Bioavailability and Toxicity Of Heavy Metals on Soil Bacteria in Agricultural Farms

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Abstract: The Impact of heavy metal toxicity on microbial diversity of three soil locations: Fallow soil (Location A) Newly cultivated (Location B) and an Old Agricultural farm (Location C) were studied in the month of November 2008. Soil samples from three locations were polluted with different concentrations of heavy metals (Cadmium 1, 10, 100, 200 ppm, Copper, 10, 10,100, 200 ppm, Lead 1, 10, 100, 200 ppm, Nickel 10, 100, 200, 500 ppm and Zinc 1, 10, 100 and 200 ppm. The effect of various concentrations of heavy metals (Cadmium, Copper, Lead, Nickel and Zinc) on bacteria of fallow soil (Location A), newly cultivated (Location B-within 8months of planting) and old farm (Location C- above 18 months of planting) was investigated over a duration of 28 days. The Physico-chemical properties of each soil were determined. Total heterotrophic bacterial counts were recorded for each farm location. Generally, there was a decrease in heavy metal concentration at the end of the period. Total heterotrophic bacteria for Location A, B and C ranged between $3.1x10^4$ to $1.87x10^6$ cfu/g, $3.6x10^6$ to $2.0x10^7$ cfu/g and $3.6x10^3$ to $1.95x10^6$ cfu/g respectively. Generally, Bacterial counts were more in the cultivated soils when compared to the fallow soil Bioavailability test was carried out to determine the level of heavy metals available for bacteria uptake. Generally, the result of the bioavailability test revealed that bacteria in heavy metal polluted soils absorbed more concentrations of heavy metals than in the control soils. It was also observed that the uptake of heavy metals by bacteria was concentration dependent and independent of location. The effect of heavy metals on microbial populations depends on the soil type and use, heavy metal concentration and type as well as exposure time.

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1. Introduction

Maintenance of good soil quality is of prime importance for sustainable agriculture. Microorganisms play vital roles in soil fertility and primary production through organic matter, decomposition and nutrient cycling. When some stress factor such as temperature, extreme pH or chemical pollution is imposed on a natural environment, soil biota and ecological processes these microorganisms regulate are affected [1]. Geologic and anthropogenic activities increase the concentration of heavy in the soil to amounts that are harmful to both plants and animals. Some of these activities include mining and smelting of metals, burning of fossil fuels, use of fertilizers and pesticides in agriculture, production of batteries and other metal products in industries, sewage sludge, and municipal waste disposal [2-5]. Elevated concentrations of these compounds are known to affect soil microbial population and their associated activities [6, 7].

Microorganisms are far more sensitive to heavy metal stress than soil animals or plants growing on the same soils [8]. Once these elements enter the soil, they persist for thousands of years there and are very difficult to eliminate their effects in the soil- plant system. Their chemical forms depend on the origin of heavy metal and main factors which affect the mobility of elements [9].

Microbial activities can play an important role in transfer and mobility of toxic element in soils but the property of microorganisms and their ability to participate in mobilization-immobilization equilibrium of heavy metals in soil plant system are often not considered [10]. The heavy metal bioavailability can be assessed and is rarely quantified, particularly in microbial investigation [8], the role of soil microorganism in heavy metal mobilization and immobilization processes in soil has been recently discussed in some papers [11, 12]. Due to intensive development, increased heavy metals contents is clearly observed in environment nowadays and may lead to physiological and biochemical changes in plants, the most common form of them could be membrane damages, changes in enzymes activity, and inhibition of root growth. The retention of heavy metals in any fraction of soil such as exchangeable carbonates, hydroxides, Fe/Mn oxides or organic matter [13] depend on soil solution pH, soil constituent and the type of heavy metals. The metals in water soluble and exchangeable fraction would be readily bioavailable in the environment, whereas the metals in the residual fraction would not be expected to be released under natural conditions [14-16]. The aim of this research is to find out the effect of heavy metals on farmed soils and the availability of these heavy metals to microorganisms.

2. Materials And Methods

2.1 Sampling sites

Soil samples were collected during the month of November from fallow soil, newly cultivated agricultural farm and old agricultural farm. (Locations A, B, and C respectively).

Location A: The study took place in a patch of land in Nkpolu-Oroworukwo area of Port Harcourt, Rivers State, Nigeria. The site is fenced to prevent animal and human encroachment and heavy metal pollution. The fallow patch of land that has been left unfarmed for 4 years. *Meligna aborrhoae*, *Elaeis guineensis* and *Panicum maximum* plants and trees were present.

Location B was a farm land that was left to fallow for 2 years but was farmed on in the month of February. The soil site comprises of *Dioscorea rotundata*, *Curcubita pepo* and *Manihot esculenta*. Crops. The distance between each plant, point of soil collection is 15-20 cm. Houses and Canteens were located around the farm, this farm served as a garden farm.

Location C was an old farm site, farmed on for 2 years. It has mature sticks of *Manihot esculentus*, *Ananas comosa* and *Panicum maximum*. The distance between soils collected and plant is 15-25 cm. This farm site was not prevented from animal encroachment. The site was located around human settlement. Soil samples for heavy metal pollution and bioavailability study was taken from all soil locations.

2.3 Experimental design

The experimental design was randomized complete block design (RCBD). Each block unit or plot was 30 cm x 30 cm. Microbial influenced agricultural soil fertility is in the range 0-15 cm depth, volume of soil per plot was 30 x 15 x 15 = 13,500 cm^3.

2.2 Sampling method

Soil samples were collected with soil auger before pollution with different concentrations of heavy metals (Cadmium, Lead, Zinc, Copper, and Nickel), 500 g of surface soil (0-15 cm depth) was collected from each plot after tilling in sterile polythene bags. Soil sample collection was collected from 6 random points per plot and then bulked to form composite samples. Small portion (10 g) of the composite sample was transferred into sterile bottles using sterile spatula for microbiological analysis. All microbial analyses were carried out within 24hrs after sample collection. All soil samples for future analysis were stored at 4 ^{o}C according to ISO and OCED standard [17].

2.3 Physicochemical analysis of soil samples

Physico-chemical characteristics of soil under study were analyzed as per method of APHA4500, APHRP, APHA4500-NC, APHA4500-P, APHA3500-Na B, APHA3500-Mg-B, APHA3120 B. The physico-chemical properties were determined before and after heavy metal application followed by nutrient amendment. Soil samples to be analyzed for physico-chemical properties were air dried, sieved through 2mm mesh and stored appropriately.

2.4 Enumeration of bacterial population in soil

A standard spread plate method with modification was used [18]. Total viable count of heterotrophic aerobic bacteria, Actinomycetes and asymbiotic nitrogen fixers were evaluated on appropriate selective media as adopted earlier [19]. All the above microbiological media were sterilized by autoclaving at 121 ^{o}C for 15 minutes.

2.5 Determination of survival of indigenous soil microorganisms

One gram of the soil sample amended with different concentration of the heavy metals (Cd- 1, 10, 100, 200), (Zn- 1, 10, 100, 200), (Pb - 1, 10, 100, 200), (Cu- 1, 10, 100, 200) and (Ni- 10, 100, 200, 500) respectively was serially diluted in normal saline solution, 0.1ml of diluted sample was spread over the surface respective culture medium on 1, 3,5, 7, 14, 21 and 28 days after incubation. The above test was carried out for all soil sites used in this work. For bacteria, incubation was for 24 hours, after which plates that had between 30 and 300 colonies [20] were counted and recorded. Colonies expressed as colony forming unit per gram.

2.6 Bioavailability Analysis

This was carried out after 28 days of soil pollution. Isolated heterogeneous bacteria (THB) was analyzed using Atomic Absorption spectrophotometer which determines the concentrations of heavy metals absorbed by the microorganisms during exposure to heavy metals.

3.0 Results And Discussion

3.1 Effect of various Heavy metal

Effect of various Heavy metal concentrations on THB counts for Location A is shown in Table 1.

In Location A, the control values ranged from 4.9 to $0.32x10^6$ cfu/g. The range of THB in Cadmium polluted soil was between $2.1x10^4$ to $3.6x10^6$ cfu/g, the highest value was observed on Day 3 at 1ppm, while the lowest count was observed on the 28th day after pollution at 200ppm. Cadmium concentrations had no significant effect on the THB count as $p>0.05$ but the number of days had a significant effect on THB count as $p<0.05$ Effect of copper concentrations on THB count was between $1.19x10^3$ cfu/g, and $9.5x10^6$ cfu/g the highest value was observed after Day 1 at 1ppm and the lowest value was observed after 28 days of pollution. Statistically $p>0.05$, the concentrations of copper did not have an effect on THB, while the number of days had an effect on THB. Effect of Lead concentrations on THB counts was between 4.5×10^3 and 2.43×10^6 cfu/g, the highest value was observed at the concentration of 10ppm after Day 1 of pollution and the lowest value was observed on the 28th day at 200 ppm. Statistically, there was significant effect on both the concentration of Lead and the number of days as $p<0.05$. Effect of Nickel concentrations on THB counts showed the highest count of 9.1×10^6 cfu/g observed on Day 3 at 10ppm, while the lowest count of 6.20×10^3 cfu/g was observed on the day 28 after pollution at 1ppm. Statistically Nickel concentrations had no significant effect on THB count as $p>0.05$ while it had effect on number of days as $p<0.05$. Effect of various Zinc concentrations on THB counts showed the range of 4.5×10^3 to 9.0×10^7 cfu/g, the highest value was observed on the day after pollution at concentration of 1ppm, while the lowest values was observed at 200ppm on the 28 day. Statistically, Zinc concentration had no significant effect on THB counts ($p>0.05$) but the number of days had a significant effect ($p<0.05$).

Summary of the effect of heavy metal concentration in Location A showed that the number of days had a significant effect on THB counts while heavy metal concentration had no effect on THB counts ($p>0.05$) except lead.

Table 1. Total viable heterotrophic bacteria count treated with different concentrations of heavy metal in Location A (cfu/g)

Metals	**Conc. (ppm)**	**Day 1** ($\times 10^6$ cfu/g)	**Day 3** ($\times 10^6$ cfu/g)	**Day 5** ($\times 10^5$ cfu/g)	**Day 7** ($\times 10^5$ cfu/g)	**Day 14** ($\times 10^4$ cfu/g)	**Day 21** ($\times 10^5$ cfu/g)	**Day 28** ($\times 10^4$ cfu/g)
Control	0	1.87	3.40	49.00	19.30	96.00	21.80	32.00
Cadmium	1	1.91	3.60	6.80	7.30	67.00	2.10	5.10
	10	1.50	3.50	5.20	3.40	47.00	1.10	2.20
	100	1.96	1.90	3.60	1.40	12.00	1.00	4.30
	200	2.18	1.60	1.80	1.80	13.00	0.81	2.10
Copper	1	9.50	5.30	1.92	1.95	16.10	1.23	1.56
	10	2.10	5.10	1.52	1.61	19.00	3.60	7.30
	100	1.42	6.20	1.86	1.19	8.60	0.49	4.20
	200	1.99	3.20	1.73	1.06	6.30	0.46	0.19
Lead	1	1.42	1.31	6.40	1.85	9.20	0.53	0.84
	10	2.43	1.29	8.70	3.60	2.40	0.39	0.63
	100	1.35	1.12	4.30	4.20	4.30	0.23	0.62
	200	1.96	1.83	2.20	1.10	2.10	O.17	0.45
Nickel	1	2.46	9.10	8.40	0.182	1.14	1.18	4.80
	10	2.32	7.30	1.29	0.316	1.05	0.93	3.10
	100	2.86	2.70	4.60	0.175	5.40	0.66	0.62
	200	1.13	2.40	6.60	0.110	4.70	0.39	0.62
Zinc	1	90.00	2.91	18.20	15.10	820.00	11.00	4.90
	10	1.92	7.60	12.00	4.70	18.00	1.60	4.50
	100	2.04	1.21	10.80	3.10	9.00	0.56	1.70
	200	2.05	1.31	7.20	2.90	8.30	0.31	0.45

3.2 The Effects of various concentrations of heavy metals on soil in Location B as shown in Table 2

Effect of Cadmium concentrations on THB counts showed that the control values ranged from 0.3 to 20.4×10^6 cfu/g, the range of Cadmium was observed between 1.1×10^4 to 5.7×10^6 cfu/g the highest value was observed at concentration 200 ppm on Day 1 after pollution and the lowest was observed on day 28 after at concentration 200 ppm. Statistically $p<0.05$, showed that cadmium concentrations had a significant effect on THB counts as well as the number of days. The THB counts ranged between 1.8×10^4 and 8.0×10^6 cfu/g, for copper polluted soil, the highest concentration observed at 200 ppm on Day 1 and lowest value on the Day 3 at concentration 100 ppm. Statistically, copper had no significant effect on THB counts as $p>0.05$ but the number of days affected the THB counts. The THB in Lead polluted soil was ranged 1.5×10^4 to 4.6×10^6 cfu/g, highest value was observed on Day 3 at 1ppm concentration and the lowest value was observed at 100/200 ppm on the 28th day, statistically, Lead concentrations had a significant effect on THB counts as $p<0.5$ while the number of days had no effect on THB counts as $p>0.05$.

Table 2. Total Viable heterotrophic bacteria count treated with different concentrations of heavy metals in Location B (cfu/g)

Metals	Conc. (ppm)	Day 1 (x10^6 cfu/g)	Day 3 (x10^6 cfu/g)	Day 5 (x10^5 cfu/g)	Day 7 (x10^5 cfu/g)	Day 14 (x10^4 cfu/g)	Day 21 (x10^5 cfu/g)	Day 28 (x10^4 cfu/g)
Control	0	20.40	1.00	8.20	58.00	86.00	21.30	36.00
Cadmium	1	2.77	5.40	2.70	2.26	4.80	1.40	2.30
	10	2.68	2.70	4.10	1.97	0.38	0.87	5.40
	100	5.00	1.50	4.30	2.80	0.23	0.23	1.60
	200	5.70	1.40	1.10	0.79	3.20	0.12	1.10
Copper	1	1.96	8.00	18.00	1.65	1.25	1.06	36.00
	10	2.95	7.10	11.00	2.80	2.30	2.20	17.00
	100	1.15	4.20	5.40	3.30	2.80	1.20	1.80
	200	8.00	2.10	4.50	1.30	2.00	0.95	3.90
Lead	1	2.93	4.60	29.00	29.00	5.50	1.43	9.10
	10	2.10	2.80	9.80	7.40	4.90	1.40	7.30
	100	1.61	1.60	8.50	6.80	3.50	1.20	1.50
	200	1.84	1.40	4.20	2.20	2.60	1.10	1.50
Nickel	1	16.30	3.20	7.20	5.10	2.40	2.30	20.00
	10	15.40	2.90	7.40	1.50	1.20	1.80	16.00
	100	50.00	3.30	5.30	2.50	3.40	1.20	9.00
	200	10.50	2.10	3.10	1.60	1.30	0.34	2.60
Zinc	1	1.05	8.80	36.00	21.00	3.20	1.18	3.90
	10	2.98	7.60	54.00	12.00	4.80	2.80	3.50
	100	1.93	1.20	1.45	7.10	1.90	0.76	1.60
	200	1.31	1.10	1.86	6.80	2.60	0.21	0.23

THB counts on nickel polluted soil was between 2.1x10^4 cfu/g and 5.0x$10^{7,}$ the highest value was observed on Day 1 at 200 ppm, while the lowest value was observed on Day 28 at 500 ppm. Statistically there was no significant difference in Nickel concentrations on THB counts while there was a significant effect for the number of days as $p<0.05$.

Effect of Zinc concentrations on THB counts was observed to be 2.3x10^3 cfu/g to 8.8x10^6 cfu/g, the highest value was observed on Day 7 and the lowest value was observed on the Day 28 at concentration 200 ppm. Zinc concentration and number of days had no significant effect on the number of THB counts as statistically $p>0.05$. Summary of the effect of heavy metals on THB counts in Locations B showed the concentrations of heavy metals had a significant effect on THB counts $p<0.05$ while the number of days did not, $p>0.05$ though, generally, the lowest counts were recorded on Day 28.

3.3 The Effects of heavy metals concentrations of on soils in Location C

The effects of heavy metal concentration on soil samples in Location C are as shown in Table 3. The effect of Cadmium concentrations on THB counts showed the control value ranged from 0.36 to 19.6x10^6. The range of THB for cadmium polluted soil ranged 1.1x10^4 to 5.5x10^6 cfu/g. The highest value was observed on Day 1 at concentration of 1 ppm while the lowest value was observed at 200 ppm on the Day 28. Statistically $p<0.05$, cadmium concentrations affected THB counts significantly as well as the number of days. The range of concentrations of copper polluted soil on THB counts was observed between 3.8x10^3 and 2.5x10^6 cfu/g. The highest value was observed on Day 1 at concentration of 1 ppm, while the lowest value was observed on Day 28 at 200 ppm. Statistically $p<0.05$ showed that copper concentration had a significant effect on THB counts as well as the number of days. The range of THB for Lead polluted soil was observed between 7.3x10^4 to 4.2x10^6 cfu/g, the highest value was observed on the Day 3 at concentration 200 ppm and the lowest value at concentration of 200 ppm on the Day 28. Statistically, the concentration of Lead had a significant effect on THB count as $p<0.05$ as well as the number of days. The range of THB for Nickel polluted soil ranged 1.2x10^5 to 50x10^6 cfu/g. The highest value was observed on Day 1 at concentration 500 ppm, while the lowest value was observed on the Day 28 after pollution at concentration 500 ppm. Statistically, Nickel concentrations had no significant effect on THB counts as $p>0.05$ as well as the number of days. The range of THB for Zinc polluted soil was 2.1x10^4 cfu/g to 9.1x10^6. The highest value was observed on the Day 1 at 200 ppm while the lowest value was observed at 10 ppm, this showed that Zinc concentration showed no significant effect on THB counts $p>0.05$ while the number of days had a significant effect on THB counts $p<0.005$.

Table 3. Total Viable heterotrophic bacteria count treated with different concentrations of Heavy metals in Location C (cfu/g)

Metals	Conc. (ppm)	Day 1 ($x10^6$ cfu/g)	Day 3 ($x10^6$ cfu/g)	Day 5 ($x10^5$ cfu/g)	Day 7 ($x10^5$ cfu/g)	Day 14 ($x10^5$ cfu/g)	Day 21 ($x10^5$ cfu/g)	Day 28 ($x10^5$ cfu/g)
Control	0	1.95	4.60	4.00	29.60	51.0	29.30	36.00
Cadmium	1	1.97	5.60	32.00	2.73	2.78	1.56	3.30
	10	1.23	4.60	45.00	2.70	1.90	1.05	3.20
	100	1.07	3.60	22.00	1.80	1.70	0.83	0.31
	200	1.72	3.30	5.80	1.70	1.50	0.83	0.11
Copper	1	2.65	2.60	9.20	7.40	4.10	1.26	0.24
	10	2.07	2.00	8.90	5.50	3.40	0.97	0.112
	100	1.92	1.60	4.90	4.10	3.20	0.39	0.38
	200	1.01	1.10	4.10	4.00	2.20	0.11	0.038
Lead	1	2.17	2.10	19.00	16.00	5.40	0.11	0.038
	10	1.71	3.20	4.30	3.20	3.20	2.40	1.90
	100	1.27	1.00	2.80	2.60	2.50	2.90	2.60
	200	1.07	4.20	2.10	2.00	1.80	1.80	1.50
Nickel	1	1.17	5.10	2.18	9.50	2.14	2.10	1.60
	10	1.21	1.50	5.10	8.30	3.80	2.14	0.54
	100	1.24	1.80	3.50	2.2	1.80	1.35	0.34
	200	9.10	1.00	2.70	1.30	1.10	4.70	0.13
Zinc	1	2.09	8.20	1.46	1.39	4.70	2.10	1.40
	10	1.71	5.30	1.36	1.01	2.30	0.82	0.21
	100	1.07	3.70	6.40	6.30	5.20	0.112	0.34
	200	9.10	3.30	4.40	3.10	8.70	0.69	0.31

Effect of heavy metal concentrations on THB in all locations as represented in Figures 4-8 showed the concentrations of cadmium had a significant effect on all three Locations as $p<0.05$, while the Locations had no effect on THB counts. Statistically, the concentrations of copper had a significant effect on THB as $p<0.05$ while the soil locations had no effect on THB counts. Similarly, Lead affected the THB counts statistically as $p<0.05$, while the Locations had no significant effect. The concentration of Nickel showed that statistically $p>0.05$ Nickel did not affect THB counts but the Locations or type of farmed soil affected it $p<0.05$. Zinc concentrations had a significant effect of THB counts, $p<0.05$, while the Locations had no effect as $p>0.05$.

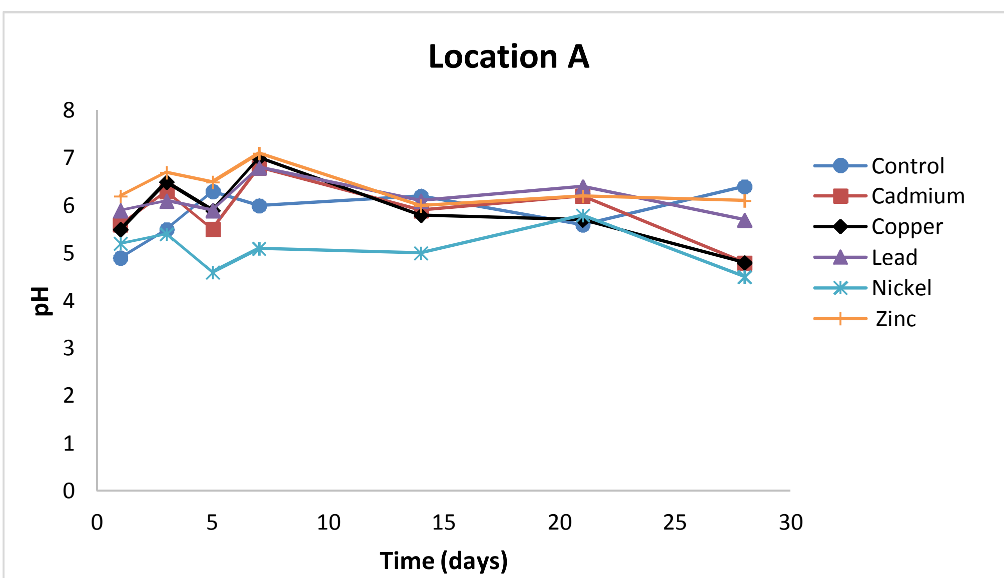

Figure 1 Effect of heavy metals on soil pH in location A

Table 4. Normal range of Heavy metals in soil and Soil critical concentration

Heavy metal	Normal range in soil (mg/kg)	*Soil critical Total concentration (mg/kg)
Ni	2 – 750	100
Cd	0.01 – 2	3 – 8
Cu	2 – 250	60 – 125
Pb	2 – 300	100 – 400
Zn	1 – 900	70 – 400

Source: Bowen [21]
***Soil critical total concentration:** Is the range of values above which toxicity is considered to be possible.

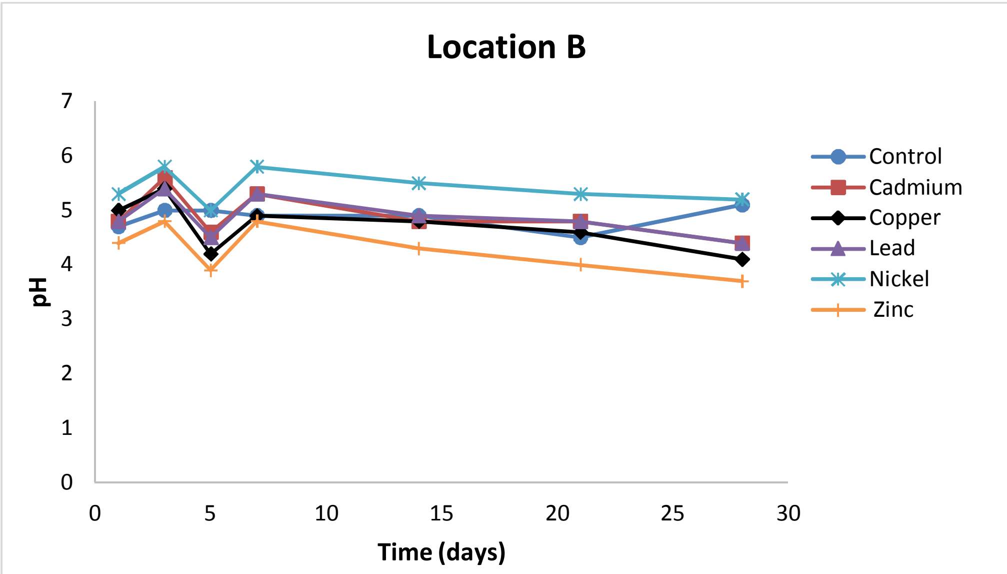

Figure 2. Effect of Heavy metal concentrations on soil pH in location B

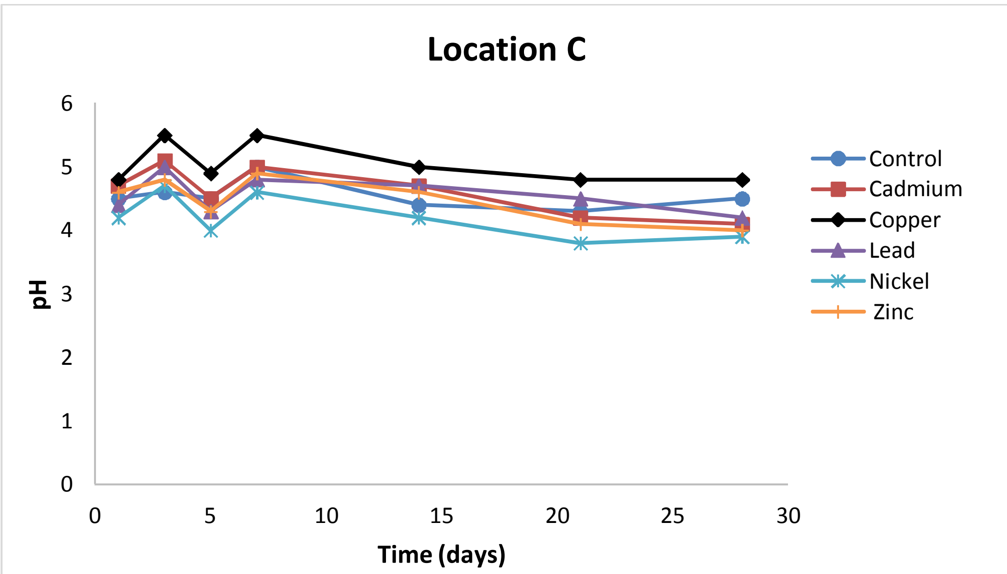

Figure 3. Effect of Heavy metal concentrations on soil pH in location C

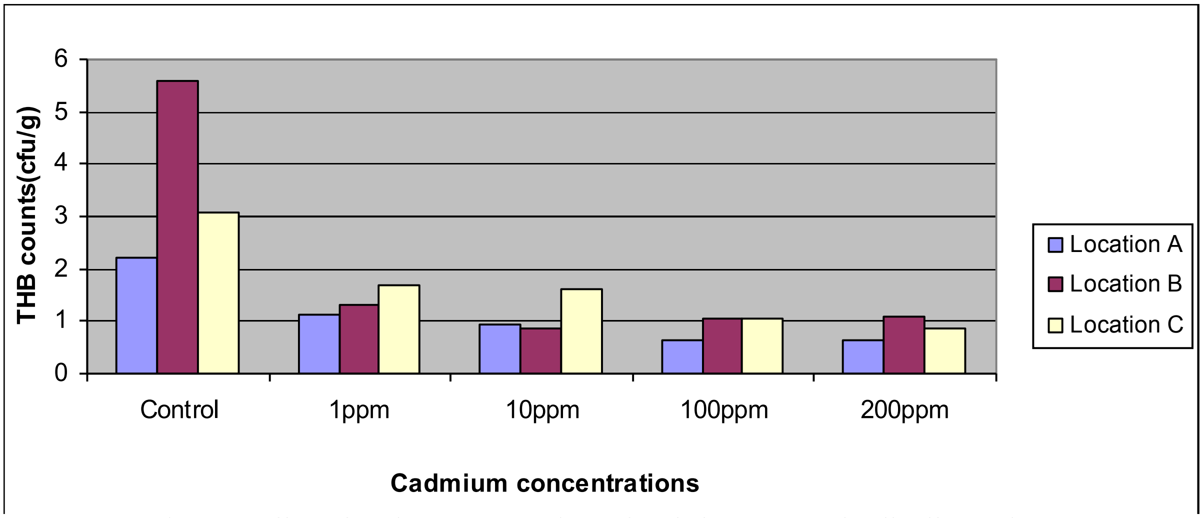

Figure 4. Effect of Various concentrations of Cadmium on THB in all soil Locations

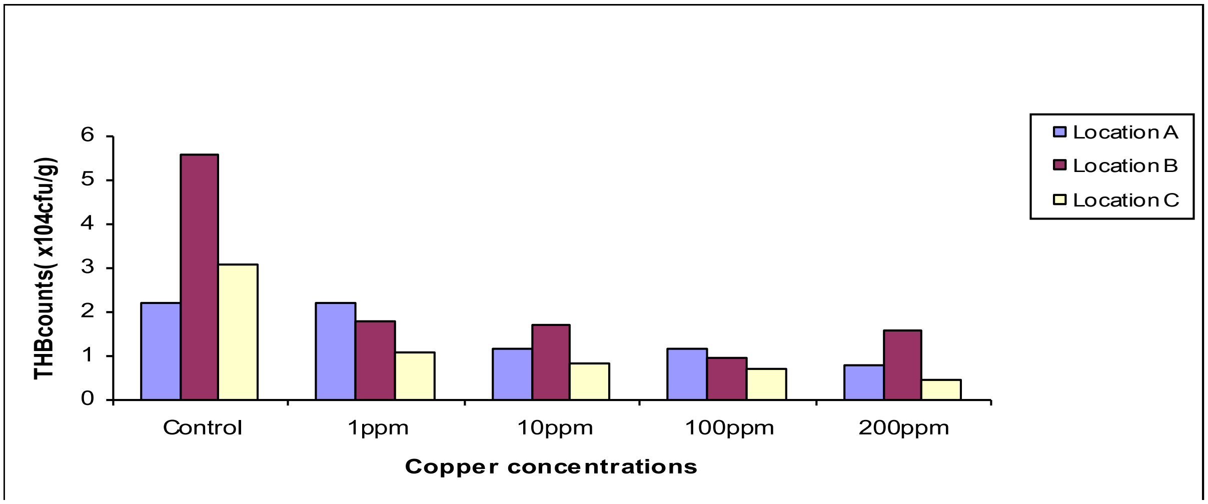

Figure 5. Effect of Various concentrations of copper on THB in all soil Locations

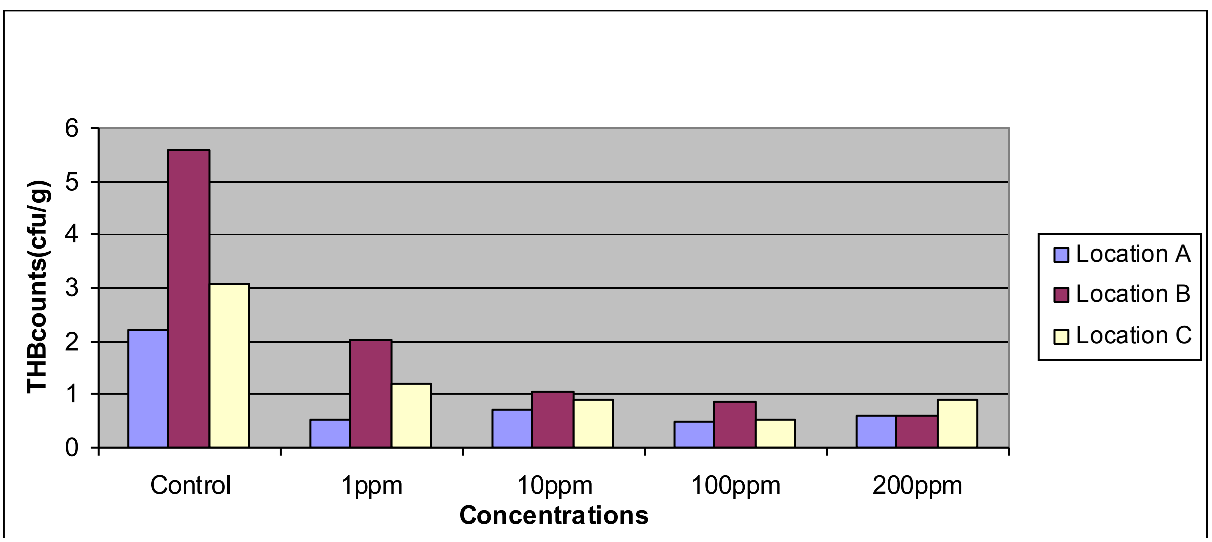

Figure 6. Effect of Various concentrations of Lead on THB in all soil Locations

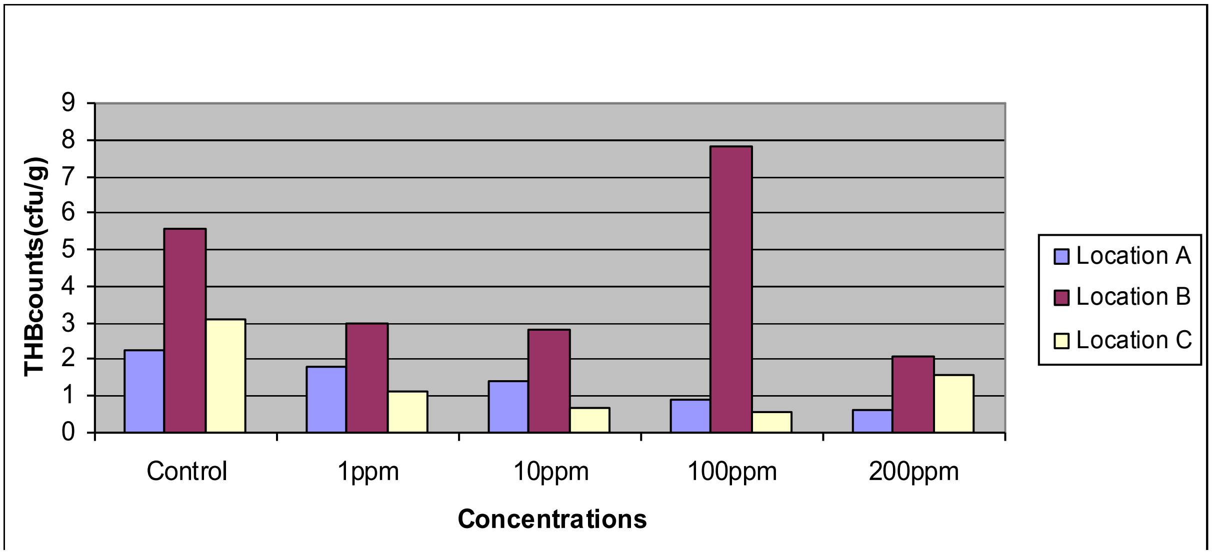

Figure 7. Effect of Various concentrations of Nickel on THB in all soil Locations

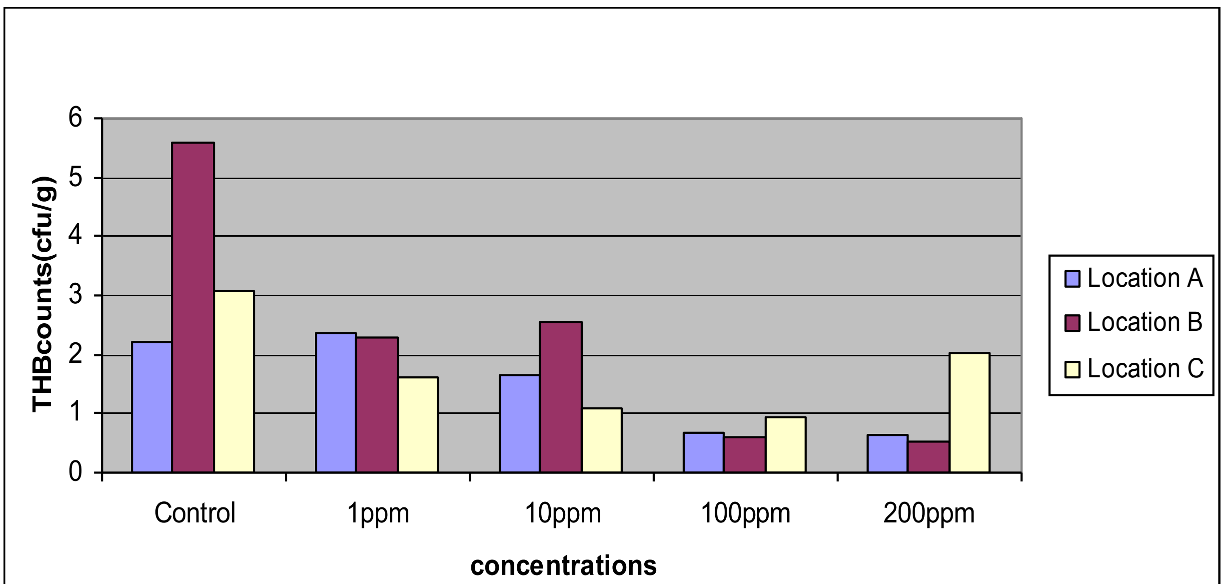

Figure 8. Effect of Various concentrations of Zinc on THB in all soil Locations

3.4 Discussion

The ecosystems are historically contaminated by toxic concentration of heavy metals, these ecosystems provide a good model for examining the effect of heavy metals on soil microorganisms since they form an integral part of the nutrient cycling and energy flow processes of the ecosystem. This study checked the effect of heavy metals on microorganisms as a result of direct pollution of the soil with various concentrations of heavy metals. Conversely, heavy metals may modify soil properties especially soil biological properties [23]. Changes in soil microbiological and biochemical properties after contamination can be used to evaluate the intensity of soil pollution at a faster rate compared with monitoring soil physical and chemical properties [24]. Heavy metals affect the number, diversity, and activities of soil microorganisms. The toxicity of these metals on microorganisms depends on soil temperature, pH, clay minerals, organic matter, inorganic anions, cations, and chemical forms of the metal [23, 25].

The pH for heavy metals polluted soils in all locations represented in Figure 1-3 is between 4.2 and 6.4 and within the range of humid soil throughout the duration of the work, the soil maintained an acidic range. At $p<0.05$ heavy metals had no effect on the pH of the soil within the period of study. pH values for

Locations A, B and C ranged 4.9 -6.4, 4.2 -5.2 and 4.2-5.4 respectively. There was no difference in pH between the metal polluted soils and control. This agreed with the work of [1] that there were no differences in pH, texture and organic matter content between two soil samples, polluted and non-polluted soils, but there were differences in heavy metal content.

Generally, the high concentrations of heavy metals in the polluted soils were reduced at the end of 28 day considerably by 50-60%. This agreed with the work of [1] that a wide range of soil properties as pH, organic matter content, clay content, iron oxide content all alter the effect of various metal levels in soil microbes. Soil properties affect metal availability in diverse ways [26]. Reported that soil pH is the major factor affecting metal availability in soil the range of pH could account for the variation in absorption of metals. Availability of Cd and Zn decreased with increases in soil pH [27]. The selectivity order of heavy metal retention in soils depends on the pH of the soil solution [28].

Total heterotrophic bacterial counts at the different Locations varied, metals react differently on microbial populations as shown in Figures 4-8, Heavy metals activity on microorganism depend differently on the soil, this agrees with the work of [29, 30]. Generally, Cadmium had most effect on bacterial populations, cadmium effect depends on the period of exposure. Soil pollution causes a pressure on sensitive microorganisms and so changes the diversity of soil microflora, representation of trophic groups of microorganisms [31]. The decrease in microbial density caused by a high level of heavy metal contamination found at the sites were examined and agrees with [32]. Locations B and C had more bacterial counts, probably because, they have acclimatized to harsh conditions through pollutions, and when soils are tilled for agricultural purposes, there is aeration and this will enhance the growth of aerobic bacteria, microbial activity is increased in agricultural soils as a result of decomposition of litters by bacteria and this provides more nutrients to the microorganisms for uptake. This agreed with the work of [33] that the influence of Cadmium and other heavy metals on the proliferation of soil microorganism is strong for light sandy soil than in clay or organic soil. The soils under study showed spatial variation in the concentrations of heavy metals in the area. [14] and [34] also reported that the adsorption properties of heavy metals by soil constituents reduce the biological impacts of the heavy metals by reducing their bioavailability. This could account for the level of bioavailability by bacteria [35], also reported that heavy metals in the soils are present in various specifications due to interactions with various soil properties. Total heavy metal concentration in soil cannot provide a precise evaluation of their influence upon soil microorganism, from this findings microorganisms take up some heavy metal that are made available to them in the environment as bacteria in the metal polluted soil absorbed more concentrations of the metal than in the control soil this agrees with the statement of [36] that the role of microbial biomass in soil fertility by serving as a reservoir of plant nutrients (especially N, P, and S) as a catalyst in the cycling of C, P and S make microbial biomass indispensable in soil nutrient flux and bioavailability.

The uptake of heavy metals by microorganisms as represented in Table 5 and Figures 9-13 shows that the uptake of heavy metals in fallow soil was low compared to the uptake in the cultivated farms, this could also be attributed to the high organic matter content in the fallow soil and this agrees with the work of [36] and [26] that high organic matter content reduces bioavailability. Bacteria have been shown to absorb little concentration of the metal and this can be transferred to plants through decomposition processes. This fact that heavy metals are made available for plant uptake by microorganisms at little concentration agrees with the work of [37] that microorganisms participate in mobilization – immobilization equilibrium..: Bioavailability was low for Locations B and C because of its high pH, this agrees with [34] that increase in pH contributes to a decrease in solubility and bioavailability of heavy metals resulting in a positive effect in the microbial communities in a remediated soil.

Bacterial tolerance to heavy metals varied according to Locations, Location A; Cd>Cu>Zn>Ni>Pb, Location B: Cu>Ni>Pb>Cd>Zn while Location C: Cd>Pb>Ni>Zn>Cu. Bacterial tolerance to heavy metals according to Location rank in the order: Cd- Locations C>A>B; Cu –Locations B>A>C; Pb-Locations C>B>A; Ni- Locations B>C>A; Zn – Locations C>A>B. This agree with the work of [38] that the ability of microbes to tolerate a definite level of the heavy metal under natural conditions might be different owing to the complex nature of the soil environment. [33, 39, 40] also reported that the number of microorganisms in the soil depend on forms of heavy metals present. It is also conditioned by several other factors such as the granulometric composition of soil, quality and quantity of organic matter, especially carbohydrate rich organic matter, pH, total exchange capacity, nutrient availability, moisture, temperature and oxygen availability. Bacteria tolerance to copper-contaminated soil varied according to Locations, as bacteria tolerance to copper increased in Locations A and B, while at Location C (Old Farm soil) most sensitive to

Cu. Though, this agrees with the findings of [41] that bacteria tolerance increases in Cu contaminated soil, but tolerance may be soil use specific.

Table 5. Bioavailability of various concentrations of heavy metal by Bacteria on all soil Locations after 28 days

Metal	Conc.	Location A		Location B		Location C	
		Control	Conc absorbed	Control	Conc absorbed	Control	Conc absorbed
Cd	1ppm		0.037		0.034		0.032
	10ppm		0.038		0.041		0.029
	100ppm	0.026	0.035	0.023	0.035	0.023	0.031
	200ppm		0.041		0.029		0.032
	Mean		0.038		0.035		0.031
Cu	1ppm		0.317		0.295		0.224
	10ppm		0.268		0.341		0.132
	100ppm	0.070	0.130	0.104	0.300	0.108	0.236
	200ppm		0.732		2.802		0.435
	Mean		0.362		0.934		0.257
Ni	1ppm		0.324		0.206		0.187
	10ppm		0.199		0.220	0.120	0.213
	100ppm	0.161	0.218	0.121	0.184		0.178
	200ppm		0.248		0.313		0.215
	Mean		0.247		0.231		0.198
Pb	1ppm		0.067		0.070		0.052
	10ppm	0.045	0.047	0.042	0.064	0.042	0.061
	100ppm		0.060		0.056		0.084.
	200ppm		0.062		0.091		0.089
	Mean		0.059				0.072
Zn	1ppm		0.816		1.059		0.842
	10ppm		1.205		0.684	0.642	0.941
	100ppm	0.655	1.048	0.636	1.176		0.981
	200ppm		1.302		1.093		1.085
	Mean		1.093		1.047		0.962

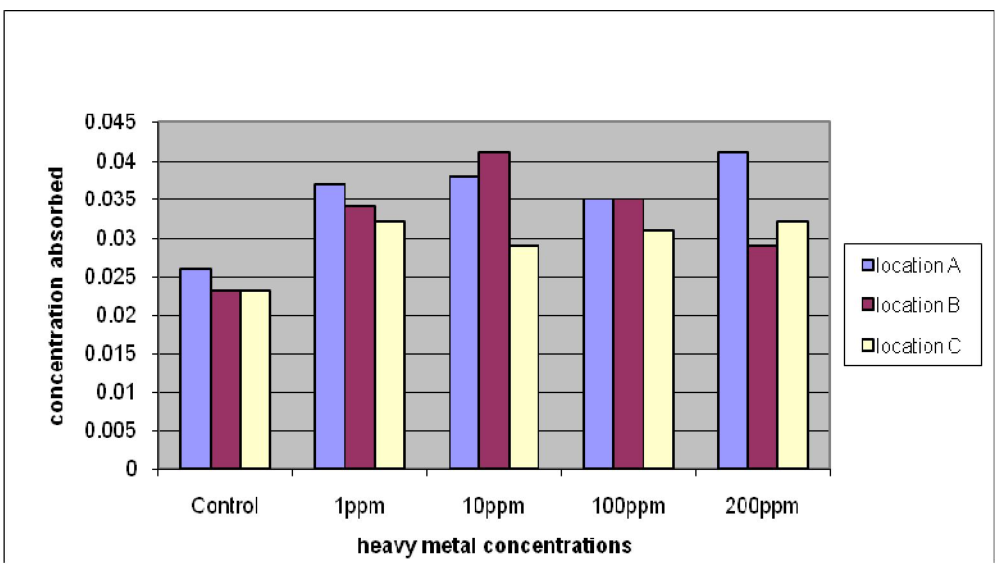

Figure 9. Absorbance level of cadmium by bacteria

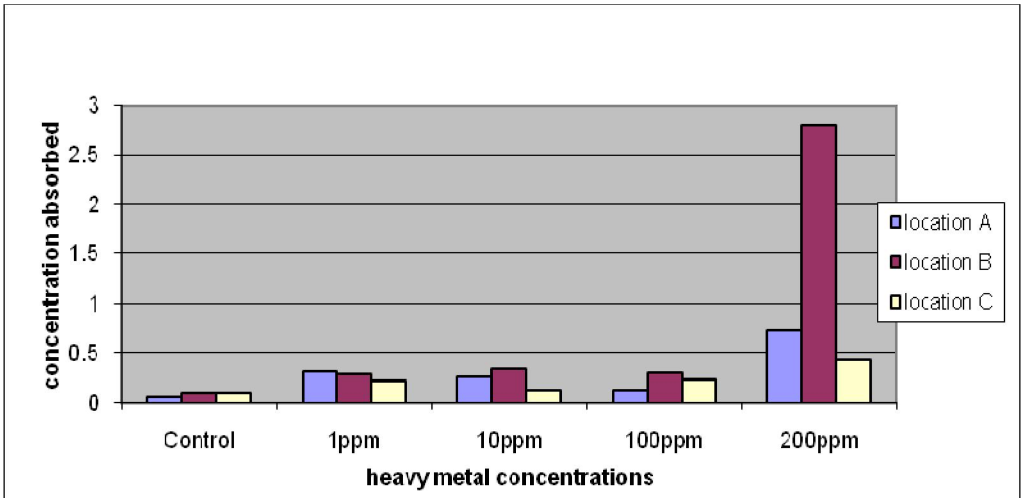

Figure 10. Absorbance level of copper by bacteria

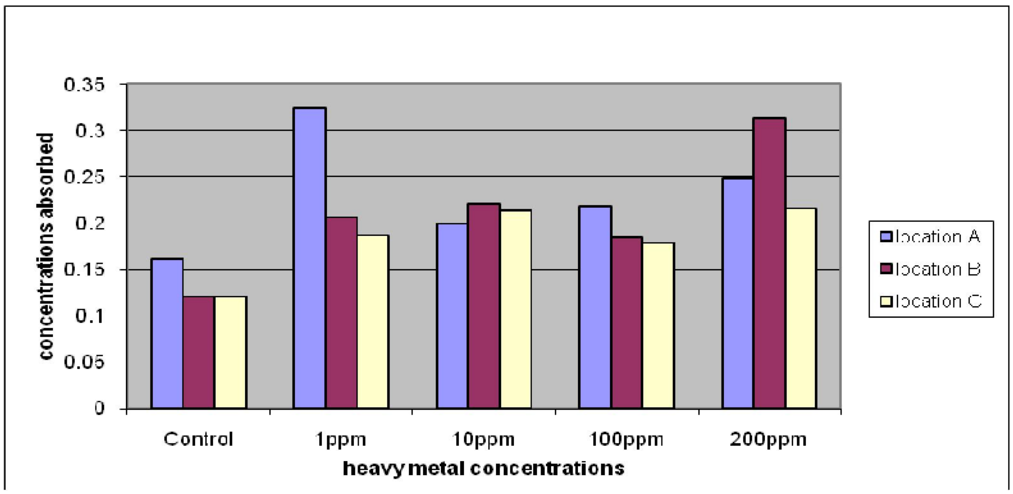

Figure 11. Absorbance level of Nickel by bacteria

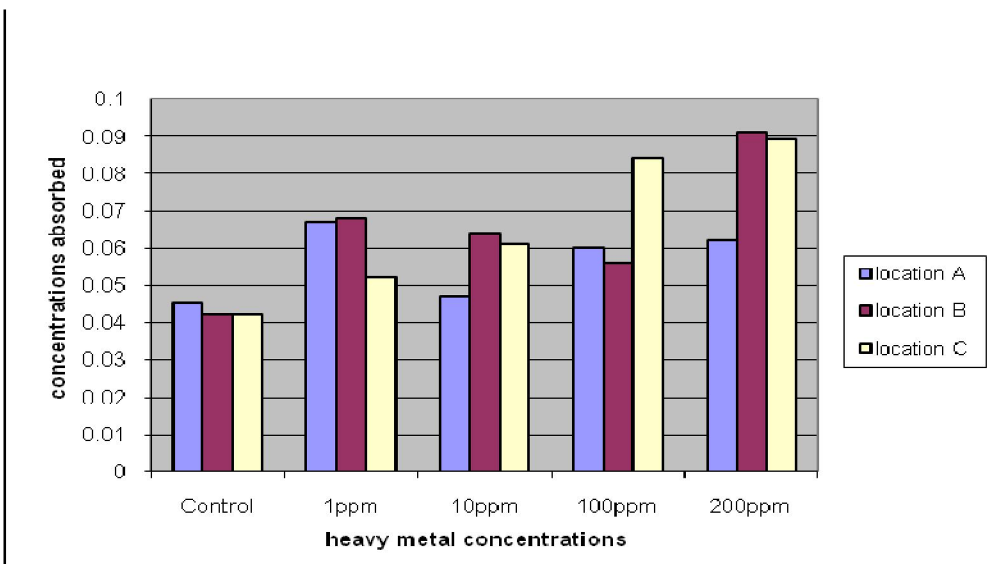

Figure 12. Absorbance level of lead by bacteria

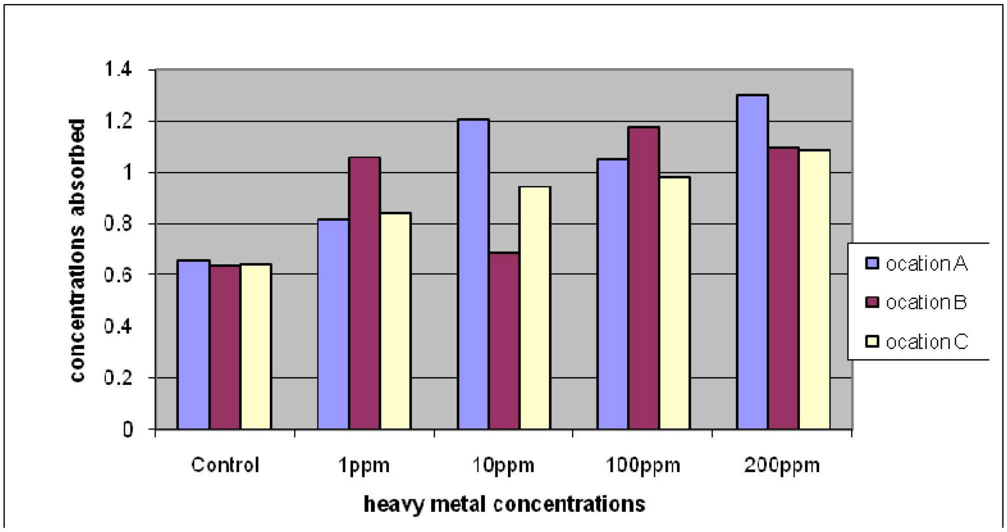

Figure 13. Absorbance level of zinc by bacteria

Comparison of the effect concentrations of individual heavy metals on all Soil Locations (A, B and C) on THB counts

Comparison of the effect of Cadmium on soil Locations on THB counts as shown in Figure 6 shows that Statistically the concentrations of Cadmium had a significant effect on all three Locations as $p<0.05$, while the Locations had no effect on THB counts. The effect of Copper on THB counts on all soil Locations as shown in Figure 7. Statistically, the concentrations of copper had a significant effect on THB as $p<0.05$ while the soil Locations had no effect on THB counts. As represented in figure 8, Statistically, $p<0.05$ the concentrations of Lead affected the THB counts, while the Locations had no significant effect. As represented in Figure 9, showed that the concentration of Nickel did not affect THB counts statistically, $p>0.05$ but the Locations affected it as $p<0.05$. Comparing the effect of Zinc on soil Locations on THB as shown in Figure 10. Statistically Zinc concentrations had a significant effect of THB counts, $p<0.05$, while the Locations had no effect as $p>0.05$.

Statistical analysis of Bioavailability of heavy metals by Bacteria

It was observed that bacterial uptake of heavy metal increased with contaminated soil, Generally, bacteria from the control soil had the least absorbed metal when compared to bacteria in heavy metal polluted soil. The bioavailability of Cadmium by bacteria as represented in Table 5 and Figures 9 – 13 showed that the concentrations of the Cadmium had significant effect on its uptake by bacteria $p<0.05$, while the locations had no significant effect on bacteria uptake of the metal. The concentrations of copper as well as the soil Location had no effect on the uptake of copper by bacteria as $p<0.05$, the concentration of Nickel affected its uptake by bacteria $p<0.05$, while the Locations did not affect the bacteria uptake of the metal $p>0.05$. The concentrations of Lead had a significant effect on bacteria uptake of the metal as $p<0.05$ while the Locations had no effect. $p>0.05$ The concentration of Zinc affected the metal uptake by bacteria statistically, $P<0.05$ while the Locations did not affect Zinc uptake by bacteria as $p>0.05$. This agrees with the findings of [42-44] that potentially available (bioavailable) heavy metals may change depending upon the surrounding physical and geochemical conditions. [34] also reported that the adsorption properties of heavy metals by soil constituents which reduce the amount of heavy metals seek to reduce the biological impacts of the heavy metals by reducing their bioavailability. This could account for the level of bioavailability by bacteria recorded in this finding. [35] also reported that heavy metals in the soils are present in various specifications due to interactions with various soil properties. Total heavy metal concentration in soil cannot provide a precise evaluation of their influence upon soil microorganism.

A summary of the effect of heavy metals on soil microbial populations indicated that heavy metal toxicity is concentration and time dependent. This agreed with the work of [22] that heavy metal toxicity was concentration and time dependent. Bacteria uptake for Cadmium is low, this is because they do not require it in their metabolic pathway, this could explain why their absorbance level is low for Cadmium. This agreed with the work of [3] that bacteria have low absorption for Cadmium.

The concentrations of heavy metals used for this work were not able to affect the decomposition activity of bacteria on total organic matter, this could explain why there was an increase in total organic matter in all soil Locations, polluted with heavy metal and this disagree with the work [44-46]. This fact that heavy metals are made available for plant uptake by microorganisms at little concentration agrees with the work of [37] that microorganisms participate in mobilization – immobilization equilibrium.

From the findings above, microbial tolerance to heavy metals depend on farm use, heavy metals used in this study did not have much effect on microbial populations in the Farmed soils (Locations B and C) when compared to the Fallow soil (Location A). This agrees with the work of [31], that the influence of Cd and other heavy metals on the proliferation of soil microorganism is strong for light sandy soil than in clay or organic soil. When the soil is polluted with heavy metals, these metals are made available for uptake by microorganisms, although the microorganisms absorb only minute quantity of the metal, heavy metals are made available to plants through decomposition processes (manure or decaying organic material) by these microorganisms, These metals are made available for plants to use, animals and man that feed on plants get exposed to these heavy metals and these metals tend to accumulate in man who is the final user thereby posing threat to human life. From these findings, heavy metals used in this study did not have much effect on microbial populations in the farmed soils (Locations B and C) when compared to the fallow soil. The findings indicate that heavy metal toxicity is concentration and time dependent. Heavy metal toxicity and bioavailability varied according to soil Locations. Long term exposure of heavy metals to microorganism affected microbial population; microorganisms' diversity should be chosen instead of total counts of bacteria. From these findings, microorganisms take up some heavy metal that are made available to them in the environment. The concentrations of heavy metals did not have much effect on soil microbial population this is because the soil parameters come into play reducing the heavy metal concentration.

Conflict of interest. Author declares there is no conflict of interest.

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