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Effect of Different Application Rates of Lead (Pb) on Urease, Phosphates and Microbial Community Structure in Uyo, Nigeria

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Abstract- Soil contamination with heavy metals occurs as a result of both anthropogenic and natural activities. This research was designed to investigate the effect of different application rates of Lead (Pb) on urease, phosphates and microbial community structure. The study was a screen house experiment, and was carried out in the Department of Soil Science, University of Uyo, between March to October 2018. The experiment was a pot experiment, and Lead nitrate ($Pb(NO_3)_2$) solution was added to wet soil sample at the rates of 0, 150, 300, and 500mg/500g soil and mixed thoroughly and incubated for 12 weeks. Samples were collected at two weeks (2wks), nine weeks (9wks), twelve weeks (12wks) after incubation for analysis of urease, acid and alkaline phosphates, and microbial populations. The results revealed that urease (URE), Acid Phosphatase (ACP), and alkaline phosphatase (ALP) were significantly lower in Pb amended soil samples than those of the control. The urease inhibition rate increased with increasing Pb concentrations. The result was more significant at two weeks of incubation,(28.33%) of the control.

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Abstract- Soil contamination with heavy metals occurs as a result of both anthropogenic and natural activities. This research was designed to investigate the effect of different application rates of Lead (Pb) on urease, phosphates and microbial community structure. The study was a screen house experiment, and was carried out in the Department of Soil Science, University of Uyo, between March to October 2018. The experiment was a pot experiment, and Lead nitrate ($Pb(NO_3)_2$) solution was added to wet soil sample at the rates of 0, 150, 300, and 500mg/500g soil and mixed thoroughly and incubated for 12 weeks. Samples were collected at two weeks (2wks), nine weeks (9wks), twelve weeks (12wks) after incubation for analysis of urease, acid and alkaline phosphates, and microbial populations. The results revealed that urease (URE), Acid Phosphatase (ACP), and alkaline phosphatase (ALP) were significantly lower in Pb amended soil samples than those of the control. The urease inhibition rate increased with increasing Pb concentrations. The result was more significant at two weeks of incubation,(28.33%) of the control. The reduction (6.31%) was more at two weeks of incubation in 500mg/500g of treatment. Bacteria, actinomycetes were depressed by 66.25% and 70%, respectively at 12wks in 500mg/g application rate, while fungi decreased in all the amended soil.

Keywords: lead, urease, phosphatase, microbial community.

I. Introduction

Soil contamination with heavy metals occurs as a result of both anthropogenic and natural activities. Heavy metal contamination in the environment is eventually deposited in soil in some forms of low solubility compounds such as pyrite (Huerta-Daiz and Morse, 1992), absorbed on surface-reactive phases, such as Fe and Mn oxides (Copper *et al*., 1970; Hamilton-Taylor *et al*., 2005). Those heavy metals could have long-term hazardous impacts on the health of the soil ecosystem and adverse influence on soil biological processes. Heavy metals can inhibit enzymatic activities by interacting with the enzyme-substrate complexes, denaturing the enzyme protein, and interacting with its active sites (Megharaj *et al*., 2003). Heavy metals can also influence microbial community, which ultimately lead to changes in soil enzymatic activities (Kandeler *et al*., 2000). Aside from long-termed metal-mediated changes in soil enzymatic activities, many reports have shown a large reduction in microbial activity due to short-term exposure to toxic metal (Hamida *et al*., 1992; Doehman and Haanstra, 1984). The bacterial activity was shown to be very sensitive to metal pollution (Diaz-Ravina and Baath, 1996). It was also observed that habitats that have high levels of heavy metal contaminations show a lower number of microbes than uncontaminated habitats (Kandeler *et al*., 2000). Lead (Pb) occurs naturally in soils, but areas impacted by human activities often have significantly elevated Pb levels (Khan *et al.,* 2007), and this affect the soil ecology and microbial depression and changes in enzymatic activities. In their conclusion, Khan *et al*. (2007) opined that heavy metals have inhibiting influences on soil enzymes as well as microbial community structure, soil enzymatic activities are considered to be a good bio-indication reflecting natural and anthropogenic disturbance and be used to evaluate soil pollution (Hinojosea *et al*., 2004; Khan *et al*., 2007). Enzymes accumulated in soils are present as free enzymes such as exo-enzymes released from living cells, endo enzymes released from disintegrated cells, and enzymes bound to cell constituents (Kandeler *et al*., 1976). Kandeler *et al.,* (1996) further stressed that the composition of the microbial community determines the potential of that community for enzyme synthesis and thus any modification of microbial community due to environmental factors.

Lead (Pb) is among the most significant toxic metal, but in normal agricultural soils is in the range of 10 – 100 mg/kg soil (Soon and AD bond, 1993), but in polluted soil, especially near mines or by sewage sludge applications, its contents are even higher than 1000mg/kg soil (Peters and Shem, 1992; Pichtal *et al*., 2000). Higher levels of Pb in soil may adversely affect the activities of soil enzymes, which in turn may result in an adverse effect on various plant parameters influencing crop quality, yield, and possibly human health through the food chain. Base on this background, this research was designed with the following objectives (i) to determine the effect of Pb on soil enzymes, (ii) determine the influence of Pb on microbial community structure.

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II. Materials and Methods

a) Study Area

The study was conducted at the University of Uyo Teaching and Research Farm, Uyo, Akwalbom State, Nigeria. The area is within Latitude 4°32' and 5°44'N and Longitude 7°35' and 8°25'E.

b) Soil Sample Collection

Soil samples were collected using a hand trowel at a depth of 0 – 15cm. There was no fixed interval for sampling, but at random. A total of six (6) location points were taken and pooled together to obtain a composite sample. The samples were taken to the laboratory. The composite samples were split into two, one half was kept for contamination with heavy metal, while the other half was used for enzymes and microbial assays.

c) Experimental Details

The experiment was a pot experiment in a screen house. The wet soil samples were added with Lead (Pb) using Pb $(NO_3)_2$ solution at the rates of 150,300 and 500 mg/kg soil. Four plastic pots were obtained, and each pot was filled with 500g soil. Pot 1 (P1) was the control, Pot 2 (P2) was thoroughly mixed with 150 mg/kg $Pb(NO_3)_2$, Pot 3 (P3) was thoroughly mixed with 300 mg/kg, and Pot 4 (P4) were mixed with 500mg/kg soil.

The contaminated soil samples with $Pb(NO_3)_2$ were incubated in the screen house for 12 weeks. During the incubation period, soil moisture contents were monitored by weighting and adjusting to 60% water holding capacity by deionized water. Samples from each pot was collected at 0,2,9 and 12 weeks for enzymes and microbial community assay.

d) Microbial Analysis

Samples from each treatment of (Pb0, Pb1, Pb2 and Pb3) representing: control (Pb0), (Pb1) 150 mg/500g, (Pb2) 300 mg/500g, (Pb3) 500mg/500g soil.

Samples were enumerated by making ten-fold dilutions of the soil samples from 10^{-1} – 10^{-3},an aliquot of 0.1ml from the 10^{-3} dilution was transferred unto plate in nutrient agar amended with nystatin (0.5-mg/ml) for isolation of bacteria, with potato dextrose agar amended with streptomycin (0.02 – 1mg/ml) was used for the isolation of fungi and Glycerol Agar was used for isolation of actinomycetes. The different cultures were incubated at different temperatures and times required for optimum growth of the microorganisms. Bacteria and actinomycetes were incubated at a temperatures of 37°C for 24 hours in an incubator, while fungi plates were incubated at 28°C for 72 hours. After the respective period of incubation, visible colonies were counted, and the microbial load determined using the formula:- Microbial load = no of colonies x reciprocal of dilution factor, and express as (CFU/g soil).

Isolated colonies were further purified by sub-culturing and identified using the biochemical tests and microscopy.

e) Identification of Isolates

Each isolate was examined for its size, margin, consistency, pigmentation, Gram reaction, and cell morphology. The isolates were characterized as described by Holt *et al*. (1999). Biochemical tests carried out included the production of catalase, indole, and oxidase enzymes. Spore production, and oxidation and formation of sugars were carried out.

f) Determination of Enzymes Activities

i. *Determination of urease*

Urease activity was determined by the method described byGu*et al*., (2009). Briefly, two grams (2g) of the moist soil sample from each pot containing 0mg, 150mg, 300mg, and 500ma of Pb weighed inside four 500ml Erlenmeyer flasks, and 2ml of toluene was measured into each flask and allow to stand for 15 minutes after stirring. The 10ml modified universal buffer (MUB) (pH 6.5) and 10ml of freshly prepared 10% urea solution were added. The flask was covered and incubated in an incubator for 24 hours at 37°C. After incubation, 4ml sodium phenol and 3ml sodium hypochlorite were added to all the Erlenmeyer flasks, and the yellow color developed. The soil solution contents were filtered through Whatman 42 filter paper. The absorbance of the released ammonium was measured using colorimeter at a wavelength of 430nm and the result recorded as NH_4 – N/g soil.

ii. *Determination of Phosphatases*

The method described by Tabatabai and Bremner (1969), Eivazi and Tabatabai (1977) were employed.

One (1) gram of the moist soil sample from the four pots was weighed into 8 Erlenmeyer flasks, (4 for acid phosphatase and 4 for alkaline phosphatase). Two (2) ml of toluene was added, 4ml modified universal buffer (pH 6.5 for assay of acid phosphatase and pH 11 for assay of alkaline phosphatase were added). Then 1ml P-nitro-phenyl phosphate was added. The flasks were covered and incubated at 37°C for 1 hour. After incubation, 1ml 0.5M calcium chloride ($Cacl_2$) and 0.5M Sodium hydroxide (NaOH) were added, and the flask swirled for few seconds to mix. The soil suspension was filtered through Whatman 42 filter paper. The yellow colour was measured using colorimeter at the wavelength of 430nm, and the results were recorded as mg formazan/g soil.

III. Results

Urease (URE), Acid phosphatase (ACP), and Alkaline phosphatase (ALP) were significantly (P=.05) lower in the Lead (Pb) amended soil samples than those of the control (Fig. 1). The enzyme inhibition extent was

clearly seen between different incubation periods and varied as the incubation proceeded, and the highest rate was detected in samples mostly at two (2) weeks of incubation.

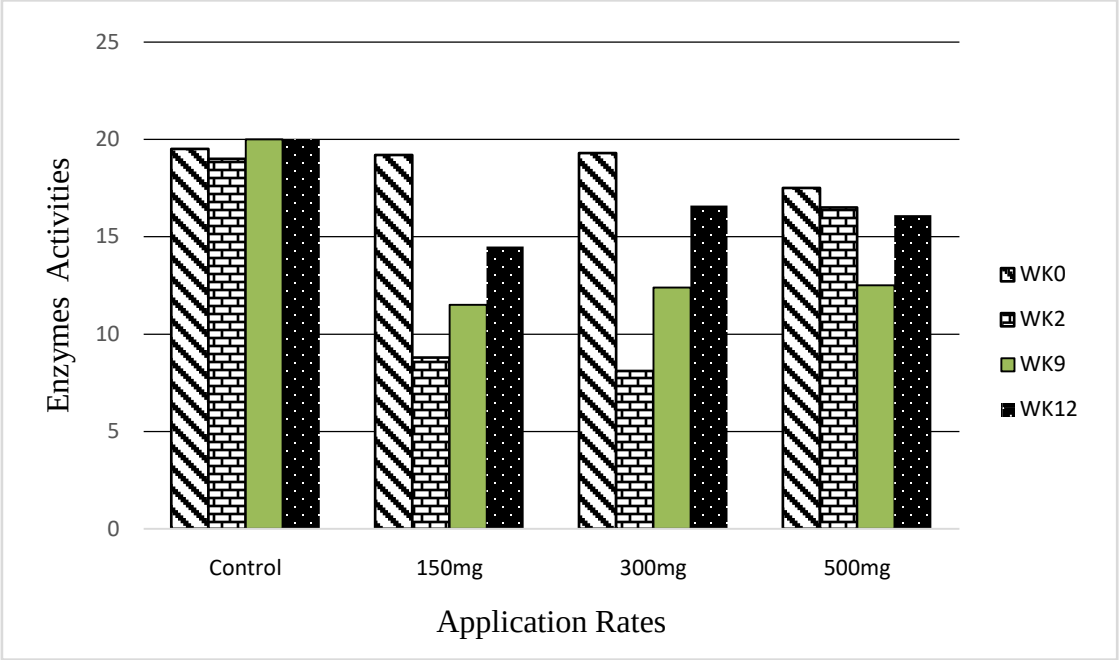

Fig. 1: Effect of Lead (Pb) on urease activity

On day zero, the mean values of urease activity were in all amended soils significantly ($P<0.05$) lower than those in control. The lowest urease activity (90.48%) of the control was found in the treatment 300ml/kg soil at two weeks. Alkaline phosphatase (ALP) activity was also significantly ($P=.05$) affected by the heavy metal (Lead). The values of this enzyme were lower in all the soil amended with lead compared to the control. The reduction was clearlyat two weeks of incubated (28.33%) of the control. Similarly, the activity of Acid phosphatase (ACP) in the lead amended soil decreased with increasing Pb levels (Fig. 2). The reduction was more pronounced at two weeks of incubation and 500ml/kg of soil (6.31%) treatment.

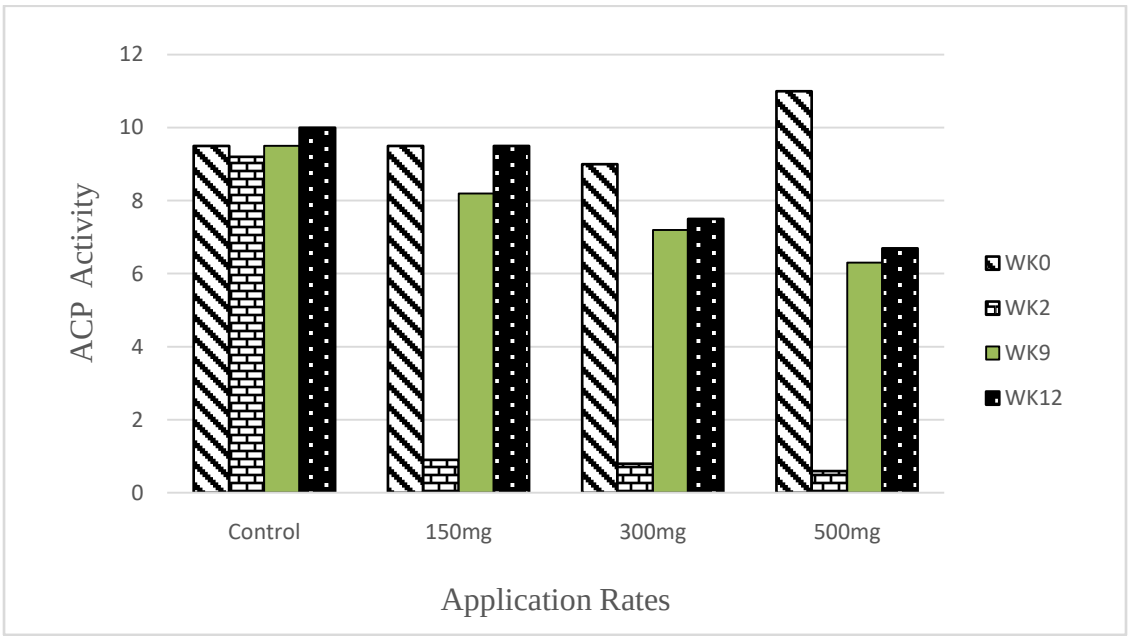

Fig. 2: Effect of Lead (Pb) on Acid Phosphatase activity

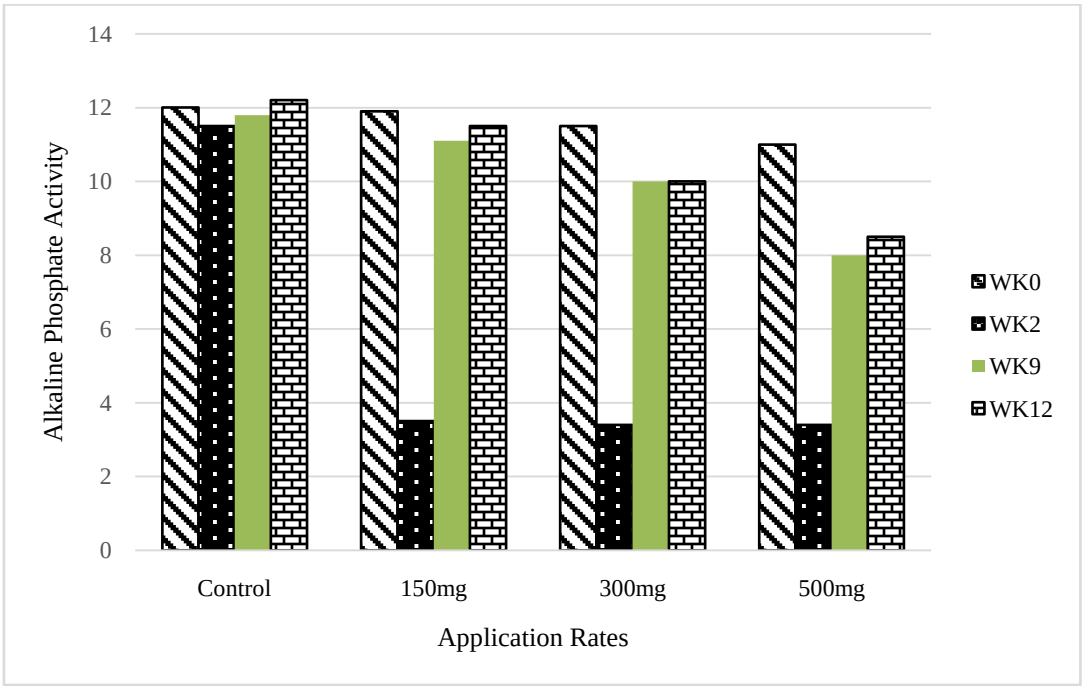

Fig. 3: Effect of Lead (Pb) on Alkaline phosphatase activity

The number of bacteria and Actinomycetes were significantly (P=.05) depressed in the heavy metal amended samples compared to the control (Fig. 4-6). The highest reduction (66.25% and 70.0%) respectively for bacteria and actinomycetes at 12 weeks with 500mg/kg application rate, while fungal cells were not significantly (P<0.05) decreased in all the amended soils compared to the control.

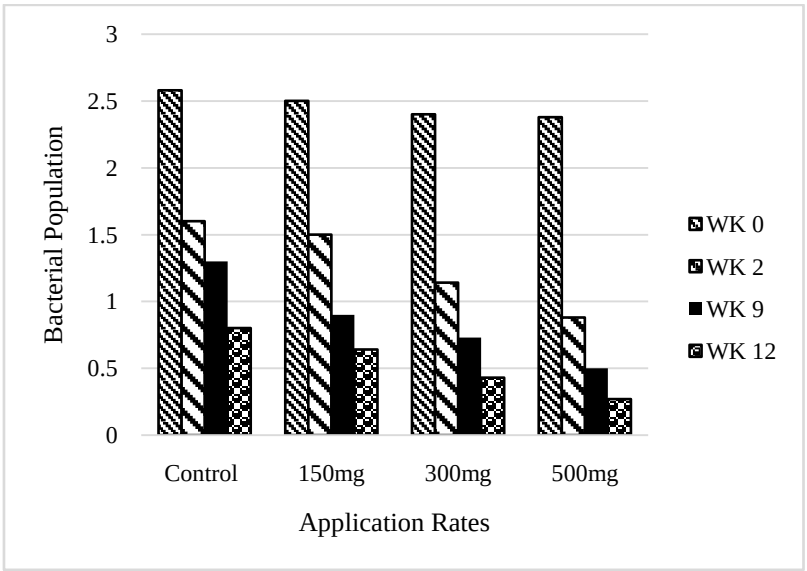

Fig. 4: Effect of Lead (Pb) on bacterial population

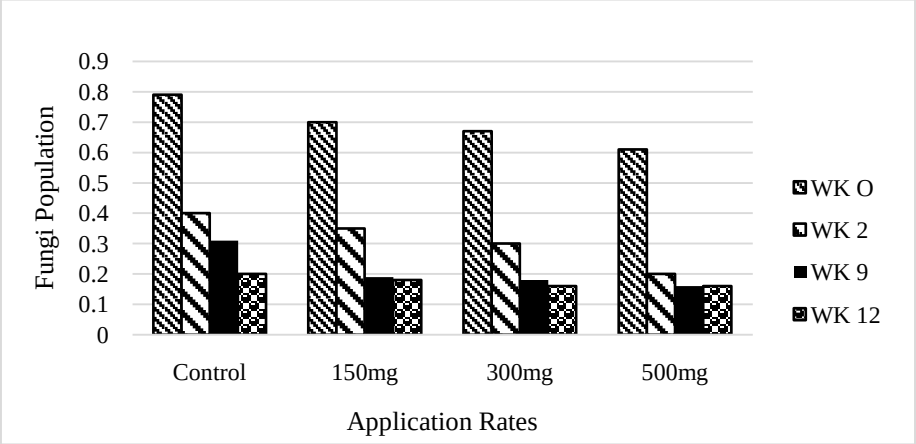

Fig. 5: Effect of Lead (Pb) on fungi population

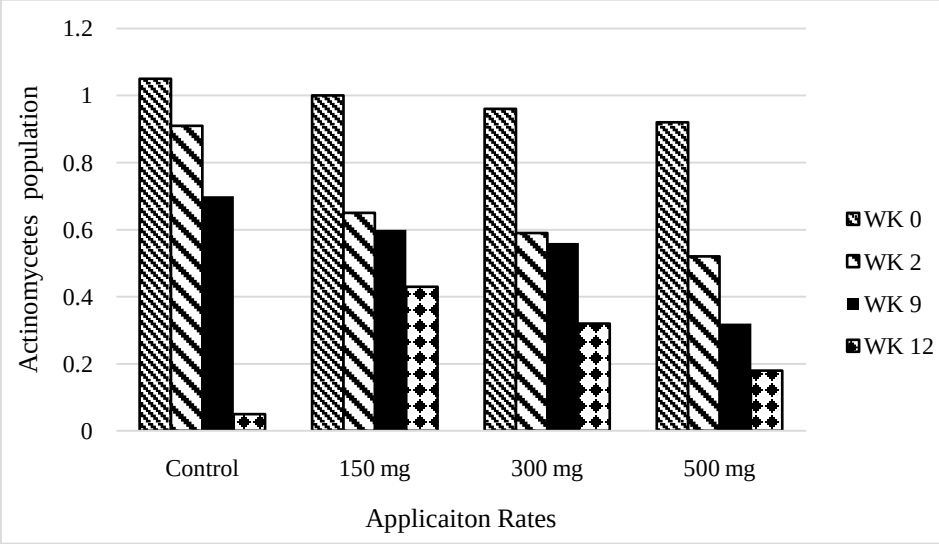

Fig. 6: Effect of Lead (Pb) on Actinomycetes population

IV. Discussion

In the soil environment, almost all reactions are catalyzed by enzymes that are largely of microbial origin and associated with viable cells (Dick *et al*., 1996). Urease enzyme is responsible for the hydrolysis of urea fertilizer applied to the soil into NH_3 and CO_2 with the concomitant rise in soil pH (Andrews *et al*., 1998; Bymes and Amberger, 1989). This in turn results in rapid N loss to the atmosphere through NH_3 volatilization (Fillery*et al*., 1984).

Soil urease originates mainly from plants (Mulvaney and Bremner, 1981). The increased inhibition of soil urease activity is likely to be related to the depression in microbial activity which is known to synthesized urease. These findings corroborated with previous reports by Khan *et al*. (2007) who reported that decrease in enzyme activity could be related to the fact that microorganisms were suddenly exposed to heavy metals. Soil phosphatases are important in soil P-cycling involving in mineralization of organic P and releasing phosphate for plants (Dick and Tabatabai, 1983; Gil-Sotres *et al*., 2005). In this study, both Alp and Acp activities were significantly inhibited with increasing concentration of Pb amendment. This inhibition may probably be due to the denaturing of the enzymes or may be due to the suppression of the microorganisms synthesizing the enzymes. These findings were in line with the studies conducted by Tyler (1974) and Kizikaja *et al*. (2004), independently stressed that enzymatic activities diminished with increasing available concentrations of heavy metals.

The rapid inhibition in enzymatic activities were found in the two weeks of incubation which should be related to the fact that the microorganisms were suddenly exposed to heavy metals. It is well documented that heavy metals react with sulfhydryl groups of enzymes and inhibit and/or inactivate the enzymatic activities. Heavy metals could also indirectly affect soil enzymatic activities by altering the microbial community which synthesizes enzymes altering the microbial community which synthesizes enzymes

(Namnipieri, 1994; Kandeler*et al*., 2000). Acid phosphatase and alkaline phosphatase are synthesized by the root of plants and microorganisms, respectively (Izaguirre-Mayoral *et al*., 2002).

In the soil ecosystem, heavy metals exhibit toxicological effects on soil microbes, which lead to a decrease in their population and activities (Yao *et al*., 2003). The results of this study suggest that the total bioactivity, richness, and diversity of microorganisms decreased with increasing levels of heavy metal concentration, because microorganisms differ in their sensitivity to heavy metal toxicity. The reduction in number of these microbes (Bacteria, Actinomycetes, and fungi) may be because these microbes were suddenly exposed to high levels of heavy metal. Similarly, Akmal *et al*., (2005) also observed changes of microbial community structure in metal amended soils in microbial activity known to synthesize urease. These findings corroborated with previous reports by Khan *et al*., (2007). Soil phosphatases are important in soil P-cycling, involving in mineralization of organic P and releasing phosphate for plants (Dick and Tabatabai, 1983; Gil-sotres*et al*., 2005). In this study, both Alp and Acp activities were significantly inhibited with increasing concentration of lead (Pb) amendment. These findingswere in line with the studies conducted by Tyler (1974) and Kizikaya*et al*. (2004), that soil enzymatic activities diminished with increasing available concentrations of heavy metals. The rapid inhibition in enzymatic activities was found in the twoweeks incubation which should be related to the fact that the microorganisms were suddenly exposed to heavy metal. It is well documented that heavy metals react with sulfhydryl groups of enzymes and inhibit and inactivate the enzymatic activities. Heavy metals could also indirectly affect soil enzymatic activities by altering the microbial community which synthesizes enzymes (Namnipieri, 1994; Kandeler*et al*., 2000). Acid phosphatase and alkaline phosphatase are produced by the root of plants and microorganisms, respectively (Izaguirrer-Magoral*et al*., 2002). In the soil ecosystem, heavy metals exhibit toxicological effects on soil microbes, which lead to a decrease in the population and activities (Yao *et al*., 2003). The results of this study suggest that the total bioactivity, richness, and diversity of microorganisms decreased with increasing levels of heavy metal concentration because microorganisms differ in their sensitivity to heavy metal toxicity. The reduction in the number of these microbes (Bacteria, Actinomycetes, and fungi) may be because these microbes were suddenly exposed to high levels of heavy metal. Similarly, Akmal*et al*. (2005) also observed change in microbial community structure in metal amended soils.

V. Conclusion

The different application rates of 0, 150, 3000, and 500mg/g soil affected soil microbial community structure, and enzyme activities. The highest inhibitory effects on soil microbial and enzyme activities were significantly at two weeks of incubation.

References Références Referencias

1. Akmal M., Wang H. Z., Wu, J. J. (2005). Changes in enzymes activity, substrate utilization pattern and diversity of soil microbial communities under cadmium pollution *J. Environ. Sci.*, 17: 802-807.
2. Andrews R.K., Bakeley R.L. and Zermer B. (1989). Urease: A Ni (II) metallic enzyme. In: *The Biomorganic Chemistry of Nickel*ed. J. R. Lancaster. pp. 141-166. VCA Publishers, New York.
3. Bymes B.H, and Amberger A. (1989). Fate of broadcast urea in a flooded soil when treated with N-(n-butyl) thiophospherictriannitride a urease inhibitor. *Fertile. Res.* 18:221-231.
4. Chen C.L., Liao M., Huang C.Y. (2005). Effect of combined pollution by heavy metals on soil enzymatic activities in areas polluted by trailing from Pb-2n-Ag mine. *J. Environ Sci.*, 17: 637-640.
5. Cooper D.C., Neal A.L., Kukkadope R.K. Brewe O., Coby A., Picardal F.W. (2005). Effects of sediment iron mineral composition on microbially mediated changes in divalent metal speciation: Importance of ferrihydrite. *Geochim. Cosmochim, Acta.* 69: 1739-1754.
6. D'Ascoli R., Rao M. Adamo P., Renella G., Landi L., Rutigliano F.A., Terribile F., Gianfreda L. (2006). Impact of river over flowing on trace element contamination of volcanic soils in South Italy: Part II. Soil biological and biochemical properties in relation to trace element speciation. *Environ Pollut.* 144: 317-326.
7. de Mora A.P., Ortega-Calvo H., Gabrera F., Maderon E. (2005). Changes in enzyme activities and microbial after "in situ" remediation of a heavy metal-contaminated soil. *Appl. Soil Ecol.* 28: 125-137.
8. Diaz-Ravina M. and Baath E. (1996). Development of metal tolerance in soil bacterial communities exposed to experimentally increase metal levels. *Appl. Environ. Microbiol*. 62: 2970-2977.
9. Dick R.P., Breakwekk, D. P., Turco R.F. (1996). Soil enzyme activities and biodiversity measurements and integrative microbial indicators (M): In methods of assessing soil quality, Madison, WI: Soil Science Society of America Publication 49, 247-271.
10. Dick W. A. and Tabatabai M. A. (1983). Activation of soil pyrophosphatase by metal ions. *J. Soil Biol. Biochem*, 15: 359-363.
11. Doehman P., Haanstra L. (1984). Short term and long term effects of cadmium, chromium, copper,

nicke, lead and zinc on soil microbial respiration in relations to abiotic soil factors. *J. Plant Soil*, 79: 317-327.
12. Effron D. de la Horra A.M., Defrieri R.L., Fontamive V., Palma P.M. (2004). Effect of cadmium, copper and lead on different enzyme activities in a native forest soil, *Comm Soil Sci. Plant Anal.* 35: 1309-1321.
13. Eick M.J., Peak J.D., Brady P.V., Peek J.D. (1999). Kinetic of Lead absorption/desorption on goethite residence time effect. *Soil Sci.* 164: 28-39.
14. Eivazi F. and Tabatabai M.A. (1977). Phosphatases in soil. *Soil Biol. Biochem.*, 9: 167-172.
15. Fillery R.P. De Datta S.K. and Crasweil E.T. (1980). Effect of phenyl phosphorous dimidiate or the fate of urea applied to wetland rice field. *Fert. Res.* 9: 251-263.
16. Gil-Sottre F., Trasar-Opeda C. and Leirros M. C. (2005). Different approaches to evaluate soil quality using biochemical properties. *J. Biol. Biochem*. 37: 877-887.
17. Gu, Y., P. Wang and Kong C.H (2009). Urease, invertase, dehydrogenase and polphenoloxidaze activities in Paddy soil influenced by allelopathic rice variety. *European J. Soil Biol.* 30: 1-6.
18. Halt J.G., N.R. Kreg, P.H.A. Sneath, J.T. Staley and S.T. Williams (1999). Bergey's manual of determinative Bacteriology. 9th edition, Baltimore U.S.A.: William and Wilkins Publishers.
19. Hamilton-Taylor J., Smith E.J., Davidson W., Sugiyama M. (2005). Resolving and modeling the effects of Fe and Mn redox cycling on trace metal behavior in a seasonally anoxic lake. *Geochim. Cosmochim, Acta.* 69: 1947-1960.
20. Hemida S. K., Omar S. A., Abdel-Mallek A. Y. (1997). Microbial populations and enzyme activity in soil treated with heavy metals. *Water Air Soil Pollut.* 95: 13-22.
21. Hinojosa M. B., Carreira J. A. and Garela-Rulz R. (2004). Soil moisture pretreatment effects on enzyme activities as indicators of heavy metal-contaminated and reclaimed soils. *J. Soil Biol. Biochem*, 36: 1559-1568.
22. Huang Q., Shimda H. (2000). Effects of copper on the activity and kinetics of free and immobilized acid phosphatase. *Soil Bio. Biochem.* 32: 1885-1892.
23. Huerta-Diaz M. D., Morse J. W. (1992), pyritization of trace metals in anocix marine sediments/ *Geochim. Cosmochim. Acta.* 56: 2681-2702.
24. Isaguirre-Mayoral M.L., Flores S., Carbailo O. (2002). Determination of acid phosphatases and dehydrogenase activities in the rhizosphere of modulated legume species native to two contrasting savannah sites in Venezuela. *Boil. Fert. Soils* 35: 470-472.
25. Kandeler E., Tcheriko D., Bruce K. D. (2000). Structure and function of the soil microbial community in microhabitats of a heavy metal polluted soil. *J. Bio. Fert. Soils*, 32: 390-40.
26. Kandeler E., Tscherko D. and Bruce K. D. (2000). Structure and function of the soil microbial community in microhabitation of a heavy metal polluted soil. *Boil. Fert. Soils*, 32: 390-400.
27. Khan S., Cao Q., Hesham AEL, Xia Y, he J (2007). Soil enzymatic activities and microbial community structure with different application rates of Cd and Pb. *J. Environ Sci.* 19: 834-840.
28. Kizikaya R., Askon T. and Bayrauch B. (2004). Microbiological characteristic of soils contaminated with heavy metals. *Eur. J. Soil Biol*, 40: 95-102.
29. Kumito T., Saeki K., Goto, S., Hayashi H., Oyaizu H., Matsumoto S. (2001). Copper and zinc fractions affecting microorganisms in long-term sludge-amended soils. *Bioresour Technol.* 79: 135-146.
30. Megharaj K.V.M., Sethunathan N., Nadu R. (2003). Bioavailability and toxicity of cadmium to microorganisms and their activities in soil: a review, *J. Adv. Environ. Res.* 8: 121-135.
31. Mulvancy, R. L. and Bremner J. M. (1981). Control of urea transformation in soils. In: Soil Biochemistry Vol. 5 (Paul, E. A., Ladd J. N. Eds) pp. 153-196. Marcel Dekker, New York.
32. Namnipieri P. (1994). The potential use of soil enzymes as indicators of productivity, sustainability and pollution. In: Soil biota management in sustainable farming systems (Pankhure C. E., Double B. M., Gapta V. V. S. R., Grace P. R. eds). Victoria, Australia: CSTRO; East Melborun, 238-244.
33. Peters R.W. and Shem L. (1992). Adsorption/ desorption characteristics of Lead on various types of soil. Environ. Progress. 11: 234-240.
34. Pitchtel J.K., Kuroiwa and A. Sawyerr (2000). Distribution of Pb, Cd and Ba in soils and plant of two contaminated sites. *Environ. Pollut.* 110: 171-178.
35. Polacco J.C. (1977). Is nickel a universal component of plant urease? *Plant. Sci. Lett.* 10: 249-255.
36. Renella G., Mench M. Gelsomino A. (2001). Functional activity and microbial community structure in soils amended with bimetallic sludge. *J. Soil Biol. Biochem*., 37: 1498-1506.
37. Tabatabai M.A. and Bremner I.M. (1969). Use of P-nitrophenyle phosphate for assay of soil phosphatases activity. *Soil Biol. Biochem* 1: 301-307.
38. Zeng L.S., Liao M., Chen C.L., Huang C.Y. (2007). Effects of Lead contamination on soil enzymatic activities, microbial biomass and rice physiological indices in soil-lead-rice (Oryza sativa L.) system. *Ecotoxicol Environ Saf* 67: 67-74.