CEDTECH International Journal of Science & Advancement in Bioconservation
Volume 5, Number 2, June 2024
http://www.cedtechjournals.org
ISSN: 2756-4614

EFFECT OF CADMIUM (Cd) POLLUTION ON MICROBIAL POPULATION AND ENZYME ACTIVITIES IN SOILS.

Godwin U. Apkan
Department of Soil Science and Land Resources Management,
University of Uyo, Uyo, P.M.B 1017 Uyo, Nigeria
Email: agumoren1@yahoo.com

ABSTRACT

Heavy metal contamination of soil occurs when heavy metal are introduced into the soil through human activities, leading to the gradual deterioration of the ecology and environment. The experiment to determine the effect of cadmium on microbial community and enzyme activities was carried out in the Department of Soil Science and land Resources Management, University of Uyo in 2022. Soil sample was collected from agricultural soil with unknown contamination history at the depth of 0 – 20 cm. The soil was contaminated with cadmium (Cd) using $CdSO_4$ solution at different application rates of (0, 150, 300, and 500mg, per 500gr soil). The amended soils (in pots) were incubated in the screen house with 60% of water holding capacity for 12 weeks. During the incubation period, samples were collected from each pot at 0, 2, 9, and 12 weeks for microbial and enzyme assays. The results showed that heavy metal (Cd) slightly inhibited bacterial and fungal populations in all the samples spiked with cadmium. Actinomycetes population was significantly inhibited by Cd. The extent of inhibition increased with increasing level of Cd concentrations, and varied with the incubation periods. Soil enzymatic activities in all the samples treated with Cd were also significantly inhibited. The level of inhibition increased significantly with increasing level of Cd concentration, and also varied with the incubation periods. Urease activity was heavily inhibited than cellulose activity.

Keynotes: *Cadmium, Microbial Population Enzyme Activities*

INTRODUCTION

Heavy metals such as Fe, Mn, Cd, Hg, and Co are introduced into the soil on a large scale through anthropogenic sources such as municipal solid waste, mining activities, use of fertilizers and pesticides in agricultural and industrial emission of waste water. Soil pollution is said to occur when the concentration of these heavy metals become significantly higher than that of the background, and therefore gradually deteriorating the ecology and

the environment (Chen 1996). Among the heavy metals, Cd pollution is the most serious. It reduces the biological activities of soil microorganisms and this affect crop yield and quality. Heavy metals like Arsenic, Cadmium, Lead, Mercury, and Chromium are common in the environment (He *et al., 2015*). They reported that these heavy metals are the most persistent pollutants of soil and water. Some of the heavy metals are not harmful as highlighted by He *et al.* (2015). They grouped heavy metals into essentials and non essentials, for example Manganese, Iron, Nickel, and Zinc are required for growth development and physiological functions of living organisms, non essential heavy metals like Cadmium, Lead, Mercury, and Arsenic are harmful, with no beneficial physiological functions. Abundance of heavy metals in soil cause reduction in food quality, adversely affect enzymatic and microbial activities, while ultimately affecting food security due to degradation (Kools *et al.,* 2005). It also lead to the contamination of surface and ground water (Hashim *et al.,* 2011; Moheenkumar *et al.,* 2016). Further, Cd is difficult to degrade and remain in the soil for a long period, eventually accumulating in the soil or plant edible parts and then poisoning animals, and humans through the food chain (Gon Liong 1992; Nioga and Pacuyrice 1998). Excessive intake of Cd can lead to prostate cancer in men, kidney cancer and other diseases (Yamagateen and Shigematsa 1976; Fan *et al.,* 2005). Microorganisms are important components of soil, their activities reflect the intensity and trend of various biochemical reactions (Tu, 1980). Changes in microbial community structure and diversity serves as an important biological indicator for evaluating the quality and status of soil (Emily *et al.,* 2009). Also soil enzymes react rapidly to the changes in the soil solution due to change in soil management practice or environmental physiochemical conditions.

This feature makes them acts as sensor for soil microbial status, soil physiochemical conditions, and for the influence of treatment on soil fertility (Cherykumali *et al.,* 2017). However, microorganisms and enzymes cannot perform these beneficial functions in the soil which quarantees optimum soil health and productivity of the soil is compromised due to pollution based on these facts, this research was therefore designed to investigate the effect of heavy metal (Cd) on soil microbial community structure and enzyme activities in soil.

MATERIALS AND METHOD

Study Area

The study was carried out at the University of Uyo teaching and Research Farm, Uyo, Akwa Ibom State, Nigeria. The area is within Latitude 4° 32 and 5° 44 N and Longitude 7° 35 and 8° 25 E. it is with the humid tropical rainforest zone.

Soil Sample Collection

Soil samples were collected using a hand trowel at a depth of 0 – 15 cm. 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 (Cadmium), while the other half used for control.

EXPERIMENTAL DETAILS

The experiment was a pot experiment in a screen house. The wet soil samples (500g) were added with Cadmium (Cd) using $Cd(SO_4)_2$ 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 (P_1) was the control, Pot 2 (P_2) was thoroughly mixed with 150 mg/kg mixed with 300 mg/kg and Pot 4 (P_4) were mixed with 500 mg/kg soil. The contamination soil samples with $Cd(SO_4)_2$ were incubated in the screen house for 12 weeks. During the incubation period, soil moisture contents were monitored by weighing adjusting to 60% water holding capacity by deionized water. Samples from each were collected at 0, 2, 9, and 12 weeks for enzymes and microbial community assay.

MICROBIAL ASSAY

Samples from each treatment of (Cd_0, Cd_1, Cd_2, and Cd_3) representing control (Cd_0), (Cd_1) 150 mg/500g (Cd_2) 300mg/500g, (Cd_3) 500mg/500g soil. Samples were enumerated by making ten-fold dilution of the soil samples from 10^{-1}-10^{-3} an aliquot of o.1ml from the 10^{-3} dilution was transferred unto plate in nutrient agar amended with mystatin (0.5 mg/ml) for isolation of bacteria, with potato dextrose agar amended with streptomycin (0.021 mg/ml) was used for the isolation of fungi and glycerol agar was used for isolation of Actinomycete. The different cultures were incubated at different temperatures and times required for optimum growth of the microorganisms. Bacteria and actinomycetes were incubated at a temperature 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 incubating visible colonies were counted and the microbial load determined using the formula **Microbial load = no. of colonies x reciprocal of dilution factor,** and expressed as (CGU/g soil). Isolated colonies were further purified by sub culturing and identification using biochemical tests and microscopy.

IDENTIFICATION OF ISOLATES

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

Determination of Enzymes Activities

Determination of Urease

Urease activity was determined by the method described by Gu *et al.,* (2009). Briefly two grams (2g) of the moist soil sample from each pot containing 0mg, 150mg, 300mg, and 500mg of Cd weighed inside four 500ml Erlemeyer flasks, and 2ml of toluence 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 hypochlorite were added to all the Erlemeyer 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 calorimeter at the wavelength of 430 nm and the result recorded as NH_4– N/g soil.

Determination of Cellulase

Cellulase was determined by the method described by Pancholy and Rice (1973). One (1 ml) milliliter toluence was added to 10 g moist soil sample in 100 ml Erlemeyer flask and allowed to stand for 15 minutes. Then 20 ml 0.5 M acetate buffer (pH 5.9), 2% 10 ml carboxymethyl cellulose (CMC), 20 ml 5 % sucrose were added to each sample from each pot containing 0 mg, 150 mg, 300 mg, and 500 mg of Cd. The samples were then incubated for 24 hours followed by centrifugation at 400 rpm for 20 minutes. The soil mixture was then filtered through Whatman No 41 filter paper and the aliquot analyzed for reducing sugars using calorimeter at wavelength of 578 nm. The result was recorded as mg reducing sugars per gram of soil.

RESULTS AND DISCUSSION

The mean values of bacterial population were lower in Cd amended samples than those in the control (Fig. 1). The inhibition rate increased with increasing Cd concentrations. The inhibition extent was also obvious between different incubation periods. The lowest mean value of bacterial population (37.06 ± 6.11 cfu/g compared to that of the control) was found in the treatment of 500 mg/ 500 g soil at 2 weeks incubation period. In the Cd treated soil, the bacterial population had a reduction ranging from 67.466 ± 2.58 to 37.06 ± 6.11 cfu/g and the highest inhibition rate from 65.00 ± 4.35 cfu/g to 43. 96 ± 7.42 cfu/g in 2 weeks, 70. 000 ± 1.37 cfu/g to 48.13 cfu/g in 9 weeks and 47.97% was observed in 500 mg/500 g soil treatment. The rapid inhibition of bacterial community was found in the 2 weeks incubation which should be related to the fact that the bacterial community were suddenly exposed to Cadmium in the soil. Similarly, Baath *et al.* (1998) has also reported that the microbial community structure changed in soil amended with Zn (359 mg/kg) and Ni (89 mg/kg) rich sludge. Akmal *et al.* (2005) has also observed the changes in microbial community structure in metal amended soils. Fungal activity fig. 2 were significantly lower ($P<0.05$) in the Cd amended samples than those in the control.

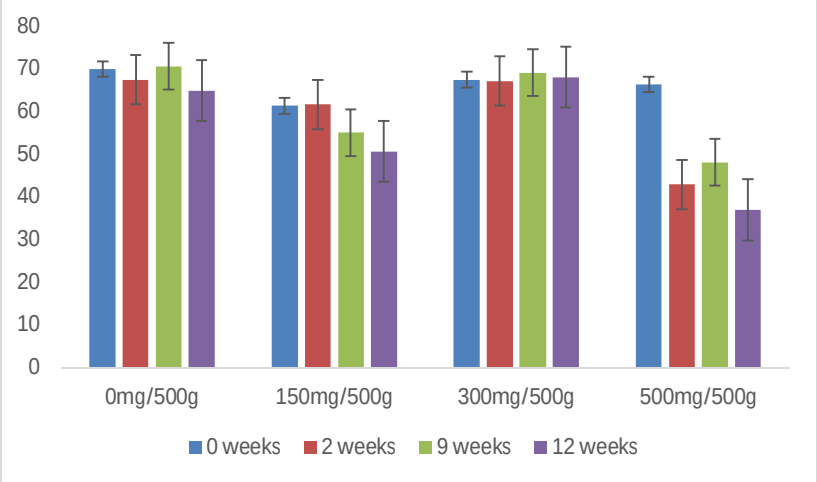

Fig 1: Effect of Cd on Heterotrophic bacteria count

The observations were obvious in 150, 300 and 500 mg/g soil and 2, 9, and 12 weeks incubation periods. In soil ecosystem, heavy metals exhibit toxicological effects on soil microbes which may lead to the decrease of their numbers and activities. This study demonstrated that toxicological

effects of heavy metals on soil fungal community structure and activity depend largely on the type and concentration of metal and incubation time.

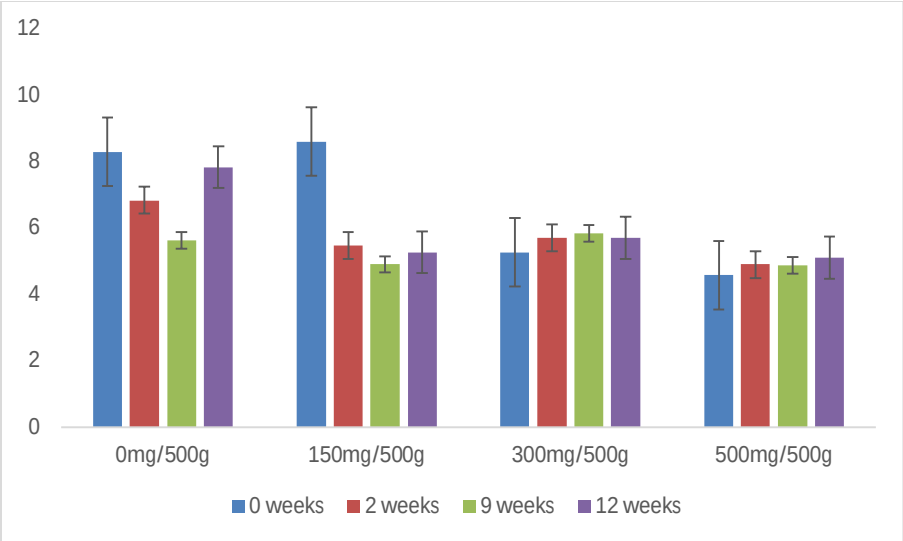

Fig 2: Effect of Cd on Total Fungal count

The incubation extent varies widely among different incubation periods for these fungi. Actinomycetes population were significantly ($P<0.05$) depressed more than bacteria and fungi, mostly on the 2 weeks and 12 weeks of incubation periods (Fig. 3), at the 150 mg, 300 mg, and 500 mg Cadmium concentrations. The rate of inhibition of actinomycetes in 2 weeks of incubation were 91.70% to 92.11% of the controls and in 9 weeks of the incubation, the rate of inhibition were between 43.66%, to 69.57% of the control, whereas in the 12 weeks of incubation, the rate of inhibition ranged from 857.55% to 4630.23% of control. The high depression of actinomycetes observed in this study may be because organic residues added to the soil are usually attacked by bacteria and fungi and later by actinomycetes, because they are slow in activity and growth than bacteria and fungi; and are said to be very sensitive to acidity/low pH.

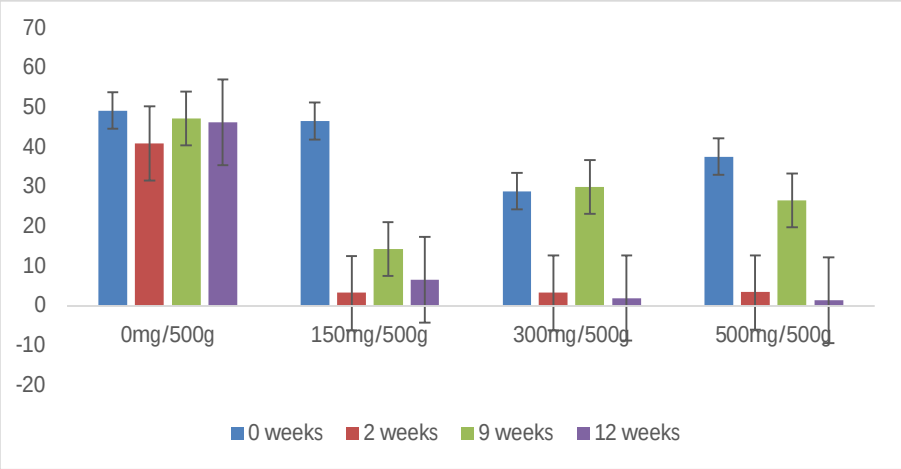

Fig 3: Effect of Cd on Total Heteviliophic actinomycetes count

In the soil environment, almost all reaction are catalyzed by enzymes that are largely of microbial origin and associated with viable cells. The mean values of Urease activity were significantly ($P<0.05$) lower in all the treatments (150 mg, 300 mg, and 500 mg) Cadmium concentrations than those in the control (Fig. 4). The Urease inhibition rate increased with increasing Cd concentrations. The reduction extent was also obvious between different incubation periods. In this study, Cadmium contaminated soil caused the reduction of Urease activity from 73.78% to 80.33%, and highest depression rate was observed in soil treated with 500 mg Cadmuim. Soil Urease originates mainly from plants (Polacco, 1977), and microorganisms found as both intra an extra-cellular enzymes (Mulvancy and Bremner, 1981). The reasons for the significant reduction of Urease activity in this study may be due to several factors such as organic matter content of the soil, soil depth, soil amendments, heavy metals, and environmental factors like temperature. These observations were in line with the reports (Tabatabai, 1977; Bremner and Mulvaney, 1978).

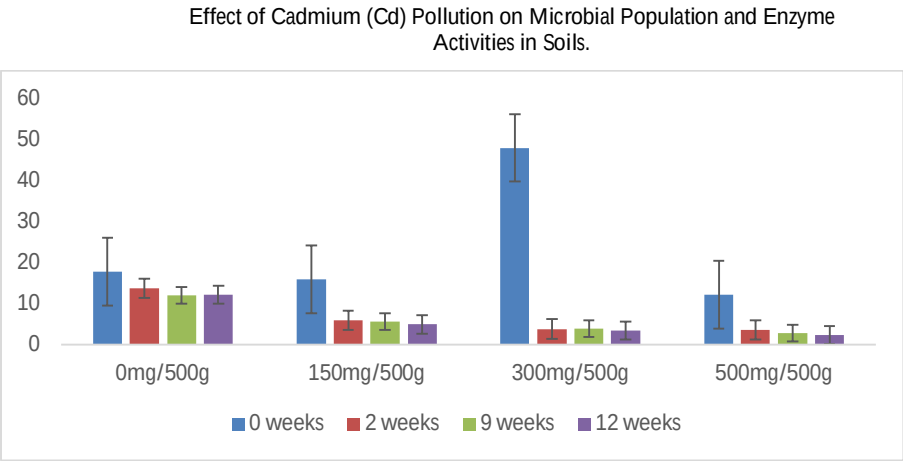

Fig 4: Effect of Cd on Urease activities

The mean values of cellulase activity also decreased significantly ($P<0.05$) with increasing levels of Cd concentrations in the soil (Fig. 5). The inhibition rate of cellulase varies in samples with different incubation periods. High depression of cellulase was observed in all the treated samples. Cellulase inhibition rate ranged from 63.13% to 77.71% in 2 weeks, from 55.38% to 75.10% in 9 weeks and from 61.83% to 79.83% in 12 weeks, respectively than those in the control. The highest inhibition effect of heavy metal on soil enzyme (cellulase) activity may be due to sudden exposure of the microbes to Cadmium in the first two weeks, and so resulted in a significant decrease in the enzyme activity. Heavy metal could also indirectly affect soil enzymatic activities by altering the microbial community which synthesizes enzymes as observed in this study in the case of bacteria, fungi, and actinomycetes, which was the most hit. Studies have shown that activities of cellulase in agricultural soils are affected by several factors. These include temperature, soil pH, water, and abiotic condition: (Rubidge, 1977; Tabatabai, 1982), and the trace elements from fungicides (Deng and Tabatabai, 1994).

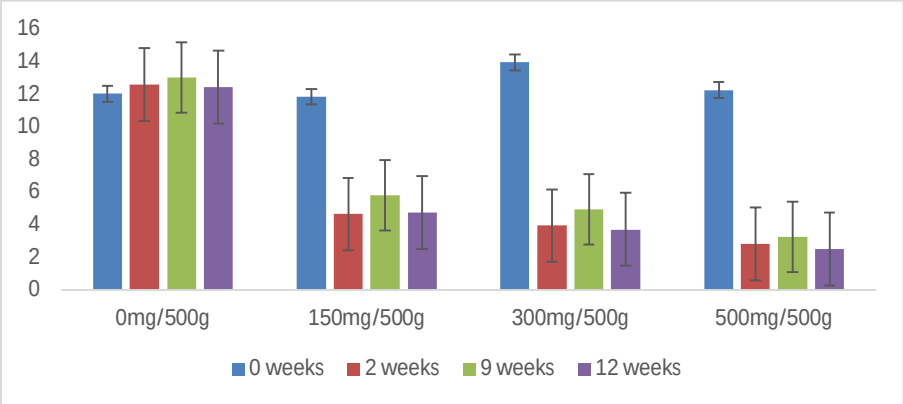

Fig 5: Effect of Cd on Cellulase activities

CONCLUSION

In this study, I can conclude that the heavy metal (Cadmium) had strong negative impact on soil microbial community structure. The highest inhibitory effect was observed in soil contaminated with 150 mg/500g soil and 500 mg/500g soil at the initial 2 weeks of incubation and also at 12 weeks of incubation periods. Actinomycetes populations were significantly decreased in all the treatments. Enzymatic activities were also inhibited across all the treatments and incubation periods. It was also observed that the heavy metal (Cadmium) had strong inhibitory effect on urease and cellulose activities, notably in 150 mg, 300 mg, and 500 mg/500g soil and across incubation periods. The highest inhibitory effect of Cadmium on soil urease and cellulose activities was on 2 weeks and 12 weeks incubation periods.

REFERENCES

Akmal M., Wang H. Z. and Wu J. J (2005) Chengen in enzymes activities, substrate utilization pattern and diversity of soil microbial communities under cadmium pollution. (J). *J. Environ. Sci.* 17 : 802 – 807

Baath E., Diaz – Ravina M. and Frostegard A. (1998). Effect of metal – rich sluge amendments on the soil microbial community (J). *Appl. Environ. Microbial*, 64 : 234 – 245

Bremner j. M. and Mulvaney R. L (1978). Urease activity in soils. *Ini. Soil Enzymes (Bum's R. G Ed.)* Pp 149 – 196 Academic press, London.

Chen H. (1996). Heavy metal pollution in soil plant system (in Chinese) MJ. *Peking Science Press* 71. 95

Cherukumali S. R (2017). Hydrolutic enzymes of some periphytic marine bacteria. *Canadian J. of Microbiology* 18 : 1483 – 1490

Deng S. P. and Tabatabai M. A. (1994). Cellulose activity of soils. *Soil Biol. Biochem.* 26 : 1347 - 1354

Emily E., Austin A. C, Side K. E., Hector F. C., Schadt C. w, and Clasen A. T. (2009). Assessment of 10 years of CO_2 fumigation on soil microbial communities and function in a sweet gum plantation. *Soil Biol. Biochem.* 41 : 514 – 520

Fan D. E., Wu X. M. and Chen B. (2005). Discussion on remediation methods of cadmium pollution in soil poisonous elements. *J. Geology* 29 (1) : 32 – 36

Gaon Liang (1992). Soil pollution and its prevention measures. *Agric. Environ. Protection.* 11 (6) : 272 – 276

Gu, Y. P., Wang and Kong C. H. (2009). Urease, invertase, dehydrogenase and polphenoloxidase activities in paddy soil influenced by allelopathic rice variety. European *J. Soil Biol.* 30 : 1 – 6

Hashim M. A., Mukhopadhyay S., Saha J . N. and Sengupta B. (2011). Remediation techniques for heavy metal contaminated ground water. *J. Environmental Management.* 92 (10) : 2355 – 88

He, Z., Shentu J., Yang X., Baligan, V.C, Zand T. and Stoffella F. J (2015). Heavy metal contamination of soils. Sources, indicators and assessment. *J. Environmental Indicators* 9 : 17 – 18

Holt J. G., N. R. Kreg, P. H. A. Shealth J. T. Staley and S. T. Williams (1999). Bergey's manual of determinative Bacteriology. 9th edition, Balthimore U S A. Williams and Williams publishers

Kools S. A. E, Van Roovert M., Van Gester C. A. M., and Van Straalen N. M. (2005). Glyphosphate degradation as a soil health indicator for heavy metals polluted soils. *Soil Biol. Biochem.* 37 (7) : 1303 – 7.

Mohankumar K., Hariharan V. and Rao N. P. (2016). Heavy metals contamination in ground water around industrial estate vs residential areas in coimbatore. *Indian J. Clinical Diagnosis. Sio.* (4) Ec 05 – 7

Mulvaney R. L., and Bremner J. M. (1981). Control of Urea transformation in soils. *Ini. Soil Biochemistry, Vol. 5 (Paul E. A, Ladd J N Eds)* Pp 153 – 196. Marcel Dekker, New York.

Polacco J. C. (1977). Is nickel a universal component of plant ureases?. *Plant Sci. Lett.* 10 : 249 – 255

Rubidge T. (1977). The effect of moisture content and incubation temperature upon the potential cellulose activity of John Innes no. 1 soil (ISSN. 0020 – 6164). *Int. Biodeterior. Bul.* 13 : 39 – 44

Tabatabai M. A (1982). Sulphur in methods of soil analysis. Part II. Chemical and microbiological properties (Page A. L., Miller R. H and P. S Keeney DR. Eds). *Soil Sci. Soc. Am. Madison,* Pp 501 – 538

Tabatabai. (1977). Effect of trace elements on urease activity in soils. *Soil Bio. Biochem.* 9 : 9 – 13

Tu C. M. (1980). Influence of five pyrethoid insecticides on microbial population and activity in soil. *Microbial Ecolo.* 5: 321 – 327

Yamagatan and Shigematsu I. (1970). *Cadmium Pollution in Perspective Bull. Inst. Public Health.* 19 (1) : 1 – 27