

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

3 \*Linus.A.Nwaogu<sup>1</sup>, Cosmos. O. Ujowundu<sup>1</sup>, Callistus.I.Iheme<sup>1</sup>, Tobias.N.I.Ezeji<sup>2</sup>  
and Donatus.C. Belonwu<sup>3</sup>.

4 <sup>1</sup>Department of Biochemistry, Federal University of Technology, Owerri, Nigeria.

5 <sup>2</sup>Department of Biotechnology, Federal University of Technology, Owerri,  
Nigeria.

6 <sup>3</sup>Department of Biochemistry University of Port Harcourt, Port Harcourt, Nigeria.

7 \*Corresponding author's Email: [nwogulinus@yahoo.com](mailto:nwogulinus@yahoo.com)

## 8 **ABSTRACT**

9 The effect of **sublethal contaminations (100 µg/dm<sup>3</sup>)** of heavy metals such as  
10 **mercury (Hg), lead (Pb) and cadmium (Cd) on soil enzyme** and physicochemical  
11 properties was investigated after one hundred and twenty days. Soil sample  
12 without heavy metal **contamination** served as the control. Results indicate that Hg,  
13 Pb and Cd at 100 µg/dm<sup>3</sup> concentration caused a significant ( $P < 0.05$ ) change in  
14 the soil pH and electrical conductivity relative to the control. There was no  
15 significant ( $P > 0.05$ ) difference in these soil physicochemical properties: moisture,  
16 phosphate, sulphate, chloride, calcium carbonate, total nitrogen and organic  
17 carbon when to the control. There were significant ( $P < 0.05$ ) decrease in soil  
18 dehydrogenase and catalase activities in all the metal-**contaminated** soil samples  
19 when compared to the control, indicating that these heavy metals increased soil  
20 acidity and electrical conductivity at this concentration and period of exposure.

21 **Keywords:** Soil properties, **contamination**, heavy metals, enzyme activity.

## 22 **1. INTRODUCTION**

23 Most problems of soil pollution are associated with large amount of heavy metals  
24 deposited on it through disposed wastes, especially sewage sludge [1, 2]. These  
25 metals which are often non biodegradable are accumulated in living organisms  
26 through the food chain when released into the environment. Apart from the  
27 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, 5, 6, 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 include 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

62 microbial activities in soil. Both dehydrogenase and catalase activities are very  
63 sensitive to heavy metal pollution [18, 19]. Their values have been suggested to be  
64 used as a simple toxicity test [20]. The aim of this study was to determine the  
65 effect of sublethal exposure to mercury, lead and cadmium at  $100 \mu\text{g}/\text{dm}^3$   
66 concentration on soil physicochemical properties and the activities of catalase and  
67 dehydrogenase.

## 68 2. MATERIALS AND METHODS

### 69 2.1 Chemicals / Reagents

70 2, 3, 5-triphenyl tetrazolium chloride (TTC) was bought from Sigma-Aldrich  
71 Chemical Company St. Louis Mo, USA. Other chemicals used were from varied  
72 local sources and of analytical grade.

### 73 2.2 Collection and preparation of soil samples.

74 Loamy soil sample was collected at a depth of 0-20 cm from a garden farm of the  
75 Federal University of Technology Owerri, Nigeria in April, 2012. The soil sample  
76 was initially analyzed for the presence of these heavy metals, using Atomic  
77 Absorption Spectrophotometer (AAS). The sample was sieved using a 2mm (No  
78 10 mesh) sieve. Coarse particles were removed and the fraction used for the  
79 experiment. One kilogram each of the sieved soil sample was weighed into four  
80 plastic pots and labeled A-D. 100 ml solutions containing  $100 \mu\text{g}/\text{dm}^3$  of nitrate  
81 salt of mercury, lead and cadmium were applied singly to the respective pots A-C,  
82 while D served as the control. Distilled water was applied periodically on the pots  
83 A-D to maintain soil moisture at constant level. They were allowed to stand for a  
84 period of one hundred and twenty days.

### 85 2.3 Soil Physicochemical Properties Analysis

86 Soil samples from pots A-D were air-dried, ground, passed through a 2 mm sieve,  
87 stored in different labeled plastic containers and used for the various analyses. The  
88 soil samples for heavy metal analysis were digested according to the method  
89 described by Ademoroti [21]. The digested samples were analyzed for the heavy  
90 metals using Atomic Absorption Spectrophotometer (AAS). Soil pH was  
91 measured in a soil-water ratio of 1:2 using a pH meter according to Davey and  
92 Conyers [22]. Moisture content of the soil was measured gravimetrically by  
93 drying 50 g of soil samples at  $105^\circ\text{C}$  for 24 hours. Phosphate, sulphate, chloride  
94 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 analyses 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 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, and the absorbance was read spectrophotometrically at 485 nm. Catalase activity of the soil samples was assayed using the method as described by Cohen et al,[28] where decomposed hydrogen peroxide was measured by reacting with excess potassium tetraoxomanganate (Vii) (KMnO<sub>4</sub>). The residual KMnO<sub>4</sub> 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. 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 (Graphpad Prism 5.3) and differences at P< 0.05 were considered as significant.

### 3.0 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  $\mu\text{g}/\text{PO}_4/\text{kg}$ , sulphate of 427.70  $\text{mg}/\text{SO}_4/\text{kg}$ , chloride 50.00

126 mg/kg, calcium carbonate of 0.061 %, total nitrogen of 5.78 %, organic carbon of  
 127 1.57 % and electrical conductivity of 107us/cm. Hg-polluted soil sample has a pH  
 128 of 4.80, moisture content of 2.26 %, phosphate 45.60  $\mu\text{g}/\text{PO}_4/\text{kg}$ , sulphate of  
 129 450.19  $\text{mg}/\text{SO}_4/\text{kg}$ , chloride of 45.60  $\text{mg}/\text{kg}$ , calcium carbonate of 0.055 %, total  
 130 nitrogen of 5.64%, organic carbon of 1.48% and electrical conductivity of 160  
 131  $\mu\text{s}/\text{cm}$ . Pb-contaminated soil sample has a pH of 4.20, moisture content of 2.45 %,   
 132 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  
 133  $\text{mg}/\text{kg}$ , calcium carbonate of 0.048 %, total nitrogen of 5.56 %, organic carbon of  
 134 1.46 % and electrical conductivity of 170  $\mu\text{s}/\text{cm}$  while Cd-contaminated soil  
 135 sample has a pH of 4.10, moisture content of 2.30 %, phosphate of 45.80  
 136  $\mu\text{g}/\text{PO}_4/\text{kg}$ , sulphate of 452.30  $\text{mg}/\text{SO}_4/\text{kg}$ , chloride of 45.80  $\text{mg}/\text{kg}$ , calcium  
 137 carbonate of 0.052 %, total nitrogen of 5.68 %, organic carbon of 1.50 % and  
 138 electrical conductivity of 168  $\mu\text{s}/\text{cm}$  respectively.

139 Table 2 presents the results of heavy metal concentrations in the soil samples  
 140 studied. The control sample has the following metal concentrations in  $\mu\text{g}/\text{kg}$ : Zn  
 141 (12.0), Cu (6.65), Ni 1.63), As (0.42), Hg (0.06), Pb (1.32), and Cd (0.24). Hg-  
 142 contaminated soil has Zn (12.0), Cu (6.65), Ni (1.65), As (0.41), Hg (0.06), Pb  
 143 (0.32) and Cd (0.25). Pb-contaminated soil has Zn (12.0), Cu (2.64), Ni (1.65), As  
 144 0.42), Hg (0.06), Pb (0.39) and Cd (0.23) while Cd- contaminated soil has Zn  
 145 (12.0), Cu (2.65), Ni (1.65), As (0.43), Hg (0.06), Pb (0.23) and Cd (0.28)  
 146 respectively indicating that natural soil contain traces of metals.

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

#### 151 4. DISCUSSION

152 The mean pH values of all the soil samples analyzed indicate that the heavy metal-  
 153 polluted soil samples had low pH values 4.10-4.30 relative to the control pH 7.20.  
 154 This revealed that the addition of these metals at these concentrations lowered the  
 155 pH of the soil sample. Soil pH is an important soil property, having great effects  
 156 on solute concentration and absorption in soil. High soil acidity creates chemical  
 157 and biological conditions which may be harmful to plants and soil micro

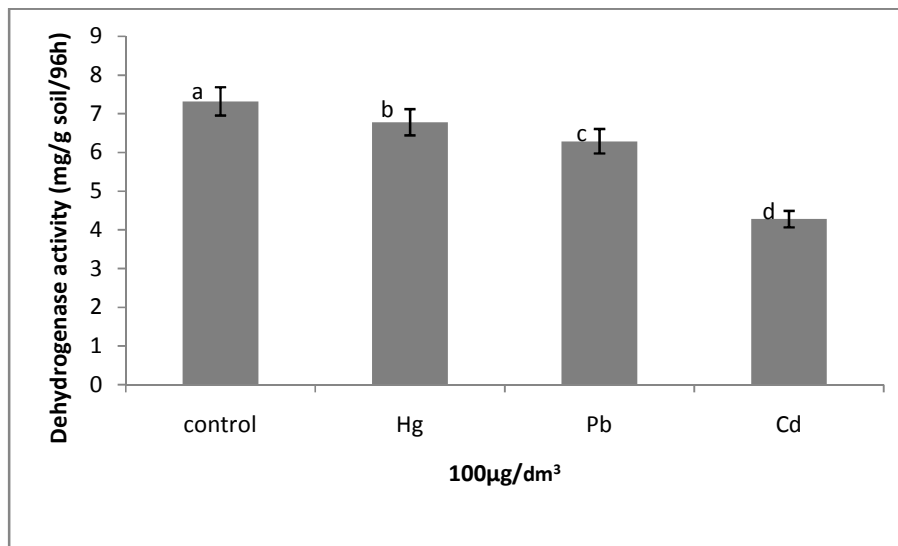
158 organisms. This observation agrees with the reports of [28, 6], who reported that  
159 heavy metals from automobile service centres decreased soil pH thus increasing  
160 the soil acidity. There was no significance ( $P>0.05$ ) difference in the mean values  
161 of moisture contents, phosphate, sulphate, chloride, calcium carbonate, total  
162 nitrogen and organic carbon obtained for all the heavy metals-polluted soil  
163 samples relative to the control. This indicates that the soil physicochemical  
164 parameters were not adversely affected. However, the results of electrical  
165 conductivity measurement was significantly ( $P<0.05$ ) higher than the control with  
166 mean values ranging from 160-168  $\mu\text{s}/\text{cm}$ . This may be due to the increase in the  
167 concentration of some soluble salts in the soil samples polluted with these  
168 individual heavy metals. This observation corroborates with the reports of [29, 4].

169 **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 ( $\mu\text{g}/\text{PO}_4/\text{kg}$ )   | 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 ( $\text{mg}/\text{kg}$ )                  | 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                       |

170 **Table 2: Heavy Metal Concentrations in Soil Samples ( $\mu\text{g}/\text{g}$ )**

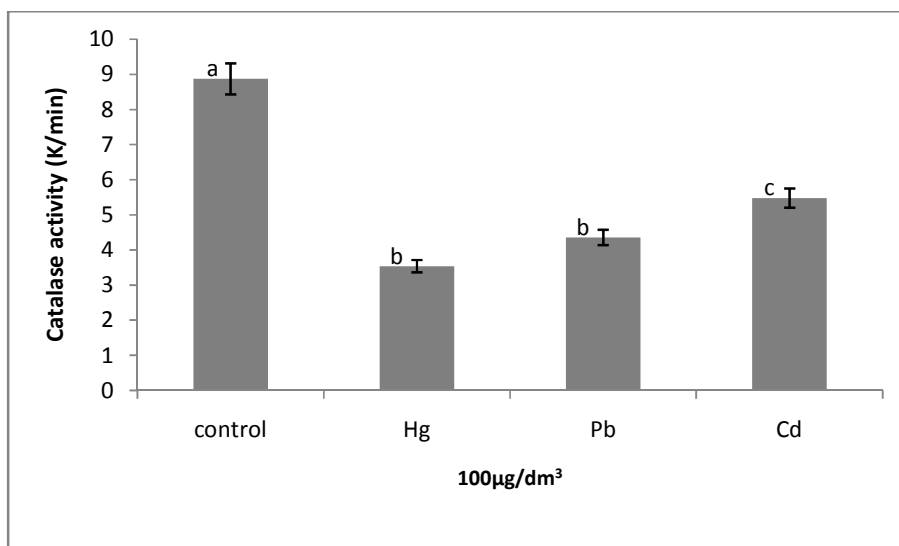
| Heavy Metals | Control | Hg- Polluted Soil Sample | Pb-Polluted Soil Sample | Cd – Polluted Soil Sample |
|--------------|---------|--------------------------|-------------------------|---------------------------|
| Zn           | 12.0    | 12.0                     | 12.0                    | 12.0                      |
| 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                      |



171 Fig 1: Effects of the heavy metals on soil dehydrogenase activity

172 Alphabets on the bars bearing the same superscript are not significantly different

173 at ( $P > 0.05$ )



174 Fig 2: Effect of the heavy metals on soil catalase activity

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

177 A study of the results of metal concentrations in soil generally revealed that soil  
 178 naturally contain trace levels of heavy metals as noticed in the control sample  
 179 where there were trace levels of Zn, Cu, Ni, As, Hg, Pb and Cd [3]. These trace  
 180 levels of heavy metals in the control sample is attributable to anthropogenic  
 181 sources that normally go on soil through human activities [1].

182 Soil enzyme activities in the samples studied revealed that **sublethal**  
 183 **concentrations** of these heavy metals **contamination** altered the soil dehydrogenase  
 184 and catalase activities. The heavy metals at this concentration significantly  
 185 ( $P < 0.05$ ) decreased soil dehydrogenase activity from  $4.28 \pm 1.73$ ,  $6.20 \pm 1.64$  and  
 186  $6.78 \pm 1.46$  mg/g dry soil / 96 hr in Pb, Hg and Cd- **contaminated** soil samples  
 187 respectively when compared to the control with  $7.32 \pm 1.20$  mg/g dry soil / 96 hr.  
 188 This observation may be as a result of decrease in microbial activity and  
 189 respiration rate as a result of these metals. **The reduction of dehydrogenase**  
 190 **activity could be ascribed to saturation of active sites in the microbial cells. Most**  
 191 **of these metals are known to be toxic to micro organisms because they are not**  
 192 **essential for biological functions and are inhibitors of microbial metabolism. The**  
 193 **results agree with the reports of [30] and [31].**

194 Soil catalase activity of the soil samples show a significant ( $P < 0.05$ ) decrease in  
 195 activity ranging from  $3.54 \pm 0.70$ ,  $4.36 \pm 0.66$  and  $5.48 \pm 0.90$  Kmin<sup>-1</sup> from Hg,  
 196 Pb and Cd- **contaminated** soil samples respectively relative to the control that has

197  $8.88 \pm 0.02 \text{ Kmin}^{-1}$ . The decrease could be because, catalase, being an enzyme, its  
198 activity is altered by unfavourable conditions such as hypoxia, unavailability of  
199 soil nutrient and change in pH. This result agrees with the reports of [11, 32].  
200 When soil enzyme activities are negatively affected by pollution, be it from  
201 petroleum hydrocarbons, heavy metals, effluents from cassava, palm oil or  
202 pharmaceutical, the physicochemical properties of the soil are affected which  
203 ultimately decreases **its** productive potential [33].

#### 204 4. CONCLUSION

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

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#### 213 COMPETING INTERESTS

214 Authors have declared that no competing interests exist.

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