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The effects of heavy metals inhibitory activity on microbial enzymes assay in polluted soil

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Abstract

The impact of Hg^{2+} and Fe^{2+} on microbial soil enzyme activity in polluted and unpolluted soil samples was assessed. Soil samples were collected from fallow land (FAL), dump site (DS), farm land (FAM) and oil impacted soil from mechanic village (MV). The soils were polluted with varied concentrations of Hg^{2+} and Fe^{2+} and their microbiological and enzyme activities were assessed before and after the addition of the metal ions. The enzymes, dehydrogenases, phosphatases, ureases, and catalases were affected by*Aspergillus* sp, *Saccharomyces* sp, *Corynebacterium* sp, *Enterococcus* sp and*Bacillus* sppafter the addition of heavy metals. However, their impact varied with concentrations of the heavy metal. Predominant bacterial isolates in polluted and unpolluted soil samples include *Bacillus subtilis, Staphylococcus aureus*, *Micrococcus roseus*, *Micrococcus luteus, Bacillus cereus, Bacillus lichieniformis* and *Pseudomonas* sp. *Penicillium notatum, Mucor* sp, *Penicillium* sp, *Cladosporium* sp, *Rhizopus nigricans, Rhizopus stolonifer, Fusarium* sp were also isolated as predominant fungi. However, varied activity was observed depending on the nature of the soil and the concentrations of Hg^{2+} and Fe^{2+}. The study concludes that heavy metals of Hg^{2+} and Fe^{2+} had inhibitory activities on soil microbial population,microbial diversity and microbial soil enzyme activity.

Keywords: heavy metals, polluted soil, microbial enzyme kinetics

Introduction

Heavy metals are inherent components of soils, but a great concern today is related to their accumulation due to anthropogenic activities. The soil is affected negatively under conditions caused by adverse anthropogenic effects such as dissemination of chemical pollutants like heavy metals. The development and biochemical activities of soil microorganisms undergo several alterations when the soil has been contaminated with heavy metals. (Filip, 2002). Many reports have shown that short-term or long-term exposure to heavy metals results in the reduction of microbial diversity and activities in soil (Akmal *et al*., 2005). Diversity and activity of microbial communities are important indices of soil quality. Soil Enzymes are synthesized by microorganisms and act as biological catalysts to facilitate different reactions and metabolic processes to

decompose organic pollutants and produce essential metabolites or intermediary metabolites for both microorganisms and plants (Moreno *et al*., 2006).Soil enzymatic activities are recognized as a more sensitive bio-indicator than plants and animals of any natural and anthropogenic disturbance (Hinojosa *et al*., 2004). The effect of heavy metals on biological activity of soil depends on the physicochemical properties of soil, particularly on its humic content. On the other hand, it is also dependent on concentrations as well as kinds of pollutants or enzymes involved (Moreno *et al*., 2001). The negative influence of most of the heavy metals on the activity of soil enzymes was reported by Wyszkowska and Kucharski (2003).

This study was therefore carried out to evaluate the effect of heavy metals (Iron and Mercury) on soil enzymatic activity of dehydrogenase, phosphatases (acidic and alkaline), urease, and catalase under controlled laboratory conditions using different soil samples as a case study.

Materials and Methods

Sample Collection and Preparation: Four composite soil samples were collected from different locations in Owerri west, Imo State. Soil samples were collected from dump sites at locations which include Eziobodo, Umuchima and Hostel D (school campus). Petroleum polluted Soil samples were also collected from mechanic village located at Naze, Imo state. The soil samples were collected using a soil auger at a maximum of 30 cm depth. The soil samples were taken to the laboratory where it was dried, crushed to remove soil lumps and sieved. Samples were air-dried at room temperature (25-35ºC); relative humidity 20-60%) and stored. The four soil samples which are collected from the dump site, mechanic village, farm land, and fallow land were designated DS, MV, FAM, and FAL respectively. Composite sampling was adopted in making a total of four samples per sampling location for statistical purposes.

Preparation of Media for Microbiological Analysis

Bushneii Haas Agar, Eosine Methylene Blue Agar, Nutrient Agar, *Salmonella Shigella* Agar, Cetrimide Agar and Potato Dextrose Agar were prepared according to the manufacturer's specification.

Preparation of Soil Samples and Inoculation

Ten grams (10g) of soil samples wereadded into a conical flask containing 90ml of sterile diluents and shaken vigorously to suspend the soil samples. Serial dilutions of the soil samples were made up to $10^{-6}$and an aliquot (0.1 ml) inoculated into the different culture media and spread uniformly prior to incubation at ambient temperature for 24 - 48 h for bacteria and 72 - 96 h for moulds. Vapour phase technique was adopted for the isolation of hydrocarbon utilizing bacteria on Bushneii Haas agar.

Assay for Soil Enzyme Activity

Soil Catalase Test

By a modified method described by Fidelis and Patrick (2014), one hundred ml of phosphate buffer, pH 7.4, was added to 10 g of soil and stirred vigorously. The soil suspension was filtered using cheesecloth. The filtrate was centrifuged at maximum speed of 7000 g for 10 min to obtain supernatant (S1). Catalase activity was determined as described by Rani *et al*. (2004). Catalase breaks down hydrogen peroxide to give oxygen that oxidises potassium dichromate. The oxidation of chromate gives a chromophore that absorbs maximally at 610 nm. The enzyme extract (0.5 ml) was added to the reaction mixture containing 1 ml of 0.05 M phosphate buffer (pH 7.5), 0.5 ml of 0.2 MH_2O_2, 0.4 ml H_2O and incubated for different time period t1, t2 and t3 for 1 minute, 2 minutes and 3 minutes respectively. The reaction was terminated after each time interval by the addition of 2 ml of

acid reagent (dichromate/acetic acid mixture) which was prepared by mixing 5% potassium dichromate with glacial acetic acid (1:3 by volume). To the control, the enzyme was added after the addition of acid reagent. All the tubes were heated for 10 min in boiling water and the absorbance was read at 610 nm. Catalase activity was expressed in terms of moles of H_2O_2 consumed/min.

Soil Dehydrogenase Assay

The dehydrogenase activity of the soil was determined by the reduction of 2, 3, 5-triphenyl tetrazolium chloride to 2, 3, 5-triphenyl formazan (TPF) as described by Casida*et al.* (1964).Soil samples (1g) were taken in 25 x 150mm capacity screwed cap glass tubes. 50mg of calcium carbonate was added followed by addition of 2.5ml of distilled water and 1ml of 3% TTC. The contents were swirled for few minutes and incubated at room temperature for 24 hours. The red precipitate of TPF was dissolved in methanol and the contents were shaken twice with 10ml portion of methanol for half an hour and filtered.The filtrate was made to 25ml with methanol and the red colour intensity was measured on a spectrophotometer at 485nm. The dehydrogenase activity was measured with respect to the amount of TPF produced and expressed as µg of TPF produced g^{-1} soil h^{-1}.

Soil Urease Test

Soil urease test was described by Tabatabai and Bremner (1972) modified by Dorich and Nelson (1983). The procedure is described below.Soil sample (5 g) was taken in 25 x 150 mm capacity screw capped tubes. 9ml of distilled water was added, the contents were gently mixed followed by addition of 1ml of 0.2M urea. The contents were then swirled and incubated at 37 ± 0.5°C for two hours in BOD incubator. The reaction was terminated by addition of 15ml of KCl- Ag_2SO_4 solution. The contents were agitated on mechanical shaker for one hour to release all NH_4^+formed and the suspension was allowed to settle. Control soil samples were run simultaneously in the same way except adding 1ml of 0.2M urea solution after termination of reaction. One ml of supernatant from the soil suspension after incubation with urea and deactivation with KCl- Ag_2SO_4 was transferred to 25ml volumetric flask. To this, 1ml of 6% EDTA was added followed by addition of 2ml of Phenol-nitroprusside and 8ml of buffered hypochlorite reagent. The volume was then made up to the mark, mixed thoroughly by inverting several times and placed in water bath maintained at 40°C. Thirty minutes were allowed for colour development. The flasks were removed and brought to room temperature and the absorbance of blue colored complex was measured at 636nm on spectrophotometer. The urease activity was measured with respect to the amount of NH_4^+ liberated and expressed as µg of NH_4^+ released g^{-1} soil h^{-1}.

Soil Phosphatase Test

Four milliliter (4 ml) of modified universal buffer pH 6.5 (for assay of acid phosphatase) and pH 11 (for assay of alkaline phosphatase) was added into glass tubes containing one gram of soil samples. This was followed by followed by addition of 1ml of 4-nitrophenyl solution. The glass tubes were swirled for few seconds to mix the contents, stoppered and incubated for one hour at 37 ± 0.5°C in BOD incubator. To these, one ml of 0.5M $CaCl_2$ was added followed by addition of 4ml of 0.5M NaOH to deactivate the enzyme and to extract the 4-nitrophenyl liberated. The glass tubes were swirled and the soil suspension was filtered through Whatman No. 42 filter paper. The absorbance of yellow colour of 4-nitrophenol liberated due to hydrolysis of the substrate by phosphomonoesterases was measured at 420nm. Controls were run simultaneously following the same procedure except adding 1ml of 4-nitrophenyl phosphate. Corrections were made for control/blank values. The method adopted here was described by Tabatabai and Bremner (1969) and Eivazi and Tabatabai (1977).

Experimental design

Soil samples were supplemented in order to imitate anthropogenic activities as shown in Table 1. After pollution of the soil samples with the different concentrations of the two heavy metals, they were left fallow for 2 weeks.

Subsequently, they were subjected to the same test and assays such as soil microbiology, soil urease test, soil dehydrogenase, soil catalase and soil phosphatase.

Table 1. Experimental design after supplementing with heavy metals

Soil Samples	Control	Ferric Chloride		Mercuric Chloride	
		10 ml	5 ml	10 ml	5 ml
Dump Site (DS)	DS	DS 10 ml, DS 10 ml	DS 5 ml, DS 5 ml	DS 10 ml, DS 10 ml	DS 5m l, DS 5 ml
Mechanic Village (MV)	MV	MV 10 ml, MV 10 ml	MV 5 ml, MV 5 ml	MV 10 ml, MV 10 ml	MV 5 ml, MV 5 ml
Fallow Land (FAL)	FAL	FAL10 ml, FAL 10 ml	FAL 5 ml, FAL 5 ml	FAL10 ml, FAL 10 ml	FAL 5 ml, FAL10 ml
Farm Land (FAM)	FAM	FAM 10 ml, FAM 10 ml	FAM 5 ml, FAM 10 ml	FAM 10 ml, FAM 10 ml	FAM 5 ml, FAM 10 ml

Results

Soil Enzymatic Activity of soil before treatment

Figure 1 shows the soil enzyme activities of the tested soil samples. The results recorded higher values of soil dehydrogenase activity in mechanic village while, the lower values of soil dehydrogenase activity were recorded in fallow land.

Similarly, the results recorded higher value of catalase activity in mechanic village while the least value for catalase activity was seen in dump site.

Furthermore, the results recorded higher values of urease activity in mechanic village and the least value were recorded in fallow land for urease activity. For acid phosphatase, fallow land recorded the highest activity followed by farm land and dump site. Mechanic village recorded the lowest acid phosphatase activity.

The highest value for alkaline phosphatase activity was recorded in dump site followed by farm land and mechanic village. Fallow land recorded the lowest amount of alkaline phosphatase activity.

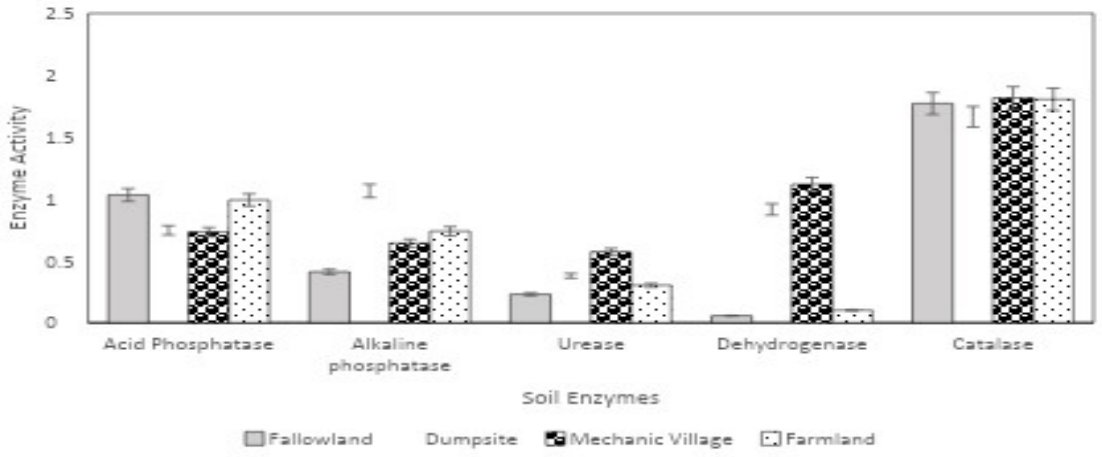

Figure 1 Mean Soil Enzymes Activities of Selected Soil Sample

Soil Microbiology - Population of cultivable bacteria in soil samples

The results of soil microbiology for the different culture were calculated as colony forming units/gram of soil sample. Table 1 displays the counts gotten from the different cultural plates. TEC stands for Total Enterobacteriaceae Count, TSSC stands for Total *Salmonella Shigella* Count, THFC stands for Total Heterotrophic Fungal Count, THBC stands for Total Heterotrophic Bacterial Count and CET represents the Cetrimide Count. For TEC, dump site recorded the highest number of colony counts while there was no single count recorded in mechanic village.

Farm land samples recorded higher number of counts in TSSC while a lower count was recorded in mechanic village. Farm land recorded the highest number of counts in THFC. Higher number of counts was also recorded in farm land for THBC.

For CET, dump site recorded higher colonial counts while fallow land recorded the lowest number of counts. In TEC, dump site recorded the highest number of counts while mechanic village had no counts.

Table 1 Colony Counts of Bacteria and Fungi on Soil Samples

Soil Samples	TEC	TSSC	THFC	THBC	CET
Fallow Land	1.0×10^{6}	7.5×10^{5}	1.5×10^{7}	7.3×10^{7}	2.5×10^{5}
Dump Site	3.5×10^{6}	7.5×10^{5}	1.2×10^{7}	4.7×10^{8}	3.5×10^{6}
Mechanic Village	0	5.0×10^{5}	1.0×10^{7}	2.8×10^{8}	7.5×10^{5}
Farm Land	2.3×10^{6}	2.8×10^{6}	3.6×10^{7}	3.9×10^{8}	2.3×10^{6}

Bushneii Haas Agar

Table 2 shows the values of the Total Hydrocarbon Utilizing Bacteria (TUB). The Total counts of Hydrocarbon Utilizing Fungi (TUF) and their percentages (TUB% and TUF %). From the results obtained, farm land recorded the highest number of HUB while lower amounts were recorded in fallow land and mechanic village.More number of HUF was recorded in farm land followed by dump site according to the results obtained. Mechanic village recorded the lowest number of HUF.

Table 2 Colony Counts of Hydrocarbon utilizing Bacteria and Fungi on BHA

Soil Samples	**HUB**	**HUF**	**%HUB**	**%HUF**
Fallow Land	7.8×10^{6}	7.0×10^{6}	61.88	46.5
Dump Site	9.3×10^{6}	1.9×10^{7}	380	252.32
Mechanic Village	8.0×10^{6}	5.0×10^{5}	61.43	6.25
Farm Land	1.9×10^{7}	9.1×10^{7}	112.5	885.55

Distribution of Bacteria and Fungi on different locations of sampling

From the results obtained in Table 3, soil samples from mechanic village and dump site recorded more bacteria than fallow land and farm land. The diversities of the bacteria is shown in the Table 3. *Bacillus, Staphylococcus, Enterococcus* species are predominant in the four locations.

Table 3 Distribution of Bacteria on Nutrient Agar

Soil Sample	Distribution of probable Bacterial isolates
Fallow Land	*Bacillus* sp, *Enterococcus* sp, *B. lichieniformis, Bacillus cereus*
Dump Site	*Serratia* sp, *Bacillus* sp, *Micrococcus* sp, *Staphylococcus* sp, *Enterococcus* sp, *Staphylococcus* sp, *Bacillus luteus, Bacillus cereus*
Mechanic Village	*Micrococcus luteus, Staphylococcus* sp, *Enterococcus* sp, *Bacillus* sp, *Micrococcus roseus, Bacillus subtilis, Micrococcus luteus, Corynebacterium* sp, *Enterococcus* sp
Farm Land	*Staphylococcus* sp, *Bacillus* sp, *Enterococcus* sp, *Micrococcus* sp, *Corynebacterium* sp, *B. cereus*

Table 4 shows the distribution of fungal isolates on the potato dextrose agar medium. The results obtained showed that *Saccharomyces* and *Mucor* species are predominant in all the soil samples. *Rhizopus stolonifer* was present in dump site while *Rhizopus nigricans* was present in farm land. *Cladosporium* species was isolated from soil in mechanic village.

Table 4 Distribution of Fungi on Potato Dextrose Agar

Soil Sample	Distribution of probable Fungal isolates
Fallow Land	*Saccharomyces* sp, *Penicillium* sp, *Mucor* sp, *P. notatum*
Dump Site	*Saccharomyces* sp, *P. notatum, Mucor* sp, *Rhizopus stolonifer, Aspergillus* sp
Mechanic Village	*P. notatum, Saccharomyces* sp, *Mucor* sp, *Penicillium* sp, *Cladosporium* sp
Farm Land	*P. notatum, Saccharomyces* sp, *Fusarium* sp, *Mucor* sp, *Rhizopus nigricans, Penicillium* sp

Soil Enzymatic Activity

Figure2 shows the soil enzyme activities of the tested soil samples after pollution with two different concentration of ferric chloride (5ml and 10ml).

5ml Concentration of Ferric Chloride: The results recorded higher values of dehydrogenase activity in mechanic village while lower values of dehydrogenase were recorded in fallow land. Urease activity was also higher in mechanic village while fallow land recorded the lowest urease activity. More catalase activity was recorded in dump site and less catalase activity was recorded in farm land. Higher values of acid phosphatase were recorded in fallow land while lower values were recorded in dump site. For alkaline phosphatase, dump site recorded the highest value while lower values were recorded in fallow land.

10ml Concentration of Ferric Chloride: Higher values of dehydrogenase activity were recorded in farm land while lower values of dehydrogenase were recorded in fallow land. Urease activity was higher in mechanic village while it was lower in fallow land. The results obtained also recorded higher values of catalase in farm land while lower values were recorded in fallow land. Higher values of alkaline phosphatase were recorded in farm land while lower values were seen in mechanic village. For the acid phosphatase, lower values were recorded in mechanic village while higher values were recorded in farm land.

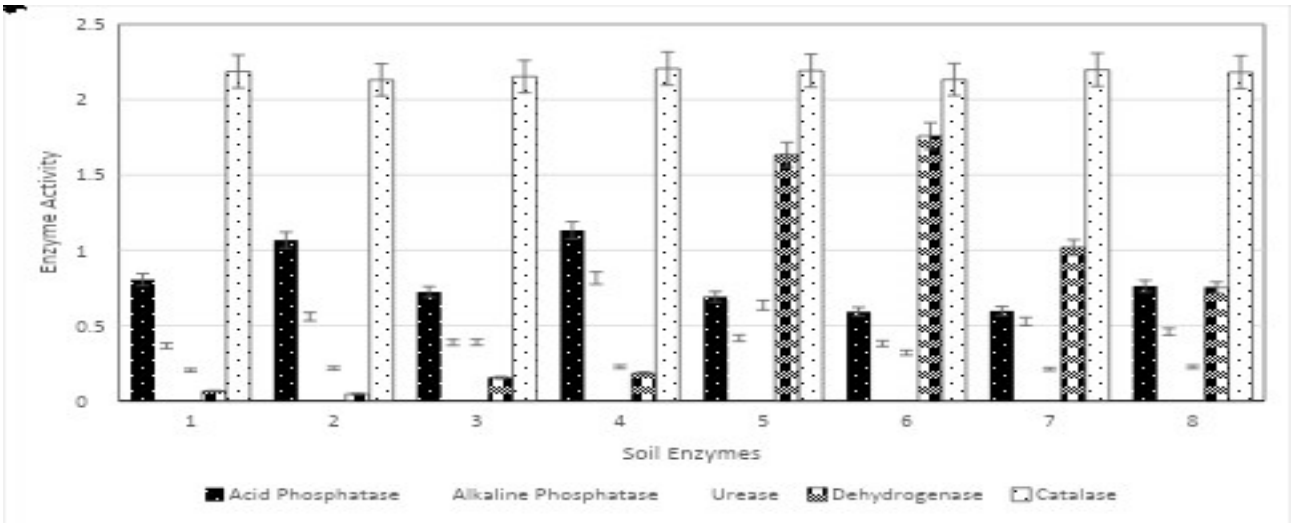

Figure 2 Mean soil enzymes activities of selected soil samples after pollution with ferric chloride

Figure 3 shows the soil enzyme activities of the tested soil samples after pollution with two different concentration of mercuric chloride (5ml and 10ml).

5ml Concentration of Mercuric Chloride: Higher values of catalase activity were recorded in dump site while the lowest was seen in fallow land. Dehydrogenase activity was present in more amounts in farm land while it was lower in mechanic village. The results obtained recorded higher values of urease activity in fallow land while it was lower in dump site. The activity of alkaline phosphatase was higher in farm land while it was lower in fallow land. More so, acid phosphatase recorded higher values in fallow land while lower values were recorded in mechanic village.

10ml Concentration of Mercuric Chloride: The results recorded higher values of dehydrogenase activity in dump site while lower values were recorded in fallow land. Urease activity was more pronounced in farm land while it was less in mechanic village. The values of catalase activity were higher in farm land while it was lower in dump site. Acid phosphatase recorded higher values in fallow land and lower values in dump site while alkaline phosphatase recorded higher values in farm land and lower values was recorded in dump site.

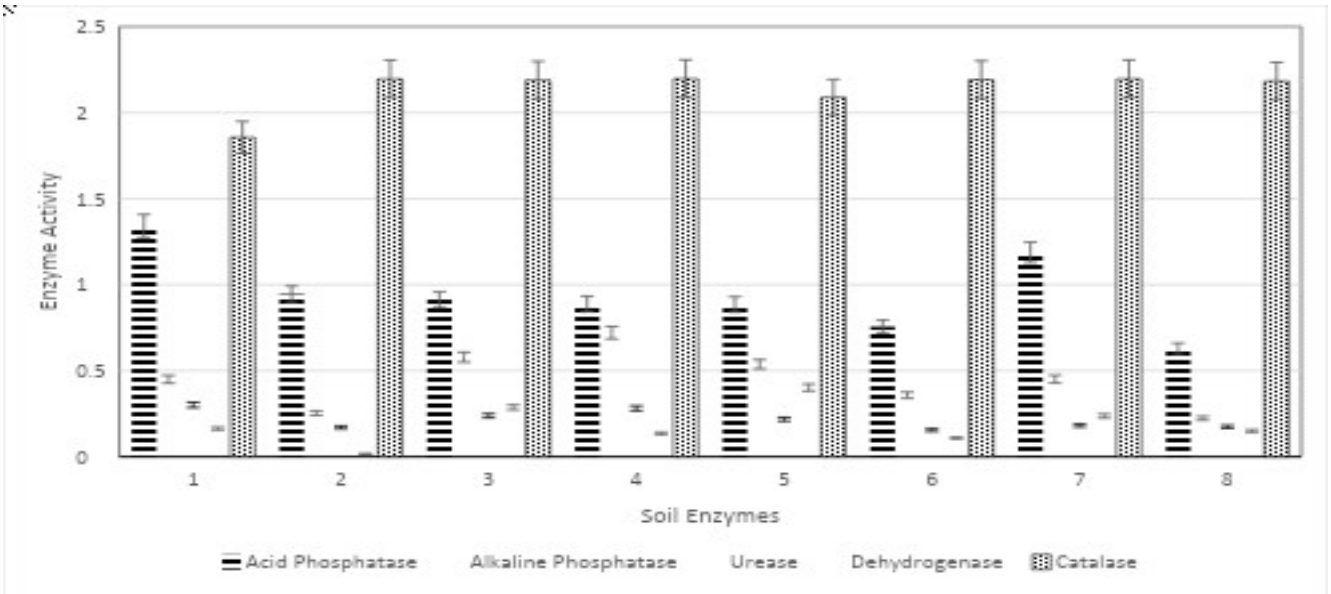

Figure 3 Mean soil enzymes activities of selected soil samples after pollution with mercuric chloride

Soil Microbiology

The results of soil microbiology for the different culture were calculated as colony forming units/gram of soil sample.

Table 5shows the total counts obtained from the different culture media after it has been polluted with different concentrations of ferric chloride notably 5ml and 10ml. From the results obtained, there was no growth in all the tested samples for TSSC and CET.

Table 5 Total Counts of bacteria and Fungi on soil treated with Ferric Chloride

Soil Samples		TEC	TSSC	THFC	THBC	CET
Fallow Land	5ml	0	0	1.7×10^{7}	3.8×10^{7}	0
	10ml	0	0	0	5.5×10^{6}	0
Dump Site	5ml	0	0	2.9×10^{7}	5.3×10^{7}	0
	10ml	0	0	0	2.2×10^{7}	0
Mechanic Village	5ml	0	0	5.5×10^{6}	4.8×10^{7}	0
	10ml	0	0	0	5.5×10^{6}	0
Farm Land	5ml	0	0	4.5×10^{6}	4.1×10^{7}	0
	10ml	3.0×10^{6}	0	0	1.1×10^{8}	0

Table 6 shows the total counts obtained from the different culture media after it has been polluted with different concentrations of mercuric chloride (5ml and 10ml). From the results obtained, there was no growth recorded in TEC for dump site, mechanic village and farm land on 5ml concentrations. In TSSC, no growth was recorded on fallow land (5ml), dump site (5ml) and farm land (5ml and 10ml). Farm land also showed no growth for CET (5ml and 10ml).

Table 6 Total Counts of bacteria and Fungi on soil treated with Mercuric Chloride

Soil Samples		TEC	TSSC	THFC	THBC	CET
Fallow Land	5ml	2.1×10^{8}	0	2.3×10^{8}	1.7×10^{8}	1.4×10^{8}
	10ml	4.1×10^{7}	9.0×10^{7}	4.1×10^{7}	4.9×10^{7}	8.0×10^{7}
Dump Site	5ml	0	1.5×10^{6}	8.5×10^{6}	1.7×10^{7}	1.5×10^{6}
	10ml	5.0×10^{5}	0	0	2.8×10^{7}	1.0×10^{6}
Mechanic Village	5ml	0	1.7×10^{7}	3.8×10^{7}	7.2×10^{7}	2.6×10^{6}
	10ml	7.0×10^{7}	3.5×10^{6}	4.8×10^{7}	4.4×10^{8}	6.9×10^{7}
Farm Land	5ml	0	0	1.1×10^{7}	1.9×10^{7}	0
	10ml	5.0×10^{5}	0	0	2.7×10^{7}	0

Bushneii Haas Agar

The percentage of hydrocarbon utilizing bacteria and hydrocarbon utilizing fungi were calculated using the counts obtained on Bushneii Haas Agar plates.

Table 7 and Table 8 shows the results of the Total Hydrocarbon Utilizing Bacteria (TUB), Total Hydrocarbon Utilizing Fungi (TUF), their percentages (TUB% and TUF %) obtained for ferric chloride and mercuric chloride respectively.

From Table 7, dump site recorded the highest count of HUB for 5ml concentration while fallow land recorded the lowest HUB. For 10ml concentration, HUB was higher in fallow land and farm land. Fallow land recorded highest counts of HUF for 5ml concentration while it was lower in farm land. For 10ml concentration, HUF was higher in farm land and lowest in mechanic village.

Table 7 Total Counts of bacteria and Fungi on soil treated with Ferric Chloride on BHA

Soil Samples		HUB	HUF	%HUB	%HUF
Fallow Land	5ml	5.0×10^{6}	9.5×10^{6}	13	47.73
	10ml	6.0×10^{6}	6.5×10^{6}	113.34	0
Dump Site	5ml	2.3×10^{7}	6.5×10^{6}	64.37	46.43
	10ml	1.0×10^{6}	5.0×10^{5}	4.17	0
Mechanic Village	5ml	1.7×10^{7}	8.5×10^{6}	38.64	141.67
	10ml	1.0×10^{6}	0	12.5	0
Farm Land	5ml	8.5×10^{6}	1.0×10^{6}	34.29	16.67
	10ml	6.0×10^{6}	8.0×10^{6}	6.38	0

From the results obtained in Table 8, fallow land soil sample recorded no growth for both HUF and HUB (5ml and 10ml). For 5ml concentration, dump site recorded higher counts of HUB and HUF. While for 10ml concentration, mechanic village recorded higher number of counts of HUB and HUF.

Table 8 Total Counts of bacteria and Fungi on soil treated with Mercuric Chloride on BHA

Soil Samples		HUB	HUF	%HUB	%HUF
Fallow Land	5ml	0	0	0	0
	10ml	0	0	0	0
Dump Site	5ml	6.0×10^{6}	3.0×10^{6}	36.11	40.39
	10ml	1.5×10^{6}	1.5×10^{6}	6.25	0
Mechanic Village	5ml	2.5×10^{6}	1.0×10^{6}	62.5	6.25
	10ml	1.6×10^{7}	3.0×10^{7}	403.98	13.02
Farm Land	5ml	3.0×10^{6}	5.0×10^{5}	13.64	8.34
	10ml	2.5×10^{6}	1.5×10^{6}	8.62	8.34

Comparative distribution of bacteria before and after treatment with pollutants (heavy metals)

Table 9 shows the distribution of bacteria before and after pollution on nutrient plates. There was an increase in bacterial diversities in all the soil samples after treatment.

Table 9 Distribution of Bacteria on NA before and after pollution

Soil Sample	Before	After
Fallow Land	*Bacillus* sp, *Enterococcus* sp, *B. lichieniformis, Bacillus cereus*	*Bacillus subtilis, B. cereus, Staphylococcus* sp, *Corynebacterium* sp, *Micrococcus* sp, *Bacillus* sp, *Enterococcus* sp
Dump Site	*Serratia* sp, *Bacillus* sp, *Micrococcus*sp, *Staphylococcus* sp, *Enterococcus* sp, *Staphylococcus* sp, *Bacillus luteus, Bacillus cereus*	*Enterococcus* sp, *Staphylococcus* sp, *Bacillus* sp, *Corynebacterium* sp, *Bacillus cereus, B. subtilis, Micrococcus* sp
Mechanic Village	*Micrococcus luteus, Staphylococcus* sp, *Enterococcus* sp, *Bacillus* sp, *Micrococcus roseus, Bacillus subtilis, Micrococcus luteus, Corynebacterium* sp, *Enterococcus* sp	*Bacillus* sp, *Staphylococcus* sp, *Pseudomonas* sp, *B. cereus, B. subtilis, Micrococcus* sp, *Enterococcus* sp
Farm Land	*Staphylococcus* sp, *Bacillus* sp, *Enterococcus* sp, *Micrococcus* sp, *Corynebacterium* sp, *B. cereus*	*B. subtilis, Enterococcus* sp, *Bacillus* sp, *Staphylococcus* sp, *Micrococcus* sp, *Micrococcus luteus*

Table 10 shows distribution of fungi the potato dextrose agar plates before and after treatment with the heavy metals. More fungal types were recorded on the fallow land after treatment with the heavy metals. Farm land recorded fewer fungal types after treatment with the heavy metals.

Table 10 Distribution of Fungi before and after pollutionon PDA after pollution

Soil Sample	Before	After
Fallow Land	*Saccharomyces* sp, *Penicillium* sp, *Mucor* sp, *P. notatum*	*Aspergillus* sp, *Mucor* sp, *Saccharomyces* sp, *Penicillium notatum*
Dump Site	*Saccharomyces* sp, *P. notatum, Mucor* sp, *Rhizopus stolonifer, Aspergillus* sp	*Cladosporium* sp, *Aspergillus* sp, *Mucor* sp, *Saccharomyces* sp, *P. notatum*
Mechanic Village	*P. notatum, Saccharomyces* sp, *Mucor* sp, *Penicillium* sp, *Cladosporium* sp	*Saccharomyces* sp, *P. notatum, Mucor* sp, *Aspergillus* sp.
Farm Land	*P. notatum, Saccharomyces* sp, *Fusarium* sp, *Mucor* sp, *Rhizopus nigricans, Penicillium* sp	*Saccharomyces* sp, *Mucor* sp, *Aspergillus* sp.

Discussion

Heavy metals are inhibitory to the various enzymatic and metabolic activities of enzymes in the soil. The different assays carried out were used as an indicator of biological activities in soil.Four enzymes were used in this study, which are catalase, dehydrogenase, phosphatase and urease. Soil dehydrogenase activity was inhibited by heavy metals in fallow land and dump site soil samples. In mechanic village soil sample, dehydrogenase activity was not inhibited by ferric chloride; it was only inhibited by mercuric chloride. There was no inhibition in farm land soil sample. The inhibition of dehydrogenase may be caused by soil contamination due to the presence of heavy metals. This affects the physiological active soil microbial biomass. Nweke *et al*. (2007) concluded that for all the metal ions (Cd^{2+}, Hg^{2+}, Co^{2+}, Zn^{2+}, Fe^{2+} and Ni^{2+}), there was progressive inhibition in dehydrogenase activity and rhizoplane microbial community with each successive increase in the concentration of metal ions. Malley *et al*.(2005) also found out that dehydrogenase activity was strongly inhibited by the addition of Cu.

Soil urease activity was strongly inhibited in all the soil samples when heavy metals were added to it. Shen *et al*. (2005) and Mallaiah (2014) reported that the order of inhibition of urease activity generally decreased according to the sequence Cr > Cd > Zn > Mn > Pb (Zheng *et al*., 1999**)**.

In all the soil samples, acid phosphatase activity was inhibited by the heavy metal pollution. Soil alkaline phosphatase enzyme was inhibited after heavy metals were added to the soil samples. Landi *et al*. (2000) also noted that the activity of phosphatase in the soil was affected by the influence of heavy metals. Soil catalase enzyme was not inhibited by the heavy metals in any of the soil samples.

In respect to the comparative distribution of bacterial and fungal organisms in the soil before and after addition of pollutants. This study shows an increase in microbial communities for both bacterial and fungal organisms. This agrees with Glick, 2010 who opined that some bacteria (*Pseudomonas, Bacillus* and *Enterobacter*) can thrive in heavy metal contaminated soils. These bacteria may develop mechanisms to resist or tolerate these heavy metals, leading to increased activity. Heavy metals can alter fungal community composition, with some species increasing in abundance while others decline. For example, *Aspergillus* can tolerate high levels of heavy metals (Adriano, 2001). It is important to note that microbial responses to heavy metals pollution can vary depending on the soil type, pH, organic matter content, metal type and concentration.

In conclusion, chronic heavy metal exposure can lead to reduced microbial diversity, which can have cascading effects on the ecosystem function.

References

Adriano, D.C. (2001). Trace elements in terrestrial environments: Biogeochemistry, bioavailability and risks of metals. *Springer*.

Akmal, M., Wang, H.Z. and Wu, J. J. (2005). Changes in enzymes activity, substrate utilization pattern and diversity of soil microbial communities under cadmiumpollution. *Journal of Environmental Sciences*, *17*(5), 802–807.

Casida, L.E., Klein, D.A and Santoro, T. (1964). Soil dehydrogenase activity. *SoilScience.98*, 371 – 376.

Dorich, R.A and Nelson, D.W. (1983). Direct colorimetric measurement of ammonium in potassium chloride extracts of soil. *Soil Science Society ofAmerican Journal*. *47*(4), 833 – 836.

Eivazi, F. and Tabatabai, M.A. (1977). Phosphates in soils. *Soil Biol. Biochem*. *9*, 167-172.

Fidelis, I. A., and Patrick, N.O. (2014). Effect of Petroleum Products on Soil Catalase and Dehydrogenase Activities. *Open Journal of Soil Science*, *4*, 399-406.

Filip, Z. (2002). International approach to assessing soil quality by ecologically-related biological parameters. *Agric. Ecosyst. Environ*, *88*, 169–174.

Glick, B.R. (2010). Using soil bacteria to facilitate phytoremediation. *Biotechnology Advances*, *28*(3), 367-374.

Hinojosa, M.B., Carreira, J.A. and Garcia-Ruiz, R. (2004). Soil moisture pre-treatment effects on enzyme activities as indicators of heavy metal-contaminated and reclaimed soils. *Soil Biol Biochem*. *36*, 1559–1568.

Landi, L., Renella, G., Moreno, J.L., Falchini, L. and Nannipieri, P. (2000). Influence of cadmium on the metabolic quotient, L: D Glutamic acid respiration ratio and enzyme activity: microbial biomass ratio under laboratory conditions. *Biol. Fert.Soils,32*(1), 8.

Malley, C., Nair, J and Ho, G. (2005). Impact of heavy metals on enzymatic Activity of substrate and on composting worms *Eisenia fetida. Biores Technol*, *97*, 1498–1502.

Mallaiah, C. (2014). Impact of heavy metals on soil microbial communities. *Journal of Environmental Science, 49*(1), 1-11.

Moreno, J.L., Garcia, C., Landi, L., Falchini, L., Pietramellara, G. and Nannipieri, P. (2001). The ecological dose value (ED_{50}) for assessing Cd toxicity on ATP content and dehydrogenase and urease activities of soil. *Soil Biol. Biochem*, *33*(4-5), 483.

Moreno, J.L., Sanchez-Marín, A., Hernández, T. and García, C. (2006). Effect of cadmium on microbial activity and a rye grass crop in two semiarid soils. *Environ*. *Manage*, *37*(5), 626–633.

Nweke, C.O., Ntinugwa, V., Obah, I.F., Ike, S.C., Eme, G.E., Opara, E.C., Okolo, J.C. and Nwanyanwu, V. (2007). *In vitro* effects of metals and pesticides on dehydrogenase Activity in microbial community of cowpea (*Vigna unguiculata*) rhizoplane. *Afr J Biotechnol*. *6*, 290-295.

Rani, P., Meena, U.K. and Karthikeyan, J. (2004) Evaluation of Antioxidant Properties of Berries. *Indian Journal of Clinical Biochemistry*, *19*, 103-110.

Shen, G., Lu, Y., Zhou, Q. and Hang, J. (2005). Interaction of polycyclic aromatic hydrocarbons and heavy metals on soil enzyme. *Chemosphere*, *61*, 1175 – 1182.

Tabatabai, M.A and Bremner, J.M. (1972). Assay of urease activity in soils. *Soil Biology and Biochemistry*. *4*, 479-487.

Wyszkowska, J. and Kucharski, J. (2003). Biochemical and physicochemical properties of soil contaminated with the heavy metals. *Zesz. Prob. NaukRol*. 492-435(in Polish).

Zheng, S.J., Ma, J.F. and Matsumoto, H. (1999). High aluminium resistance in buckwheat. *Plant Physiology*, *121*(2), 557-564.

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