

Effect of Subchronic Exposure of Heavy metal Pollution on Soil Physicochemical Properties, Catalase and Dehydrogenase Activities.

Abstract

The effect of subchronic exposure of heavy metal-pollution mercury (Hg), lead (Pb) and cadmium (Cd) at 100 $\mu\text{g}/\text{dm}^3$ concentration on soil physicochemical properties (pH, moisture, phosphate, chloride, sulphates, calcium carbonate, nitrogen, organic carbon, electrical conductivity as well as the activities of selected enzymes (catalase and dehydrogenase) was investigated after one hundred and twenty days. Soil sample without heavy metal pollution served as control. Results indicate that Hg, Pb and Cd at 100 $\mu\text{g}/\text{dm}^3$ concentration pollution caused a significant ($p < 0.05$) change in the soil pH and electrical conductivity relative to the control. There was no significant ($p < 0.05$) difference in the other soil physicochemical properties (moisture, phosphate, sulphate, chloride, calcium carbonate, total nitrogen and organic carbon) relative to the control. There was a significant ($p < 0.05$) decrease in catalase activity in all the metal-polluted soil samples in contrast to a significant ($p < 0.05$) increase observed in the dehydrogenase activity when compared to the control, indicating that these heavy metals increased soil acidity, electrical conductivity and alters soil enzyme activities at this concentration and period of exposure.

Keywords: Soil properties, pollution, heavy metals, enzyme activity.

1. INTRODUCTION

Most problems of soil pollution are associated with large amount of heavy metals deposited on it through disposed wastes, especially sewage sludge (Nouri, 1980; Singh et al., 2005). These metals which are often not biodegradable are accumulated in living organisms when released into the environment through the food chain. Apart from the contribution from anthropogenic sources and disposed wastes, soil naturally contain trace level of heavy metals (FEPA, 1999). Soil pollution by heavy metal have been investigated by various authors (Oviasogie and Omoruyi, 2007; Nwuche and Ugoji, 2008; Onweremadu et al., 2007;

Aiyesanmi, 2005) implicated large amount of anthropogenic inputs. This can have serious health implication especially with regards to crops/vegetables grown on such soils (Nwaogu et al., 2012a). Most of these heavy metals are necessary for growth and normal functions for both plants and animals at trace amount in which they constitute essential part of the metabolizing and / or detoxifying enzymes, but large amount of any of them may cause acute or chronic toxicity (WHO, 1996).

The most common problem causing cationic metals (metallic elements whose forms in soil are positively charged cations) are lead, mercury, cadmium, copper, zinc, chromium while the most common anionic compounds whose forms in soil are combined with oxygen and are negatively charged are arsenic, molybdenum, selenium and boron (USDA,2000).

Excess heavy metal accumulation in soil is toxic to humans, animals and micro organisms. Long term effect of heavy metal exposure to human and higher animals include lead that precipitates mental lapse, cadmium affects the kidneys and central nervous system.

Micro-organisms in the soil are responsible for nitrogen fixation, assimilation and degradation of organic matter to release nutrients (Baath, 1989). When heavy metals are retained in the soil by repeated and uncontrolled addition, through pollution, they interfere with microbial key biochemical processes, which alter biological balance. Toxic effects of heavy metals on micro organisms manifest in numerous ways such as decrease in litter decomposition and nitrogen fixation, less efficient nutrient cycling, impaired enzyme synthesis and activity in soil (Baath, 1989). These consequently affect the soil physicochemical properties, thereby affecting the plants that grow on it. Excessive concentration of heavy metals in plants can cause among other things oxidative stress (Nwaogu et al., 2012) and stomatal resistant (Shah et al., 2009). It can also affect photosynthesis and chlorophyll florescence processes (Monni et al., 2001). Lead reduces chlorophyll production, arsenic interferes with metabolic process, copper, inhibits photosynthesis and reproductive processes while zinc and tin affect the growth of leaves and shoots, thereby making plant growth limited or impossible (Ling et al., 2009; Ayari et al., 2000).

Dehydrogenases (EC.1.1.1.1) are enzymes which catalyse the removal of hydrogen atom from different metabolites (Nelson and Cox, 2005). Active dehydrogenases are considered to exist in the soil as an integral part of intact cells. They conduct a broad range of oxidative activities that are responsible for degradation of soil organic matter. Soil dehydrogenase

activity can reflect changes in the respiratory activity of a given population size in response to changes in the soil environment (Margesin et al., 2000). Catalase(EC.1.11.1.6) is an iron porphyrin enzyme which catalyses very rapid decomposition of hydrogen peroxide to water and oxygen (Nelson and Cox, 2005). Catalase is widely present in nature, which account for its diverse activities in soil. Catalase activity alongside with the dehydrogenase activity are used to give information on the microbial activities in soil. Both dehydrogenase and catalase activities are very sensitive to heavy metal pollution (Naplekovn and Bulavka, 1983; Perez and Gonzalez, 1987). Their values have been suggested to be used as a simple toxicity test (Roger and Li, 1985). The aim of this study was to determine the effect of subchronic exposure to mercury, lead and cadmium at $100 \mu\text{g}/\text{dm}^3$ concentration on soil physicochemical properties and the activities of catalase and dehydrogenase.

2. MATERIALS AND METHODS

2.1 Chemicals / Reagents

2, 3, 5-triphenyl tetrazolium chloride (TTC) was bought from Sigma-Aldrich Chemical Company St. Louis Mo, USA. Other chemicals used were from varied sources and of analytical grade.

2.2 Collection and preparation of soil samples.

Soil sample was collected at a depth of 0-2s0 cm from a garden farm of the Federal University of Technology Owerri, Nigeria in April, 2012. The soil sample was analyzed for the presence of these heavy metals before use, using Atomic Absorption Spectrophotometer (AAS). The sample was sieved using a 2mm (No 10 mesh) sieve. Coarse particles were removed and the fraction that passed through the 2mm sieve was the fine soil used for the experiment. One kilogram each of the sieved soil sample was weighed into four plastic pots and labeled A-D. 100 ml solutions containing $100 \mu\text{g}/\text{dm}^3$ of each heavy metal; mercury, lead and cadmium as nitrates of penta-hydrates were applied singly to the respective pots A-C, while D served as the control. Distilled water was applied periodically on the pots A-D to maintain soil moisture at constant level. These were allowed to stand for a period of one hundred and twenty days.

2.3 Soil Physicochemical Properties Analysis

Soil samples from pots A-D were air- dried, ground, and passed through a 2 mm sieve and stored in different labeled plastic containers and used for the various analyses. The soil samples for heavy metal analysis were digested according to the method as described by Ademoroti (1996). The digested samples were analyzed for these heavy metals using Atomic Absorption Spectrophotometer (AAS). Soil pH was measured in a soil-water ratio of 1:2 using a pH - meter according to Davey and Conyers (1988). Moisture content of the soil was measured gravimetrically by drying 50 g of soil samples at 105°C for 24 hours. Phosphate, sulphate, chloride and calcium carbonate of the soil samples will be determined according to the method described by Page et al, (1982). Total soil nitrogen was determined by the Kjeldahl digestion according to Bremner (1965). Total organic carbon was determined by the method described by Nelson and Somers (1982), while electrical conductivity was determined by the method of Chopro and Kanzar (1988). All the analyses were done in triplicates.

2.4 Assay for selective soil enzyme activities

Soil dehydrogenase activity was assayed using the method as described by Tabatabai (1982). Dehydrogenase convert 2, 3, 5-triphenyl tetrazolium chloride to formazan. The absorbance of formazan was read spectrophotometrically at 485 nm. One gram of sieved soil sample was placed in 15 test tubes (15 x 100 mm), mixed with 1ml of 3% aqueous v/w 2,3,5- triphenyl tetrazolium chloride and stirred with a glass rod. After 96 hrs of incubation at 27°C, 10ml of ethanol was added to each test tube and the suspension was vortexed for 30 seconds. The tubes were then incubated for 1hr to allow suspended soil to settle. The resulting supernatant (5ml) was carefully transferred to clean test tubes using Pasteur pipettes. Absorbance was read spectrophotometrically at 485 nm was evaluated using extinction coefficient at 15433 Mol cm⁻¹.

Catalase activity of the soil samples was assayed using the method as described by Cohen et al, (1970) where decomposed hydrogen peroxide was measured by reacting with excess potassium tetraoxomanganate (Vii) (KMnO₄) and residual KMnO₄ was measured spectrophotometrically at 480 nm.

2.5 STATISTICAL ANALYSIS

Data generated were expressed as mean $\pm$ SEM. All results were compared with respect to the control. Comparism between the concentrations of physicochemical properties, activities of selected soil enzymes and the control were made by using Analysis of Variance (ANOVA) and differences at $p < 0.05$ were considered as significant.

3.0 RESULTS

Table 1 shows the results of the physicochemical properties of the control soil, Hg-polluted soil, Pb-polluted soil and Cd-polluted soil samples respectively. The soil type used was loamy soil. The control soil sample has a pH of 7.26, moisture content of 2.44 %, phosphate of 46.50 $\mu\text{g}/\text{PO}_4/\text{kg}$, sulphate of 427.70 $\text{mg}/\text{SO}_4/\text{kg}$, chloride 50.00 mg/kg , calcium carbonate of 0.061 %, total nitrogen of 5.78 %, organic carbon of 1.57 % and electrical conductivity of 107 $\mu\text{s}/\text{cm}$. Hg-polluted soil sample has a pH of 4.80, moisture content of 2.26 %, phosphate 45.60 $\mu\text{g}/\text{PO}_4/\text{kg}$, sulphate of 450.19 $\text{mg}/\text{SO}_4/\text{kg}$, chloride of 45.60 mg/kg , calcium carbonate of 0.055 %, total nitrogen of 5.64%, organic carbon of 1.48% and electrical conductivity of 160 $\mu\text{s}/\text{cm}$. Pb-polluted soil sample has a pH of 4.20, moisture content of 2.45 %, phosphate of 46.10 $\mu\text{g}/\text{PO}_4/\text{kg}$, sulphate of 455.21 $\text{mg}/\text{SO}_4/\text{kg}$, chloride of 46.10 mg/kg , calcium carbonate of 0.048 %, total nitrogen of 5.56 %, organic carbon of 1.46 % and electrical conductivity of 170 $\mu\text{s}/\text{cm}$ while Cd-polluted soil sample has a pH of 4.10, moisture content of 2.30 %, phosphate of 45.80 $\mu\text{g}/\text{PO}_4/\text{kg}$, sulphate of 452.30 $\text{mg}/\text{SO}_4/\text{kg}$, chloride of 45.80 mg/kg , calcium carbonate of 0.052 %, total nitrogen of 5.68 %, organic carbon of 1.50 % and electrical conductivity of 168 $\mu\text{s}/\text{cm}$ respectively.

Table 2 presents the results of heavy metal concentrations in the soil samples studied. The control sample has the following metal concentrations in $\mu\text{g}/\text{kg}$: Zn (12.0), Cu (6.65), Ci 1.63), As (0.42), Hg (0.06), Pb (1.32), and Cd (0.24). Hg-polluted soil has Zn (12.0), Cu (6.65), Ni (1.65), As (0.41), Hg (0.26), Pb (1.32) and Cd (0.25). Pb polluted soil has Zn (12.0), Cu (6.64), Ni (1.65), As 0.42), Hg (0.06), Pb (2.39) and Cd (0.23) while Cd- polluted soil has Zn (12.0), Cu (6-65), Ni (1.65), As (0.43), Hg (0.06), Pb (1.23) and Cd (0.28) respectively indicating that natural soil contain traces of metals.

Figure 1 shows the activities of the two selected soil enzymes (dehydrogenase and catalase). There was a significant $p < 0.05$ difference in activity in the two enzymes. The figure shows

that the presence of these heavy metals affected the activities of these enzymes. For dehydrogenase, its activity increased while that of catalase decreased relative to the control.

4. DISCUSSION

The mean pH values of all the soil samples analysed indicate that the heavy metal-polluted soil samples had low pH values 4.10-4.30 relative to the control pH 7.20. This revealed that the addition of these metals at that concentration lowered the pH of the soil sample. Soil pH is an important soil property, having great effects on solute concentration and absorption in soil. High soil acidity creates chemical and biological conditions which may be harmful to plants and soil micro organisms. This observation agrees with the reports of Akpoveta et al, (2010) and Onweremadu et al, (2007), who reported that heavy metals from automobile service centres decreased soil pH thus increasing the soil acidity. There was no significance ($p < 0.05$) difference in the mean values of moisture contents, phosphate, sulphate, chloride, calcium carbonate, total nitrogen and organic carbon obtained for all the heavy metals-polluted soil samples relative to the control indicating that the soil samples contain almost the same proportion of these physicochemical parameters which in part, indicate that these heavy metals at this concentration did not have serious adverse effect on these soil properties. However, the results of electrical conductivity measurement from heavy metal-polluted soil samples was significantly ($p < 0.05$) higher than the control with mean values ranging from 160-168 $\mu\text{s}/\text{cm}$. This may be due to the increase in the concentration of some soluble salts in the soil samples polluted with these individual heavy metals. This observation corroborates with the reports of Osakwe and Otuya (2008); Oviasogie and Omoruyi (2007).

Table 1: Physicochemical Properties of Soil Samples from control, Hg, Pb and Cd – Pollution

Soil Properties	Control Sample	Hg- Polluted Soil Sample	Pb- Polluted Soil Sample	Cd – Polluted Soil sample
pH	7.26	4.80	4.20	4.10
Moisture Content (%)	2.44	2.26	2.45	2.30
Phosphate ($\mu\text{g}/\text{PO}_4/\text{kg}$)	46.50	45.60	46.10	45.80
Sulphate ($\text{Mg}/\text{SO}_4/\text{kg}$)	457.70	450.10	455.21	452.30
Chloride (mg/kg)	50.00	45.60	46.10	45.80
Calcium Carbonate (%)	0.061	0.055	0.048	0.052
Total Nitrogen (%)	5.78	5.64	5.56	5.68
Organic Carbon (%)	1.57	1.48	1.46	1.50
Electrical Conductivity ($\mu\text{s}/\text{cm}$)	107	160	170	168

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215 **Table 2: Heavy Metal Concentrations in Soil Samples**

Heavy Metals	Control	Hg- Polluted Soil Sample	Pb-Polluted Soil Sample	Cd – Polluted Soil Sample
Zn	12.0	12.0	12.0	12.0
Cu	6.65	6.65	6.64	6.65
Ni	1.63	1.65	1.65	1.65
As	0.42	0.41	0.42	0.43
Hg	0.06	0.26	0.06	10.06
Pb	1.32	1.32	2.39	1.23
Cd	0.24	0.25	6.23	6.28

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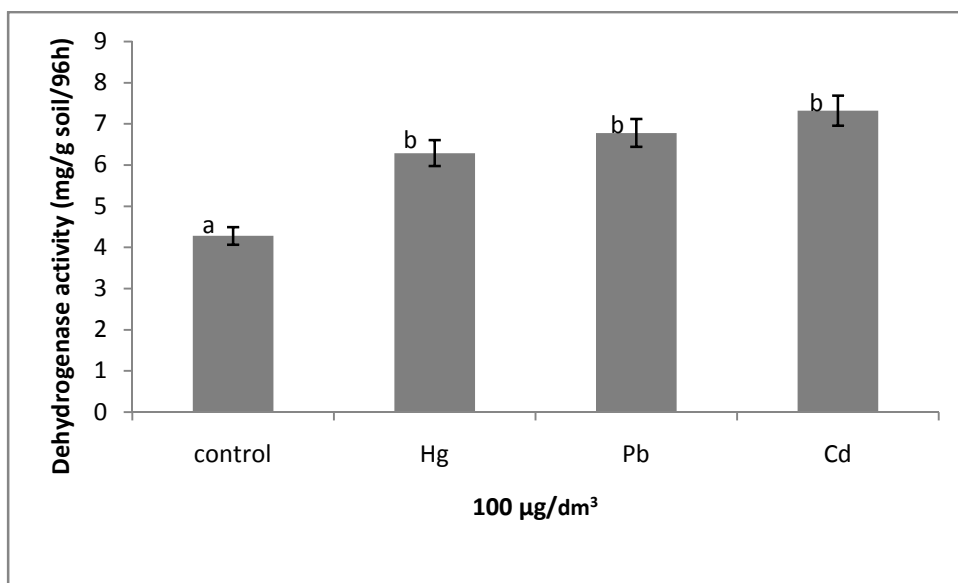


Fig 1: Effects of heavy metals ($100\mu\text{g}/\text{dm}^3$) concentration on soil dehydrogenase activity

Alphabets on the bars bearing the same superscript are not significantly different at ($p>0.05$)

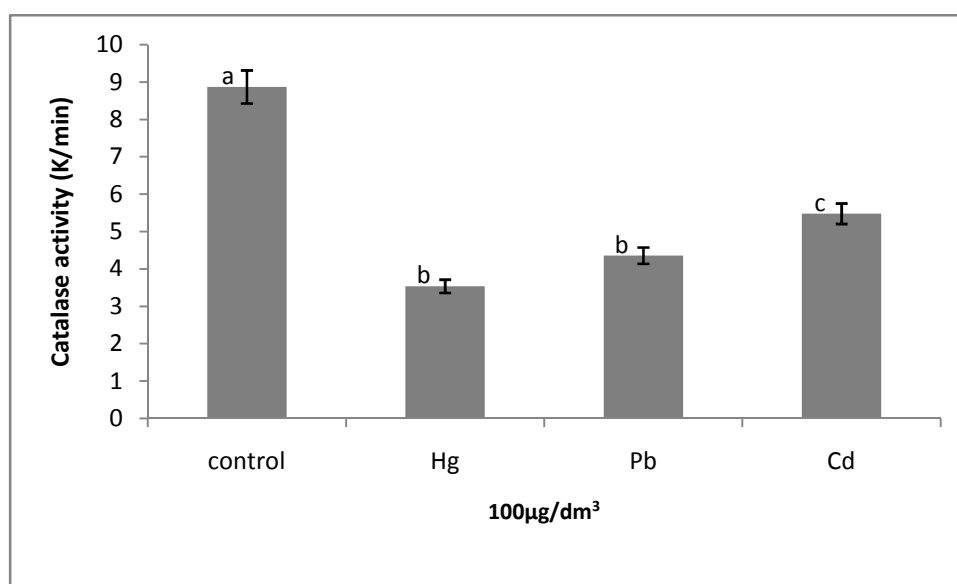


Fig 2: Effects of heavy metals ($100\mu\text{g}/\text{dm}^3$) concentration on soil catalase activity

Alphabets on the bars bearing the same superscript are not significantly different at ($p>0.05$)

A study of the results of metal concentrations in soil generally revealed that the concentrations of the metals in soil are appreciable in both the control and heavy metal polluted samples, although not significant. The results also revealed that soil naturally contain trace levels of heavy metals as noticed in the control sample where there were trace levels of Zn, Cu, Ni, As, Hg, Pb and Cd (FEPA, 1999). These trace levels of heavy metals in the control sample is attributable to anthropogenic sources that normally go on on soil through human activities (Nouri, 1980).

Soil enzyme activities in the samples studied revealed that subchronic exposure of these heavy metals pollution altered the soil dehydrogenase and catalase activities. The heavy metals at this concentration significantly ($p<0.05$) increased soil dehydrogenase activity from 6.78 ± 1.64 , 6.20 ± 1.46 and 7.32 ± 1.20 mg/g dry soil / 96 hr in Pb, Hg and Cd- polluted soil samples respectively when compared to the control with 4.28 ± 1.73 mg/g dry soil / 96 hr. This observation may be as a result of increase in microbial activity and respiration rate as a result of these metals. The increase in dehydrogenase activity may in part be due to the

involvement of specific micro organisms in the metabolism of these metals in the soil. The results agree with the report of Achuba and Peretiemo-Clarke (2008).

Soil catalase activity of the soil samples show a significant ($P>0.05$) decrease in activity ranging from 3.54 ± 0.70 , 4.36 ± 0.66 and 5.48 ± 0.90 Kmin^{-1} from Hg, Pb and Cd – polluted soil samples respectively relative to the control that has 8.88 ± 0.02 Kmin^{-1} . The decrease could be because, catalase, being an enzyme, its activity is altered by unfavourable conditions such as hypoxia, unavailability of soil nutrient and change in pH. This result agrees with the report of Nwaogu et al, (2012b); Achuba and Peretiemo-Clarke (2008). When soil enzyme activities are negatively affected by pollution, be it from petroleum hydrocarbons, heavy metal effluents from cassava, palm oil or pharmaceutical wastes, the physicochemical properties are affected which ultimately decreases soil productive potential (Osuji and Nwoye, 2007).

4. CONCLUSION

Our investigation revealed that subchronic exposure of heavy metals Hg, Pb and Cd at $100 \mu\text{g}/\text{dm}^3$ after one hundred and twenty days, adversely affected the pH, electrical conductivity, as well as the activities of catalase and dehydrogenases of the soil.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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