follow; mineral lattices> Iron (Fe) and Manganese (Mn) oxides> organics> metal organic complexes> carbonates> exchangeable and concluded that heavy metals lose bioavailability the longer they are in contact with soil.

The available forms of a metal are significant when attempting to understand its toxicity and the available forms correlate with its chemical forms in soil **Wang *et al.*, (2007a, b)**. A water or NH_4NO_3 extraction can be used to determine the solubilities of metals by releasing heavy metals into a soil solution (water extraction) or by removing soluble and exchangeable metals (NH_4NO_3 extraction) **Munoz-Melendez *et al.*, (2000)**. A soluble form of a heavy metal is considered the most readily available to microorganisms and enzymes **Huang and Shindo, (2000)**. In their study, **Bhattacharyya *et al.*, (2008)** found that metals in water-soluble and exchangeable forms inhibited soil enzyme activity strongly. A study by **Chaperon and Sauve, (2007)** concluded that metals were more toxic to enzymes studied in agricultural soil because of higher dissolved metal concentrations. Availability of metals mainly depends on the present of heavy metal fractions (total, soluble, or extractable). **Wang *et al.*, (2007 a, b)** found significant negative correlations between soil *phosphatase* activity and copper (Cu) and zinc (Zn) (soil solution, NH_4 NO_3-extracted, and total fractions).

4. Conclusion

Soil enzymes are crucial components of the soil ecosystem, as they regulate organic matter decomposition, nutrient mineralization, and overall soil fertility. However, their activities are highly sensitive to environmental disturbances, particularly heavy metal contamination. The type of metal, its concentration, chemical form, and bioavailability largely determine the extent of enzyme inhibition. Among the metals, cadmium, lead, zinc, copper, and arsenic are reported to cause significant negative impacts on enzymes such as *urease*, *phosphatase*, *dehydrogenase*, and *arylsulfatase*, ultimately disturbing microbial activity and nutrient cycling. Additionally, soil properties like pH, clay content, and organic matter influence the interaction between heavy metals and enzymes, sometimes reducing or amplifying toxicity. Therefore, monitoring changes in soil enzyme activity under heavy metal stress provides a reliable indicator of soil quality and ecological stability. Such assessments are essential for managing soil health, preventing long-term degradation, and ensuring sustainable agricultural productivity in polluted environments.

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