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Effect of Sublethal Concentration of Heavy Metal Contamination on Soil Physicochemical Properties, Catalase and Dehydrogenase Activities

Linus A. Nwaogu[1*], Cosmas O. Ujowundu[1], Callistus I. Iheme[1], Tobias N. I. Ezejiofor[2] and Donatus C. Belonwu[3]

[1]Department of Biochemistry, Federal University of Technology, Owerri, Nigeria.
[2]Department of Biotechnology, Federal University of Technology, Owerri, Nigeria.
[3]Department of Biochemistry University of Port Harcourt, Port Harcourt, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author LAN designed the study, wrote protocol and the first draft of the manuscript. Authors COU and CII performed the statistical analysis of the study. Authors TNIE and DCB managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

The effect of sublethal contaminations (100 μg/dm^3) of heavy metals such as mercury (Hg), lead (Pb) and cadmium (Cd) on soil enzyme and physicochemical properties was investigated after one hundred and twenty days. Soil sample without heavy metal contamination served as the control. Results indicate that Hg, Pb and Cd at 100 μg/dm^3 concentration 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 these soil physicochemical properties: moisture, phosphate, sulphate, chloride, calcium carbonate, total nitrogen and organic carbon when compared to the control. There were significant ($P<0.05$) decrease in soil dehydrogenase and catalase activities in all the metal-contaminated soil samples when compared to the control, indicating that these heavy metals increased soil acidity and electrical conductivity at this concentration and period of exposure.

**Corresponding author: Email: nwogulinus@yahoo.com;*

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1. INTRODUCTION

Most problems of soil pollution are associated with large amount of heavy metals deposited on it through disposed wastes, especially sewage sludge [1,2]. These metals which are often non biodegradable are accumulated in living organisms through the food chain when released into the environment. Apart from the contribution from anthropogenic sources and disposed wastes, soil naturally contains trace level of heavy metals [3]. Soil pollution by heavy metal have been investigated by various authors [4-7] who implicated large amount of anthropogenic inputs. This can have serious health implication especially with regards to crops/vegetables grown on such soils [8]. Most of these heavy metals are necessary for growth and normal functions of both plants and animals at trace amount, but large amount of any of them may cause acute or chronic toxicity [9]. 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 includes mental lapse, kidney failure and central nervous system disorder [9].

Micro-organisms in the soil are responsible for nitrogen fixation, assimilation and degradation of organic matter to release nutrients [10]. When heavy metals are retained in the soil through repeated contamination, they interfere with microbial key biochemical processes. 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 [10]. 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 [11] and stomatal resistance [12]. It can also affect photosynthesis and chlorophyll synthesis [13]. 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 [14,15].

Dehydrogenases (EC.1.1.1.1) are enzymes which catalyse the removal of hydrogen atom from different metabolites [16]. 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 [17]. Catalase (EC.1.11.1.6) is an iron porphyrin enzyme which catalyses very rapid the decomposition of hydrogen peroxide to water and oxygen [16]. 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 [18,19]. Their values have been suggested to be used as a simple toxicity test [20] .The aim of this study was to determine the effect of sublethal exposure to mercury, lead and cadmium at 100 $\mu g/dm^3$ 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 local sources and of analytical grade.

2.2 Collection and Preparation of Soil Samples

Loamy soil sample was collected at a depth of 0-20 cm from a garden farm of the Federal University of Technology Owerri, Nigeria in April, 2012. The soil sample was initially analyzed for the presence of these heavy metals, using Atomic Absorption Spectrophotometer (AAS). The sample was sieved using a 2mm (No 10 mesh) sieve. Coarse particles were removed and the fraction 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 $\mu g/dm^3$ of nitrate salt of mercury, lead and cadmium 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. They 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, passed through a 2 mm sieve, 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 described by Ademoroti [21]. The digested samples were analyzed for the 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 [22]. 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 were determined according to the method described by Page et al, [23]. Total soil nitrogen was determined by the Kjeldahl digestion according to Bremner [24]. Total organic carbon was determined by the method described by Nelson and Somers [25], while electrical conductivity was determined by the method of Chopro and Kanzar [26]. All the readings were done in triplicates.

2.4 Assay for Selected Soil Enzyme Activities

Soil dehydrogenase activity was assayed using the method as described by Tabatabai [27]. Dehydrogenase convert 2,3,5-triphenyl tetrazolium chloride to formazan. 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 27ºC, 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, and the absorbance was read spectrophotometrically at 485 nm. Catalase activity of the soil samples was assayed using the method as described byCohen et al. [28] where decomposed hydrogen peroxide was measured by reacting with excess potassium tetraoxomanganate (Vii) ($KMnO_4$). The residual $KMnO_4$ was measured spectro photo-metrically at 480 nm.

2.5 STATISTICAL ANALYSIS

Data generated were expressed as mean±SEM. All results were compared with respect to the control. Comparison between the concentrations of physicochemical properties, activities of selected soil enzymes and the control were made by using one way Analysis of Variance (ANOVA) with the aid of a computer-based statistical package (Graph pad Prism 5.3) and differences at $P<0.05$ were considered as significant.

3. RESULTS

Table 1 shows 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 μg/PO_4/kg, sulphate of 427.70 mg/SO_4/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 107us/cm. Hg-polluted soil sample has a pH of 4.80, moisture content of 2.26%, phosphate 45.60 μg/PO_4/kg, sulphate of 450.19 mg/SO_4/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 μs/cm. Pb-contaminated soil sample has a pH of 4.20, moisture content of 2.45%, phosphate of 46.10 μg/PO_4/kg, sulphate of 455.21 mg/SO_4/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 μs/cm while Cd-contaminated soil sample has a pH of 4.10, moisture content of 2.30%, phosphate of 45.80 μg/PO_4/kg, sulphate of 452.30 mg/SO_4/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 us/cm respectively.

Table 1. Physicochemical properties of soil samples from control, Hg, Pb and Cd – contamination

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 (μg/ PO_4/kg)	46.50	45.60	46.10	45.80
Sulphate (Mg /SO_4/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 (μs/cm)	107	160	170	168

Table 2 presents the results of heavy metal concentrations in the soil samples studied. The control sample has the following metal concentrations in μg/kg: Zn (12.0), Cu (6.65), Ni 1.63), As (0.42), Hg (0.06), Pb (1.32), and Cd (0.24). Hg-contaminated soil has Zn (12.0), Cu (6.65), Ni (1.65), As (0.41), Hg (0.06), Pb (0.32) and Cd (0.25). Pb-contaminated soil has Zn (12.0), Cu (2.64), Ni (1.65), As 0.42), Hg (0.06), Pb (0.39) and Cd (0.23) while Cd-contaminated soil has Zn (12.0), Cu (2.65), Ni (1.65), As (0.43), Hg (0.06), Pb (0.23) and Cd (0.28) respectively indicating that natural soil contain traces of metals.

Table 2. Heavy metal concentrations in soil samples (µg/g)

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	2.65	2.65	2.64	2.65
Ni	1.63	1.65	1.65	1.65
As	0.42	0.41	0.42	0.43
Hg	0.06	0.05	0.06	0.06
Pb	0.32	0.32	2.39	0.23
Cd	0.24	0.25	0.23	1.28

Fig. 1 shows the activities of the two selected soil enzymes (dehydrogenase and catalase). There were significant $P<0.05$ difference in activities in the two enzymes. The figure shows that the presence of these heavy metals decreased the activities of these enzymes relative to the control.

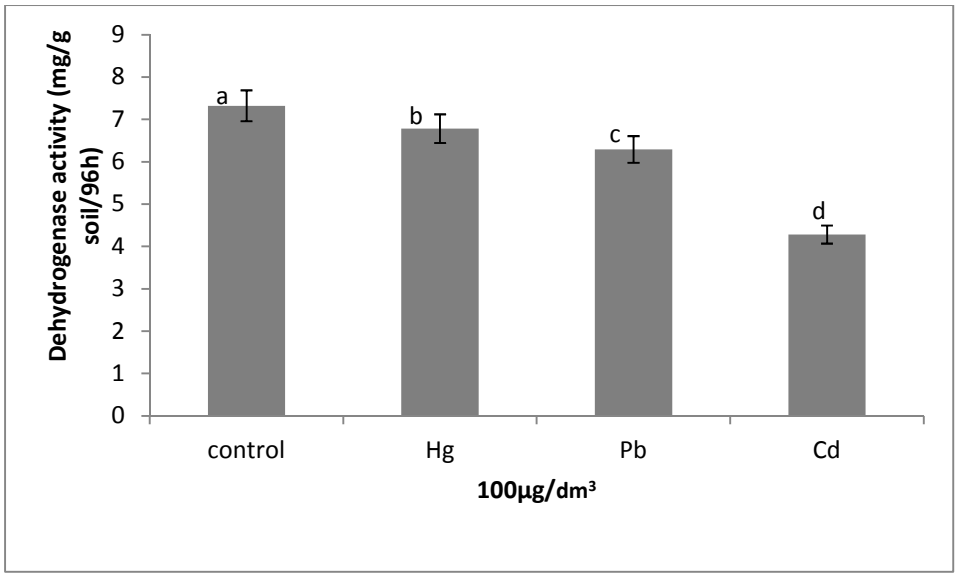

Fig. 1. Effects of the heavy metals on soil dehydrogenase activity
Alphabets on the bars bearing different superscript are significantly different at (P<0.05)

4. DISCUSSION

The mean pH values of all the soil samples analyzed 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 these concentrations 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 [28,6], 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. This indicates that the soil physicochemical parameters were not adversely affected. However, the results of electrical conductivity measurement was significantly ($P<0.05$) higher than the control with mean values ranging from 160-168 μs/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 [29,4].

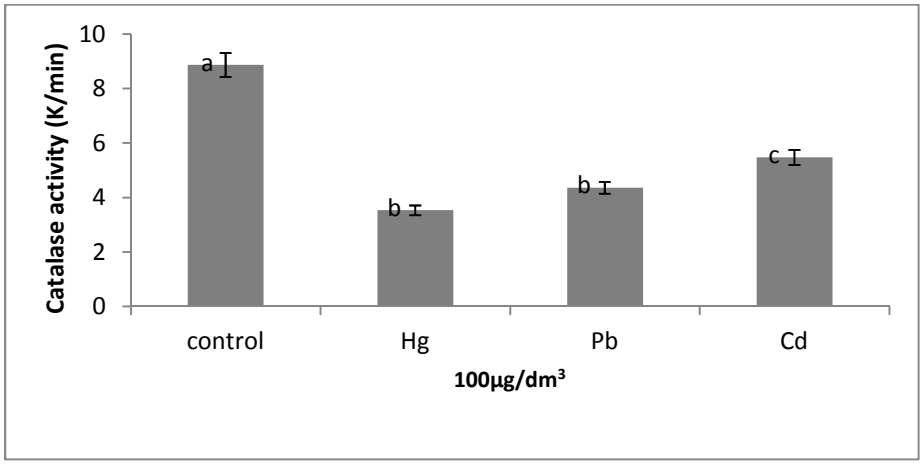

Fig. 2. Effect of the heavy metals on soil catalase activity

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 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 [3]. These trace levels of heavy metals in the control sample is attributable to anthropogenic sources that normally go on soil through human activities [1].

Soil enzyme activities in the samples studied revealed that sublethal concentrations of these heavy metals contamination altered the soil dehydrogenase and catalase activities. The heavy metals at this concentration significantly ($P<0.05$) decreased soil dehydrogenase activity from 4.28±1.73, 6.20±1.64 and 6.78±1.46 mg/g dry soil / 96 hr in Pb, Hg and Cd-contaminated soil samples respectively when compared to the control with 7.32±1.20 mg/g dry soil / 96 hr. This observation may be as a result of decrease in microbial activity and respiration rate as a result of these metals. The reduction of dehydrogenase activity could be ascribed to saturation of active sites in the microbial cells. Most of these metals are known to be toxic to micro organisms because they are not essential for biological functions and are inhibitors of microbial metabolism. The results agree with the reports of [32] and [33].

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-contaminated soil samples respectively relative to the control that has 8.88±0.02 $Kmin^{-1}$ (Fig. 2). 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 reports of [30,3]. When soil enzyme activities are negatively affected by pollution, be it from petroleum hydrocarbons, heavy metals, effluents from

cassava, palm oil or pharmaceutical, the physicochemical properties of the soil are affected which ultimately decreases its productive potential [34].

4. CONCLUSION

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

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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