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## Research Article

# Effects of Heavy Metal Stress on Certain Bacterial Populations and Microbial Activities in Soil

Ojiagu DK* and CN Umeh

Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, P.M.B. 5025, Awka, Anambra State, Nigeria
***Corresponding author:** davidkingsley77@yahoo.com

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**ABSTRACT**

Heavy metal stress adversely affects microbial activities at elevated levels but information on response of indigenous soil bacterial populations and the sensitivity of whole microbial activities is poorly understood. Thus, the effects of heavy metals on certain soil bacterial populations and microbial activities were investigated over a four-week period. Sulphate salts of zinc (Zn), cooper (Cu), and nickel (Ni) were added to the soil samples at 5 g/kg of soil. The rates of microbial carbon (total carbon, organic matter and oxidizable organic carbon), nitrogen mineralization, respiration, pH were measured. Total colony-forming units (CFU) of aerobic heterotrophic bacteria, total and faecal coliforms were taken. The results indicated that the effects of metals on the assayed parameters were generally significant ($P<0.05$) with marked positive correlations ($P<0.01$). The rates of carbon accumulated by the 4$^{th}$ week of post-treatment were high in% Cu and Zn treatments (6.38, 11.00, 4.78%; and 6.34, 10.93, 4.75% respectively), but insignificant in% Ni. There were accumulated levels of nitrogen, showing rates of 0.51, 0.53 and 0.48% in samples treated with Zn, Cu, and Ni respectively, compared to 0.38-0.40% at zero time. Respiration of the soil's microbial populations was inhibited from an initial rate of 0.24g of C/g to 0.11g of C/g in the Zn treated soil; and to 0.05g of C/g in the Cu treated soil; and to 0.09g of C/g in the Zn treated soil respectively by the 4$^{th}$ week. Plate counts of total aerobic heterotrophic bacteria, total and faecal coliforms were significantly inhibited. Inhibitory additive effects of microbial activities and population caused by the heavy metals were recorded.

**Key words:** Heavy metals, mineralization, respiration, bacterial population

## INTRODUCTION

Environmental pollution with toxic metals has markedly increased since the onset of the industrial revolution (Hogan, 2010). Pollution of soil by heavy metals, such as Cd, Pb, Cr, Cu, Ni, Zn etc, is an environmental problem of concern (Duruibe *et al*., 2007). Although heavy metals are naturally present in soils, contamination can occur from a range of sources, including: industry, agriculture (irrigation with polluted waters, sewage sludge and fertilizers, contaminated manure, pesticides and herbicides containing heavy metals), waste incineration, combustion of fossil fuels, and long-range transport of atmospheric pollutants. Steady heavy metals inflow and accumulation in soil can lead to irreversible reduction of soil microbial process rate (Niklińska *et al*., 2006). Generally, heavy metals can be found at trace levels in soil, which can serve as vital microelements. These however, have toxic effects on microorganisms at high content levels.

The effect and extended consequences on ecology by pollution in general, have led to the heightened interests to study the pollutants–environment–microbiota interactions. The concentration of a toxic metal that affects the growth and survival of different microorganisms varied widely. The tremendous microbial diversity makes it difficult to study the whole range of population. The relationship of microorganisms to heavy metal soil pollution is entirely a complex process of diverse associations and relationships. However, artificial contamination of soil of known physicochemical characteristics with metal salts and enumeration of the surviving indigenous populations, in a short term study, may reveal the occurrence of microbes in a particular soil sample with intrinsic ability to tolerate metals (Hayat *et al*., 2002), where conditions may be different with the conditions of the soil in the natural state.

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Therefore, the aim of the study is to examine the effects of added metal concentrations on certain indigenous bacterial populations and microbial activities in soil when treated with known concentrations of metal ions of zinc, copper and nickel.

## MATERIALS AND METHODS

### Collection, preparation and treatment of soil samples

Ten kilograms (10 kg) of loamy soil sample were collected from a remote land within the premises of Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. The soil was covered with vegetation at the time of sampling and was collected from a depth of 5 – 15 cm using a properly disinfected hand trowel after carefully removing surface vegetation. The hand trowel was washed with soap and water, rinsed repeatedly with distilled water and finally swabbed thoroughly with ethanol (70% v/v). Large soil particles in the samples were removed by handpicking them out of the samples wearing gloves. The samples were then sieved (mesh size < 2 mm); plant debris and any visible soil fauna were removed. The soil was then transferred into a large disinfected plastic bowl, and thoroughly mixed using a disinfected hand trowel and allowed to stabilize for 7 days by incubating at room temperature to permit the disturbance caused by sampling and sieving to subside (Friedlova, 2010). One kilogram (1 kg) each of the soil sample was distributed into 4 plastic pots disinfected by alcohol swabbing; labeled A-D (A = control, B = zinc, C = copper, D = nickel). Solutions of sulphate salts (5 g) of zinc, copper and nickel dissolved in 200 ml of distilled water were applied to the respective pots singly. The soil sample that received no metal amendment served as a control. All samples were kept in the plastic pots at room temperature.

### Determination of soil microbial activities

The soil microbial activities were determined by measuring the following: total carbon content of soil, soil organic matter, oxidizable carbon content, nitrogen content and soil respiration. Soil pH and moisture content were monitored.

Total organic carbon, organic matter and oxidizable organic carbon contents of the soil were determined by the Walkley-Black dichromate extraction with titrimetric quantitation method (Walkley and Black, 1934). The rate of nitrogen mineralization was determined by the Kjeldahl method (Kjeldahl, 1883). The soil microbial respiration was determined using the soda-lime $CO_2$ efflux technique, and gravimetrically determining the weight differences (Keith and Wong, 2006). Soil pH was determined using the combined pH electrode after 5 g of dried soil sample was mixed with deionized water, and allowed to stand for 1 hr. Moisture content was measured gravimetrically (Haney and Haney, 2010).

### Determination of the survival of certain indigenous bacteria in the heavy metal amended soils

Aliquots of 0.1 ml of the 10-fold serially diluted soil samples were spread plated on the respective growth media at the 0, 1st, 2nd , 3rd, and 4th week. Total viable count of total aerobic heterotrophic bacteria, total and faecal coliforms were determined on Nutrient and MacConkey agar plates at 37 ºC and 44.5 ºC for 24 hours respectively, according to standard methods.

### Statistical analyses

The results were analyzed by two-way analysis of variance (ANOVA) at 95% confidence interval to determine significant differences between the means of control and other treatments ($P<0.05$). Pearson correlational analyses were also performed to determine correlational strength between the various parameters assayed.

## RESULTS AND DISCUSSIONS

The result showed that carbon was accumulated in the soil during the four-week treatment period with heavy metals (Table 1). This accumulation showed that the microbial process of carbon mineralization was inhibited to various levels by the metals as measured by total carbon content of the soil *cum* organic matter and oxidizable organic carbon content of the soil. Among the treatments, Cu had the highest rate at 6.38, 11.00 and 4.78% followed by Zn which was 6.34, 10.93 and 4.75% by the end of the experiment for total carbon, organic matter and oxidizable organic carbon contents respectively. The rate of carbon accumulation in the nickel amended soil sample was insignificant. Cu amendment at the beginning of the experiment gave carbon accumulation rates of 6.22% total carbon, 10.72% organic matter, and 4.66% oxidizable organic carbon. But steadily, it rose to 6.38% total carbon, 11.00% organic matter, and 4.78% oxidizable organic carbon by the 4th week. The 1st week of experiment did not show a marked interference from the beginning of the experiment as the level was insignificant ($P>0.05$). However, there was a marked interference to the microbial process of carbon mineralization from the 2nd week down to the 4th week. Equally, the Zn treatment was 6.21% total carbon, 10.71% organic matter, and 4.66% oxidizable organic carbon, but tipped to 6.32, 10.93, and 4.75% respectively.

However, the 1st week of experiment similarly did not show a perceptible interference from control as the level was likewise insignificant ($P>0.05$). From the statistical result *vis-à-vis* Pearson Correlation, total carbon, organic matter and oxidizable organic carbon contents of the soil showed strong positive correlations ($P<0.01$) which indicate an agreeable measure of the mineralization of carbon in the amendments.

The data pertaining to the rate of nitrogen mineralization indicated that nitrogen was accumulated in the heavy metal treated soils. The Zn, Cu, and Ni treated soils did not show any significant interference with microbial nitrogen mineralization in the 1st week. By the 4th week however, Zn, Cu, and Ni had significantly interfered with microbial nitrogen mineralization giving values of 0.51% for Zn, 0.53% for Cu, and 0.48% for Ni (Table 2).

As evidenced by $CO_2$ evolution, the soil respiration declined significantly (Table 2). The Cu-amended soil showed the highest rate of decrease in respiration with 0.05 g of C/g by the 4th week. Likewise, the rate of respiration steadily declined in both Zn and Ni treated

**Table 1:** Determination of total carbon, organic matter, and oxidizable organic carbon contents of the soil

| | Total carbon content % | | | | Organic matter % | | | | Oxidizable organic carbon % | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Amendment-week | Ctrl | Zn | Cu | Ni | Ctrl | Zn | Cu | Ni | Ctrl | Zn | Cu | Ni |
| 0 | 6.21 | 6.21 | 6.22 | 6.20 | 10.71 | 10.71 | 10.72 | 10.69 | 4.66 | 4.66 | 4.66 | 4.65 |
| 1 | 6.21 | 6.25 | 6.28 | 6.23 | 10.71 | 10.78 | 10.83 | 10.74 | 4.66 | 4.69 | 4.71 | 4.67 |
| 2 | 6.23 | 6.28* | 6.31* | 6.24 | 10.74 | 10.83* | 10.88* | 10.76 | 4.67 | 4.71* | 4.73* | 4.68 |
| 3 | 6.21 | 6.32* | 6.35* | 6.28 | 10.71 | 10.90* | 10.94* | 10.83 | 4.66 | 4.72* | 4.76* | 4.71 |
| 4 | 6.22 | 6.32* | 6.38* | 6.31 | 10.72 | 10.93* | 11.00* | 10.83 | 4.66 | 4.75* | 4.78* | 4.73 |

*significantly different from control (P<0.05)

**Table 2** Determination of nitrogen content (%) and respiration of the soil (g of C/g)

| | Rate of nitrogen mineralization (%) | | | | Soil respiration (g) | | | |
|---|---|---|---|---|---|---|---|---|
| Amendment- week | Ctrl | Zn | Cu | Ni | Ctrl | Zn | Cu | Ni |
| 0 | 0.38 | 0.40 | 0.40 | 0.38 | 0.25 | 0.24 | 0.25 | 0.26 |
| 1 | 0.39 | 0.43 | 0.44 | 0.41 | 0.27 | 0.17* | 0.13* | 0.15* |
| 2 | 0.39 | 0.45* | 0.46* | 0.44* | 0.25 | 0.14* | 0.10* | 0.13* |
| 3 | 0.38 | 0.48* | 0.49* | 0.46* | 0.26 | 0.12* | 0.08* | 0.10* |
| 4 | 0.39 | 0.51* | 0.53* | 0.48* | 0.25 | 0.11* | 0.05* | 0.09* |

*significantly different from control (P<0.05)

**Table 3** Determination of pH and moisture content of the soil

| | pH | | | | Moisture content (%) | | | |
|---|---|---|---|---|---|---|---|---|
| Amendment-week | Ctrl | Zn | Cu | Ni | Ctrl | Zn | Cu | Ni |
| 0 | 7.8 | 7.6 | 7.7 | 7.7 | 58 | 55 | 53 | 57 |
| 1 | 7.6 | 6.2 | 5.8 | 6.6 | 57 | 49 | 48 | 52 |
| 2 | 7.5 | 5.7 | 5.4 | 6.1 | 56 | 45 | 43 | 48 |
| 3 | 7.7 | 5.3 | 5.1 | 5.7 | 57 | 42 | 41 | 45 |
| 4 | 7.6 | 5.1 | 4.8 | 5.3 | 57 | 37 | 34 | 42 |

*significantly different from control (P<0.05)

**Table 4** Determination of the survival of total aerobic heterotrophic bacteria, total and faecal coliforms (cfu/g) in soil amended with heavy metals

| | Aerobic heterotrophic bacteria ($x10^7$) | | | | Total coliform ($x10^6$) | | | | Total faecal coliform ($x10^6$) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Amendment-week | Ctrl | Zn | Cu | Ni | Ctrl | Zn | Cu | Ni | Ctrl | Zn | Cu | Ni |
| 0 | 2.88 | 2.71 | 2.67 | 2.81 | 5.9 | 5.4 | 5.2 | 5.6 | 4.2 | 3.9 | 3.8 | 4.0 |
| 1 | 2.84 | 1.93* | 1.46* | 2.01* | 5.7 | 3.9* | 3.7* | 4.1* | 4.0 | 3.3 | 2.9 | 3.6 |
| 2 | 2.86 | 1.74* | 1.13* | 1.87* | 5.2 | 3.3* | 2.4* | 3.8* | 4.2 | 2.8* | 2.2* | 3.2* |
| 3 | 2.81 | 1.54* | 1.01* | 1.79* | 5.4 | 2.3* | 1.9* | 2.7* | 4.1 | 2.3* | 1.9* | 2.9* |
| 4 | 2.85 | 1.26* | 0.88* | 1.52* | 5.3 | 1.7* | 1.3* | 2.0* | 4.2 | 1.8* | 1.4* | 2.1* |

*Significantly different from control (P<0.05).

soils, 0.11 and 0.09 g of C/g respectively by the 4th week. The pH of the amended soils declined (Table 3).

There was a steady decrease in the number of total aerobic heterotrophic bacteria, total and faecal coliforms in the soils. By the end of the 4th week, total aerobic heterotrophic bacteria were 1.26 x $10^7$, 0.88 x $10^7$, and 1.52 x $10^7$ cfu/g of soil; total coliform, 1.7 x $10^6$, 1.3 x $10^6$, and 2.0 x $10^6$ cfu/g of soil; and total faecal coliform, 1.8 x $10^6$, 1.4 x $10^6$, and 2.1 x $10^6$ cfu/g of soil respectively for Zn, Cu, and Ni treated soils (Table 4). However, the Cu treated soil showed the highest decrease in total aerobic heterotrophic bacteria, total coliform, and total faecal coliform counts. The data obtained showed significant differences (P<0.05) in total aerobic heterotrophic bacteria, total and faecal coliforms from the control for Zn, Cu, and Ni treated soils; and also there are strong positive correlations between the three different counts (P< 0.01).

Toxicity of heavy metals has been related to their total concentrations in soil which leads to the accumulation of carbon and organic matter (Wang *et al*., 2007; Friedlova, 2010). The available form however, in which metal is present in soil determines its toxicity (Wang *et al*., 2007). Friedlova (2010) suggested that pH is the most important parameter controlling distribution of heavy metals in soils. Toxicity also is determined by solubility, which is a function of pH. Apparently, the result showed steady decline in pH of the soils with the Cu-amended soil recording the lowest pH.

Analysis of soil respiration helps to quantify the effects of metals on the microbial activity of soils because impeded soil respiration is a common feature of heavy metal polluted soils (Marschner and Kalbitz, 2003). The levels of inhibition however, are determined by the rate of carbon and nitrogen mineralization. Thus, under heavy metal pollution, the rate of such activities is impaired and carbon and nitrogen accumulate in the soil. Addition of heavy metal salts to soils causes an immediate decrease in respiration rates, but responses are determined by the properties of both the metal and the soil. In this work, the respiration rates in the Zn, Cu and Ni- treated soils decreased significantly, with the Cu-treated soil showing the highest rate, followed by the Ni-amended soil and then Zn.

There was significant inhibition in the plate counts of total aerobic heterotrophic bacteria, total and faecal coliforms. Also, the reason as to why Ni-amended soil showed insignificant interference in carbon mineralization remains unclear, as certain biochemical processes may have contributed to non-accumulation of carbon.

**Conclusions**

Results of the present study indicate that heavy metals could reduce the activities of soil microorganisms and such reduction could be as a result of additive/ inhibitory effects caused by pollution. This is a function of the concentration of heavy metals applied to the soil which is key in ascertaining the long-term effects of land receiving heavy metal contamination through sludge/wastewater runoffs.

Because of the large unexplored reservoir of microbial diversity, plate counts may not be sufficient to determine the level of microbial diversity in relation to the toxicity caused by heavy metals in soil. Finally, due to the problem of non-culturability of viable microorganisms, molecular techniques will help to provide a richer insight into the ecology of microbes in soil under heavy metal stress.

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