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Evaluation of the Effects of Cadmium in Soil on the LC_{50} of Soil Bacteria and Fungi for Environmental Monitoring

***[1, 4]WANJALA, MP; [1]ODOKUMA, L; [2]ETELA I; [3]RAMKAT, R, [4]BLESSING, AO, [5]BOADU, KO**

**[1]Department of Microbiology, [2]Department of Animal Science, University of Port Harcourt, Nigeria*
[3]Center of Excellence in Phytochemicals, Textiles and Renewable Energy, Moi University, Eldoret, Kenya
[4]Department of Plant Science and Biochemistry, University of Port Harcourt, Nigeria
[4]Department of Physical and Biological Sciences, Bomet University College, Bomet, Kenya
[5]Industrial Chemistry Unit, Department of Chemistry, University of Cape Coast, Cape Coast, Ghana
**Corresponding Author Email: piwanjala@gmail.com, Tel: +254725891794, +2348101149649*
Co-authors Email: Lucky.Odokuma@uniport.edu.ng; ibisime.etela@uniport.edu.ng; chirirose@yahoo.com; blessing.odogwu@uniport.edu.ng; koboadu@ucc.edu.gh

ABSTRACT: Contamination of soil with heavy metals by is currently of global concern. Cadmium (Cd) is one of the metals of concern. In the current study, LC_{50} of Cd to soil bacteria and fungi was used to assess the impact of anthropogenic activity in development of Cd tolerance in soil microorganisms. Levels of Bio-physicochemical parameters in soil were determined. Results show that the concentration of Total Petroleum Hydrocarbon (TPH) and Cd in soil ranged between 5.09±0.33 to 9261.94±287.67, and 0.023±0.015 to 0.057±0.012 ppm respectively. There was significant difference ($p = 0.001$) in LC_{50} for fungi between the study and control samples. Pearson correlation showed that there was significant relationship ($r = 0.30$) between LC_{50} for bacteria and TPH. There was significant difference ($p = 0.017$) in LC_{50} values among the study and control samples for fungi. Anthropogenic activities influenced the concentrations of TPH soil but did not influence levels of Cd.

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Spillage of petroleum, petroleum products and other domestic wastes to the environment is a common scenario in the modern world. Increased spillage of petroleum has been linked to increased demand for petroleum and its products in urban, industrial and agricultural areas (BP, 2015). Further, heavy metals have been linked by studies to petroleum spillages (Egborge, 1991; Osuji and Onojake, 2004; Lima *et al*., 2014). Consequently, accumulation of heavy metals in the environment has gained momentum and is followed by bioaccumulation of the toxins in organisms which pick the metals from the environment (Nwabunike, 2016). Presence of elevated concentrations of pollutants above recommended limits in the environment is a health risk. This is because pollutants can be leached through surface water and seapage to underground waters (Todd *et al*., 1999; Wanjala *et al*., 2017). World Health Organization (WHO) has put into alert some of the metal pollutants and public caution has been put with specific permitted levels of exposure. For example; Cadmium (Cd), Zinc (Zn), Copper (Cu), Chromium (Cr), Lead (Pb) and Nickel (Ni) have specified desirable maximum levels of 0.8, 50, 36, 100, 85, and 35 ppm (mg/kg) respectively (WHO, 1996). Some of the microorganisms have developed tolerance against such metal pollutants while others still remain intolerant and vulnerable to the chemical intoxication (Mohamed *et al*., 2015), and therefore presence of elevated concentrations of metals in soil can compromise populations and diversity of microorganisms in soil and further compromise important ecosystem processess. Microorganisms find their ways to survive under harsh environmental conditions which makes them resilient. For example, Cd finds its way inside bacteria by cell transport system for divalent cations (Tynecka *et al.*, 1981; Laddaga and Silver, 1985). There are several ways by which the microorganisms tolerate Cd in their environment, which include; energy-dependent efflux mechanisms (Silver, 1996). For example, active Cd efflux (Tynecka *et al.*, 1981), enhanced transpiration of metalothionein genes (Mc Entee *et al.*, 1986), alteration of cell wall and plasma membrane complexes (Mitra & Berstein, 1977) and amplification of genes (Beach & Palmiter, 1981). Several genes

**Corresponding Author Email: piwanjala@gmail.com, Tel: +254725891794/+2348101149649*

have been assiciated with the characteristic of tolerance of microorganisms to elevated levels of Cd. For example cadB in *S. aureus*, cadX in *S. lugdunensis* (Crupper *et al.*, 1999), smt in *Synechococcus sp*. strain PCC 6301 (Robinson *et al.*, 1990) and in strain PCC 7942 (Huckle *et al.*, 1993). Soil is composed of large microbial diversity estimated at 10^7 to 10^9 species for bacteria and 1.5 million species for fungi (Narendrula - Kotha & Nkongolo, 2017). Research shows that contaminants influence levels and activities of microorganisms in soil (Bouskill *et al.*, 2010; Sheeba *et al.*, 2017). Abundance and distribution of soil microorganisms are influenced by natural environmental conditions as well as anthropogenic activities (Unimke *et al*., 2017; Wu *et al.*, 2018). Soil microbial abundance and diversity vary relative to environmental conditions and is unique among different ecosystems (Wang *et al.*, 2015; Li et al., 2017; Luo *et al.*, 2017). The rate of uptake of pollutants by organisms from soil is dependent on the levels of concentration of the pollutants in the respective niches (Liu *et al.*, 2015). Lethal Concentration 50 (LC_{50}) is the concentration that causes a 50% reduction of organisms in test after exposure to a toxin in test (Rotini *et al.*, 2017). Determination LC_{50} in organisms at individual or population levels have been applied in ecotoxicological assessments in other places (Thangavel *et al*., 2013; Mohamed *et al.*, 2015; Manguilimotan & Bitacura, 2018), but has not been extensively adopted in the current study areas. Several studies indicate a clear association between effects of pollutants at individual levels and effects at population levels (Kuhn *et al*., 2000) whereas others indicate little or no association between toxicities at individual and population levels (Herbert *et al*., 2004; Stark *et al*., 1997). Hence, there is little consensus about how specific effects at the individual level will forecast impacts at population level. To contribute on this discourse, this study used LC_{50} to evaluate the impact of pollution of soils by Cd to formation tolerance among bacteria and fungi (Mortensen *et al.*, 2018) on a population basis. High concentrations of LC_{50} of Cd showed that of contamination of soil had influenced the tolerance levels to Cd while low concentrations of LC_{50} showed that there was minimal influence of environment to development of tolerance among microorganisms in the study areas.The findings marked out areas that were inhabited with Cd tolerant fungal and bacterial communities.

MATERIALS AND METHODS

This study was conducted in 9 selected test and 3 control sites in Port Harcourt, the Capital of Rivers State, Nigeria. The study sites were grouped into 3 areas including; urban (GRA phase 2, Diobu- Mile 1 and Mguoba), Industrial (Eleme which hosts the NNPC Refinery, Agbada-SPDC- flow station) and agricultural (Aluu, Oquwi- Eleme, Emuoha- Eu). The study sites were characterized with different economic activities associated with oil (flow stations, oil companies and urban settlements). Composite samples were collected by random sampling from each of the three areas; urban, industrial and agricultural in the wet (April to October 2018) and dry (December 2018 to March 2019) seasons. Five (5) individual samples were collected following a random pattern around each test field. The five individual samples were thoroughly mixed by coning and quartering in a sterile container to attain a homogenous composite mixture. A total of 12 composite samples; A1, A2, A3, I1, I2, I3 U1, U2 and U3 as test samples, and CA, CI and CU as control samples (Table 1), were collected from the topsoil within a depth of 0 to 15 cm using a standard auger 3 times in the rainy season. Homogenized composite samples (400 gm) were then packed in polyethylene bags using a sterile wooden shovel. Samples for microbial analysis were collected using pre-sterilized materials to prevent contamination of the samples. Locations of the sampling sites were identified using a GPS and the GPS readings recorded. Samples were transported to the laboratory for analysis.

Levels of Cadmium (Cd) were determined using Atomic Absorption Spectrum [AAS] (APHA, 1995 {APHA, 301A}). Two grams of soil was weighed separately from each sample and transferred into Kjeldahl flasks; 20 ml of concentrated nitric acid (HNO_3) was added and the samples pre-digested by heating gently for 20 mins. Additional 10 ml nitric acid was thereafter added to each Kjeldahl flask and digestion was continued for further 30-40mins, until when a clear digest was obtained. The flask was cooled to room temperature and the content was transferred into 50 ml volumetric flask and brought to volume using distilled water. The resulting solution was analyzed for heavy metals using the Atomic Absorption Spectrophotometer (AAS). The heavy metals were then analyzed at the following wavelength of 228.9. Preparation of stock solution and dilutions of metal salts was done. Cadmium chloride ($CdCl_2$) [2.0360g] was disslved in 250 ml deionised water, and was there after diluted to 1 litre in a volumetric flask to constitute1000 ppm for stock solution for each. Dilution formula (1) was applied to prepare all concentrations of; 10 ppm, 25 ppm, 50 ppm, 75 ppm, 100 ppm and 150 ppm. The prepared solutions of were then sterilized using bacteriological vacuum filtration through Whatman membrane filter paper (0.22 micron) [ASTM F838-05] standard methods. The

prepared concentrations were then added to sterilized growth medium.

$$C_1V_1 = C_2V_2 \qquad (1)$$

Where C = Concentration; V = Volume; 1 and 2 are initial and final conditions

Table 1: Table showing study areas and economic activities

No	Selected Study Areas	Study Area Coding (Locations)	Coordinates N latitude E Longitude	Characteristic and main activities
		Agricultural Areas	4° 56' 11.160'	Flow station
1	Aluu	A1	6° 57' 52.248	Village close to refinery
			4° 44' 09.874'	
2	Eleme	A2	7° 08' 58.494'	Flow station
			5° 00' 00.018'	>1 km away from suspected areas
3	Emuoha	A3	6° 49' 13.032'	
			5° 00' 21.384'	
	Control	CA	6° 49' 00.000'	
		Industrial Areas	4° 46' 00.402'	Hosts the NNPC Refinery
1	Onne	I1	7° 05' 43.092'	
			4° 56' 03.444'	Hosts SPDC- flow station in a rural setting
2	Agbada	I2	6° 58' 42.060'	
			4° 48' 20.455'	
3	Trans-Amadi	I3	7° 02' 17.646'	Schlumberger/, Hallburton
			4° 47' 13.788'	
	Control	CI	7° 07' 44.620'	>1 km away from suspected areas
		Urban Areas	4° 49' 53.574'	
1	GRA Phase 2	U1	6° 59' 45.552'	Inhabited areas
			4° 47' 20.382'	Perecuma street
2	Diobu-Mile 1	U2	7° 00' 13.164'	
			4° 50' 39.864'	Petroleum refinery
3	Mgbuoba	U3	6° 58' 20.232'	
			4° 49' 17,040'	NTA
	Control	CU	6° 59' 24.168'	>1 km away from suspected areas

Cadmium tolerant bacteria were enumerated by modification of APHA *et al.*, 1998 method. One gram of soil sample was weighed into a 9 ml sterile diluent (0.85 % NaCl) under aseptic conditions. The sample was then homogenized using a laboratory vortex mixer (Model: 10101001, IP42) and serially diluted. Then 0.1 ml aliquot of inoculum was inoculated on Mineral Salt Agar (MSA) which was then acidified using 0.1 % lactic acid. This acidification inhibits the growth of hydrocarbon fungi. Fixed dose procedure was used, where microorganisms were dosed in a stepwise procedure using the fixed doses of; 10 ppm, 25 ppm, 50 ppm, 75 ppm, 100 ppm and 150 ppm of Cadmium (Cd). The initial dose levels were selected on basis of preliminary study as the dose expected to produce toxicity effects. The dosing continued until the dose causing evident toxicity of zero growth was observed. Plates were then incubated in an inverted position at room temperature for 5 to 7 days. Colonies were counted in order to obtain colony forming units per gram of soil. Cadmium tolerant fungi were enumerated by modification of APHA *et al.*, 1998 method. One gram of soil sample was weighed into a 9 ml sterile diluent (0.85% NaCl) under aseptic conditions. The sample was then homogenized using a laboratory vortex mixer (Model 10101001, IP42) and serially diluted. Then 0.1 ml aliquot of inoculum was then inoculated on Potato Dextrose Agar (PDA). Fixed dose procedure was used, where microorganisms were dosed in a stepwise procedure using the fixed doses of; 0 ppm, 10 ppm, 25 ppm, 50 ppm, 75 ppm, 100 ppm and 150 ppm of Cadmium (Cd). The initial dose levels were selected on trial basis of sighting study as the dose expected to produce toxicity effects, without causing complete mortality. Microorganisms were then dosed at higher or lower fixed doses, depending on the presence or absence of signs of toxicity or mortality. The dosing was continued until the dose causing evident toxicity was observed. Plates were then incubated in an inverted position at room temperature for 5 to 7 days. Colonies were counted in order to obtain colony forming units per gram of soil. Percentages of dead organisms (bacteria and fungi) in each study concentration was determined, which were then converted to probits using Finney's table (Table 2). Regression analysis was done, where the output of probit analysis was used to compare the amount of chemical required to create responces among microorganisms of the various study areas to different concentration of Cd in the culture medium (Vincent,

1980). Areas that reorded smaller values of LC_{50} were considered most toxic as compared to areas that recorded higher values of LC_{50} (Vincent, 1980). Microorganisms in areas that showed higher values of LC_{50} were considered more tolerant to exposure to Cd. LC_{50} was determined by calculating the corresponding x value for a probit of 5.00 and then taking the inverse log of the concentration it is associated with (Vincent, 1980).

$$y=ax+c \ldots\ldots\ldots (2)$$

Where y=5 From Finney's table; a= Calculated coefficients, x= Unknown value, c = Calculated coefficients , LC_{50}= Antilog of x

Table 2: Transformation of percentages to probits

%	0	1	2	3	4	5	6	7	8	9
0	-	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33
-	0.00	0.10	0.20	0.30	0.40	0.50	0.60	0.70	0.80	0.90
99	7.33	7.37	7.41	7.46	7.51	7.58	7.65	7.75	7.88	8.09

Finney's table (Finney 1952)

Data was analysed using SPSS, ANOVA was used to determine the difference between the LC_{50} among the study and control sites. Pearsons correlation analysis was used to determine the relationship between the values of LC_{50} and the values of metal contamination in the study sites.

RESULTS AND DISCUSSION

The mean concentration of Cd in the soils of the study areas ranged between 0.023±0.015 to 0.057±0.012 ppm (Table 3). All the values were within WHO permissible limits of 0.8 ppm but were varied among the study sites. The mean values of Total Petroleum Hydrocarbon (TPH) ranged between 5.09±0.33 to 56.70±44.63 ppm in wet season and 0.00±0.00 to 9261.94±287.67 ppm in the dry season (Table 3). The highest levels of TPH were recorded in A2 with a mean value of 56.70±44.63 ppm in wet season and I3 with 9261.94±287.67ppm in the dry season.

Table 3: Mean concentrations±SD of Cd and TPH in study sites

Area of sites	Cd (ppm)	TPH (ppm) Wet season	TPH (ppm) Dry season
CI	0.057±0.012	5.09±0.33	174.16±12.26
I1	0.037±0.006	7.61±1.38	3483.01±2919.55
I2	0.040±0.020	8.55±0.09	471.44±57.27
I3	0.047±0.015	13.89±9.94	9261.94±287.67
CA	0.043±0.023	15.76±5.77	16.18±0.22
A1	0.027±0.006	5.52±1.12	0.00±0.00
A2	0.023±0.015	56.70±44.63	1.45±0.89
A3	0.037±0.011	6.36±0.06	165.51±20.52
CU	0.040±0.000	5.16±1.77	236.71±52.57
U1	0.043±0.006	12.60±5.91	1251.44±529.54
U2	0.037±0.012	7.20±2.26	2548.59±287.50
U3	0.027±0.006	7.72±0.25	113.55±55.06

The values in Table 4 represent the mean values of three independent tests (See supporting data). The reductions in fungi actively replicating and forming colonies as compared to the control showed a reduction in CFUs/g of soil with increase in concentrations of Cd. Results reveal variation in tolerance of fungi to Cd among the study sites. The least lithal concentration 50 (LC_{50}) was observed in CU at 50 ppm and 1.03×10^3 CFUs/g of soil (Table 4). In U1 maximum growth was recorded at 75 ppm, while in CI, I1, I3, U2 and U3 maximum observable growth was observed at 100 ppm. The highest concentration of Cd that showed growth was 150 ppm which was observed at I2, CA, A1, A2 and A3 where the fungal populations ranged between 1.00×10^2 to 1.00×10^2 CFUs/g of soil (Table 4).

The values in Table 5 represent the mean of three independent tests (See supporting data). The reductions in bacteria actively replicating and forming colonies as compared to the control showed a reduction in CFUs/g of soil with increase in concentrations of Cd.

There was variation in maximum tolerable concentrations among bacteria in the study sites ranging from 100 ppm to 150 ppm. Sites I2, CU, U2 and U3 showed growth at a maximum concentration of 100 ppm and the bacterial population ranged between 1.03×10^2 to 1.45×10^2 CFUs/g of soil, while CI, I1, I3, CA, A1, A2, A3 and U1 showed growth at maximum concentration of 150 ppm where the bacterial populations ranged from 3.33×10^1 to 1.47×10^2 CFUs/g of soil (Table 5).

Table 4: Variation in mean fungal CFU/g of soil in increasing concentrations of Cd

Study location	0 ppm	10 ppm	25 ppm	50 ppm	75 ppm	100 ppm	150 ppm
CI	5.21×10^{4}	6.37×10^{3}	3.50×10^{3}	6.73×10^{2}	2.23×10^{2}	1.35×10^{2}	0
I1	6.17×10^{5}	1.85×10^{4}	2.90×10^{3}	1.33×10^{3}	1.17×10^{3}	1.00×10^{3}	0
I2	1.32×10^{4}	4.23×10^{3}	3.00×10^{3}	1.83×10^{3}	1.40×10^{3}	1.00×10^{2}	1.00×10^{2}
I3	7.50×10^{4}	6.23×10^{4}	9.77×10^{3}	5.27×10^{3}	3.50×10^{3}	2.20×10^{3}	0
CA	6.18×10^{3}	5.53×10^{3}	4.23×10^{3}	3.24×10^{3}	2.93×10^{3}	2.30×10^{3}	1.00×10^{2}
A1	1.45×10^{5}	9.31×10^{3}	2.80×10^{3}	5.97×10^{2}	5.03×10^{2}	1.20×10^{2}	1.00×10^{2}
A2	3.27×10^{4}	2.42×10^{3}	2.13×10^{3}	1.37×10^{3}	1.22×10^{3}	1.10×10^{3}	1.00×10^{3}
A3	8.23×10^{3}	6.73×10^{3}	5.53×10^{3}	4.63×10^{3}	4.30×10^{3}	2.87×10^{3}	2.67×10^{2}
CU	7.70×10^{4}	4.73×10^{3}	3.14×10^{3}	1.03×10^{3}	0	0	0
U1	1.44×10^{4}	2.98×10^{3}	9.03×10^{2}	1.93×10^{2}	1.00×10^{2}	0	0
U2	6.60×10^{4}	1.17×10^{4}	5.18×10^{3}	3.53×10^{3}	2.54×10^{3}	1.03×10^{2}	0
U3	6.23×10^{4}	5.34×10^{4}	5.07×10^{3}	3.02×10^{3}	1.77×10^{3}	1.30×10^{2}	0

Table 5: Variation in mean bacterial CFU/g of soil in increasing concentrations of Cd

Study location	0 ppm	10 ppm	25 ppm	50 ppm	75 ppm	100 ppm	150 ppm
CI	7.17×10^{7}	2.44×10^{7}	1.66×10^{5}	2.34×10^{4}	9.07×10^{3}	1.67×10^{3}	1.23×10^{2}
I1	1.37×10^{7}	6.13×10^{6}	1.27×10^{5}	2.05×10^{4}	1.46×10^{3}	9.00×10^{2}	1.03×10^{2}
I2	7.43×10^{6}	9.93×10^{5}	5.33×10^{4}	3.22×10^{4}	3.00×10^{3}	1.03×10^{2}	0
I3	3.37×10^{7}	5.53×10^{6}	1.71×10^{5}	2.60×10^{4}	2.77×10^{3}	1.49×10^{3}	6.67×10^{1}
CA	6.17×10^{7}	1.29×10^{7}	2.21×10^{5}	1.73×10^{5}	3.33×10^{4}	3.50×10^{3}	1.47×10^{2}
A1	1.30×10^{6}	8.10×10^{5}	2.16×10^{5}	3.47×10^{4}	6.50×10^{3}	1.88×10^{3}	1.17×10^{2}
A2	7.07×10^{7}	9.90×10^{6}	3.84×10^{5}	3.18×10^{4}	2.34×10^{3}	1.60×10^{2}	6.33×10^{1}
A3	5.73×10^{7}	7.23×10^{6}	1.69×10^{5}	1.40×10^{5}	2.50×10^{4}	1.93×10^{3}	4.00×10^{1}
CU	3.33×10^{6}	5.50×10^{5}	1.32×10^{5}	4.80×10^{4}	2.20×10^{4}	1.19×10^{3}	0
U1	4.57×10^{7}	1.67×10^{7}	2.01×10^{5}	1.40×10^{5}	1.35×10^{4}	1.61×10^{3}	3.33×10^{1}
U2	1.88×10^{7}	5.30×10^{6}	1.03×10^{5}	1.32×10^{4}	1.91×10^{3}	1.07×10^{2}	0
U3	1.96×10^{7}	5.67×10^{6}	1.69×10^{5}	1.35×10^{4}	3.30×10^{3}	1.45×10^{2}	0

The values in Table 6 represent the mean (±SD) of three independent LC_{50} tests. The values ranged from 0.47±0.06 ppm to 33.90±18.69 ppm of Cd for fungi and 0.48±0.35 ppm to 1.17±0.70 ppm of Cd for bacteria. Fungi showed higher tiolerance to Cd with CA and A3 recording 28.19±25.03 ppm and 33.90±18.69 ppm respectively (Table 6). There was significant difference in LC_{50} for fungi among the study sites, p=0.001, however there was no significant difference in LC_{50} for bacteria among the study and control sites p=0.999 (Table 6).

There was significant difference in LC_{50} among the study areas (urban, industrial and agricultural) and control areas, ANOVA, $F(5,30) = 3.297$ $p = 0.017$ for fungi and no significance for bacteria ANOVA, $F(5,30) = 0.246$ $p = 0.939$. Pearson Correlation analysis of metals in the study sites against LC_{50} of Cd reveal that there was significant relationship between LC_{50} for bacteria and TPH (r=0.30). The LC_{50} for fungi in agricultural area was 11.96±18.93 ppm as compared to higher value in control site 28.19±25.02 ppm (Figure 1). The LC_{50} for bacteria in agricultural area was 1.07±0.65 ppm as compared to lower value in control site 0.83±0.44ppm (Figure 1). The LC_{50} for fungi in industrial area was 2.11±1.81 ppm as compared to lower value in control site 0.82±0.08 ppm. The LC_{50} for bacteria in industrial area was 0.99±0.48 ppm as compared to higher value in control site 1.17±0.70 ppm. The LC_{50} for fungi in urban area was 1.37±0.52 ppm as compared to lower value in control site 0.93±0.49 ppm. The LC_{50} for bacteria in urban area was 0.92±0.43 ppm as compared to lower value in control site 0.85±0.24 ppm (Figure 1).

Table 6: Variation in LC_{50} values for fungi and bacteria exposed to Cd among the test and control sites

Sampling Site	Fungi	Bacteria
CI	0.82±0.08	1.17±0.70
I1	0.47±0.06	0.48±0.35
I2	2.71±2.32	1.05±0.72
I3	3.14±1.26	0.97±0.54
CA	28.19±25.03	0.83±0.44
A1	0.69±0.45	1.12±0.54
A2	1.27±0.06	1.14±0.91
A3	33.90±18.69	0.95±0.72
CU	0.93±0.49	0.85±0.24
U1	0.87±0.15	0.82±0.36
U2	1.22±0.16	1.09±0.54
U3	2.01±0.17	0.86±0.48
F	$F(11,24) = 4.949$	$F(11,24) = 0.151$
df	35	35
P Value	0.001	0.999

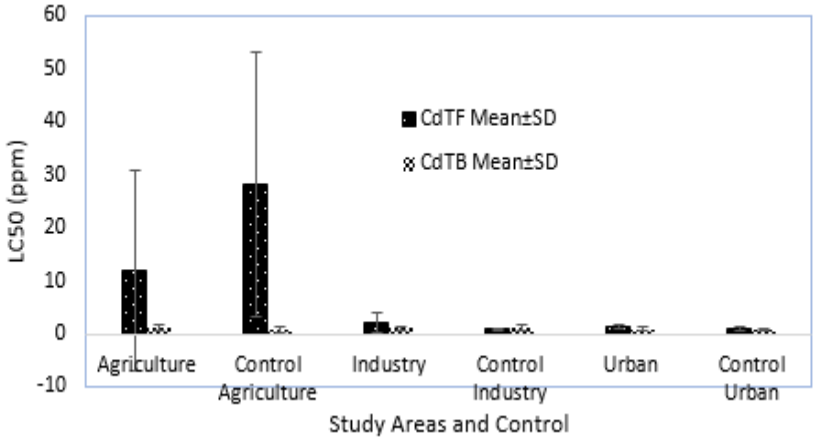

Fig 1: Levels of LC_{50} on fungi and bacteria in agricultural, industrial and urban areas (CdTF is Cadmium Tolerant Fungi, CdTB is Cadmium Tolerant Bacteria)

This study is an ecotoxicological assessement that monitors impacts of preexposure soil fungi and bacteria to Cd pollution by using bioassay with mixed fungi and bacteria in natural terrestrial ecosystems. This bioassay uses inability of bacterial and fungal cultures to survive in varied concentrations of Cd. The approach of the study is a modification of that of (Rotini *et al.*, 2017) who used *Vibrio anguillarum* to evaluate acute toxicity of environmental contaminants in marine ecosystem. As opposed to Rotini *et al.*, (2017), the current study used mixed bacterial and fungal populations to assess the impact of pollutants on soil ecosystem integrity. As per the findings in Table 4 (CA and A3), there were detectable CFUs/g of soil at 150 ppm ranging from 1.00×10^2 to 1.00×10^3, an indication that there are fungi in the area that are associated with tolerance in cadmium (Cd). Areas CA and A3 showed LC_{50} of 28.19±25.03 ppm and 33.90±18.69 ppm respectively for fungi, making the two areas as the areas with fungi that is tolerant to high Cd levels. The areas are characterised with petroleum flow stations where presence of Cd in the soils can be associated with leaks or spillage of petroleum and petroleum products (Osuji & Onojake, 2004), however, this was refuted by the findings of this study. Cadmium can also be associated with natural occurrence which maybe the case in the current study. Further, it can be associated with mining and smelting (Zhou *et al.*, 2018). Accumulation of cadmium in the environment can also be attributed to application of phosphate fertilizers in farms (Schipper *et al.*, 2011; Smolders and Mertens, 2013; Reiser *et al.*, 2014; Salmanzadeh *et al.*, 2016; Salmanzadeh *et al.*, 2017) which has extensively been used in agricultural production. As Cd is higher in agricultural areas, this can be the modest explanation of the findings in the study areas. In order to understand the fate and impact of Cd in the soil ecosystem integrity, it is necessary to identify areas with high vulnerability and possible sources of Cd in the study areas. Maximum tolerable concentration of Cd in the current study was 150 ppm, which was an indication that fungi and bacteria in the study areas could tolerate high levels of Cd. Microbial populations are varied among different locations and research reveals that increased activity can be related to zones with elevated particulate organic matter, animal manure, in rhizospheres, and growth factors (Sexstone *et al.*, 1985; Parkin, 1987;Petersen *et al.*, 1996; Lynch, 1990; Pinton *et al.*, 2001; Renella, 2017). The findings of this study are in agreement with those of Bin, (2018), who reports decrease in microbial diversity and microbial enzyme activities with increase in concentration of Cd. There were significant correlation between LC_{50} for Cd and TPH (r=0.30). The correlation suggests that TPH is a source of Cd in the soils and contributes to the development of Cd tolerance among fungi and bacteria in the study areas. However, this was to a small extent. Currently in Nigeria, there is scarce knowledge on the quantity of Cd that is being released into the environment. Estimates by Agency for Toxic Sabstance and Disease Agency, (ATSDR, 2012) show that 115 tons of Cd were released into the soils of US in 2009. Without close monitoring agencies in Africa, it is difficult to have a clear record of magnitude of pollution be Cd and its influence on soil microorganisms. Therefore, the findings of this study are relevant for future monitoring of Cadmium (Cd) levels in the study areas. The findings of this study reveals that microbial activity is severely affected by Cd among the study areas and is highest in the agricultural areas. It is concluded that agricultural areas (CA and A3 {Emuoha}) among the study areas as vulnerable to pollution with Cd while the industrial areas receive higher concentrations of TPH specifically over the dry season. The study concludes

that fungi in the agricultural areas have higher tolerance to Cd as compared to those in industrial and urban areas and as compared to bacteria. Further, the study concludes that TPH is not a contributing factor to contamination of the environment with Cd in the current study sites as the high values of TPH over the dry season did not show significant correlation to concentration of Cd. Bacteria in all study areas are intolerant to Cd intoxication and are vulnerable to low doses of Cd which is a health risk. Determination of Lethal Concentration 50 (LC_{50}) is a robust population-based method of environmental monitoring, which can be a crucial indicator of endangered habitats.

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