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Assessment of Ni Toxicity to Fungi and Bacteria in Oil Tainted Soils in Greater Port Harcourt Area, Nigeria

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ABSTRACT: Intensified urbanization and industrialization are rapidly triggering the release of pollutants to the environment. This study determined the extent of soil contamination with Nickel (Ni) in oil mining areas and its effect on the levels of Ni tolerance by fungi and bacteria. The total CFUs/g of soil were enumerated after a culture period of 7 days at 28°C and LC_{50} was determined using probit and regression analysis. The mean values of Ni were 1.38±0.23 in industrial area, 1.41±0.36 ppm in agricultural area and 1.02±0.64 in urban area. The mean values of Total Petroleum Hydrocarbon (TPH) were 4,405.46 ppm in industrial area, 55.65 ppm in agricultural area and 1,304.53 ppm in urban area. Nickel's peak concentration indicating growth of both fungi and bacteria at 150 ppm. There was significant difference ($p \leq 0.000$) in the mean levels of LC_{50} for fungi among the study sites. There was no significant correlation between the concentration of TPH in soil and LC_{50} of fungi ($r = -0.169$) and bacteria ($r = 0.042$). In conclusion, TPH influenced the levels of fungi and bacteria tolerance to Ni in soils. Moreover, it was observed that LC_{50} can be a reliable method for monitoring chemically resistant microorganisms directly in the environment to improve the use of microorganisms for the bioremediation of oil contaminated soils and in monitoring of antibiotic resistant microorganisms in natural ecosystems.

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Intensified urbanization and industrialization are increasingly releasing heavy metals to the environment. Some of the anthropogenic activities in urban and industrial areas that contribute to excessive release of metals to the environment include; smelting, application of fertilizers and pesticides, burning of fossil fuels, industrial and domestic wastes and mining (Singh, 2001). Increased global demand for crude oil and its products has intensified efforts in petroleum prospecting, mining and transportation processes which go together with spillage and contamination of the environment (Nduka, *et al*., 2006). Oil spillage is a common occurrence in Nigeria and the worst occurrences are in Niger Delta which is the epicentre of oil mining (Nduka *et al*., 2006). Some of the metals that are associated with crude oil include; Al, As, Cd, Cr, Cu, Hg, Ni, Pb, Se and Zn (Shtangeeva, 2006). Despite Nickel (Ni) being an essential micronutrient, it may be toxic to animals and humans if consumed in quantities above the recommended thresholds (McLaughlin *et al*., 1999). Nickel found in the environment has not only been linked to petroleum, but also to other various sources including; electroplating, zinc base casting and battery industry (Paul, 2017). Nickel is added to gasoline and has been associated with soils on the roadside (Arslan, 2006). Further, Nickel levels in soil vary depending on geology and anthropogenic activities specific to an area. Bacteria can be tolerant to intoxication by nickel, for example; *Cupriavidus species* (ATHA£), *Klebsiella oxytoca* (ATHA6) and *Methylobacterium spp* (ATHA7) have been associated with tolerance for Ni (Alboghobeish, *et al*., 2014). Smith, (1967), used plasmid (pMOL28) to demonstrate Ni tolerance in bacteria. There are various ways through which microbes use to absorb metals from the environment. For instance, Ni enters bacteria and fungi (*Saccharomyces cerevisiae*) by CorA system (Hmiel *et al*., 1989). Further, ATP-binding cassette and secondary permeases of NiCo T family have been

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identified as Ni transporters in microbial cells for example in *Yersinia pseudotuberculosis* and *Brucella suis* (Jubier-Maurin *et al*., 2001; Sebbane *et al*., 2002; Eitinger *et al*., 2005). The rcnA (yohM) gene has been demonstrated to be responsible for Ni efflux (Rodrigue *et al*., 2005). In some microorganisms eg. *Helicobacter pylori*, Czn operons have been associated with resistance to Ni (Stahler *et al*., 2006). Therefore, fungi can survive in environments contaminated with heavy metals and can detoxify toxins by valence transformation, active uptake, precipitation inside the cells and biosorption (Manguilimotan & Bitacura, 2018). Microorganisms are non-vertebrates and provide an alternative approach to use of animals in environmental monitoring. This approach is gaining popularity because they are relatively quick, replicable, cheap and do not attract ethical issues (Rotini *et al*., 2017; Mortensen *et al*., 2018).

There is variation in terms of availability and abundance of basic requirements for flourishment of microorganisms in various microhabitats and is a major reason for the varying niches with diverse taxa (Grayston, 2001). The type and number of organic substrates available in soil directly translates to abundance of soil microorganisms as well as their diversity in soil (Grayston, 2001). However, there are other factors that contribute on availability and resilience of microorganisms in soil. For instance, spore forming bacteria are more resilient in harsh conditions as they exist in form of spores and can undergo dormancy during the harsh conditions. When conditions are normal, they propagate once again (McKinney, 2004). Lethal Concentration 50 (LC_{50}) can be used to study ecotoxicological processes, but hardly has it been applied in environmental monitoring. In toxicology, LC_{50} is determined where 50 % of organisms die on exposure to a toxic substance (Rotini *et al*., 2017). The toxic range of a substance may be tested to determine its toxicity range. In this case dose-response relationship is determined followed by determination of LC_{50}. Several methods are available for determination of LC_{50} (Finney, 1952; OECD, 1981; Paul & Clark, 1996). This study adopted the Finney, 1952 method where percentage deaths of CFUs/g of soil were determined and linear regression analysis was used to determine LC_{50} (Manguilimotan & Bitacura, 2018). The objective of this study is to determine the impact of oil spills to Ni levels on the Greater Port Harcourt Area soils.

MATERIALS AND METHODS

Description of the Study Site: This study was conducted in 9 selected areas of Port Harcourt, the Capital of Rivers State, Nigeria (Figure 1). The study areas were selected with reference to the economic activities carried out in the areas. The study areas were categorized into 3; (1) urban, (2) Industrial and (3) agricultural. The urban areas included; GRA phase 2, Diobu- Mile 1 and Mguoba, Agricultural areas included; Aluu, Oquwi- Eleme, Emuoha- Eu. Industrial areas included; Eleme which hosts the NNPC Refinery, Agbada-SPDC- flow station in a rural setting and Trans-Amadi. Economic activities (Table 1) conducted in the study areas included; drilling and mining, fishing, fish farming, horticulture, dairy farming and crop farming, industrial processing. The study areas were assigned codes as in Table 1.

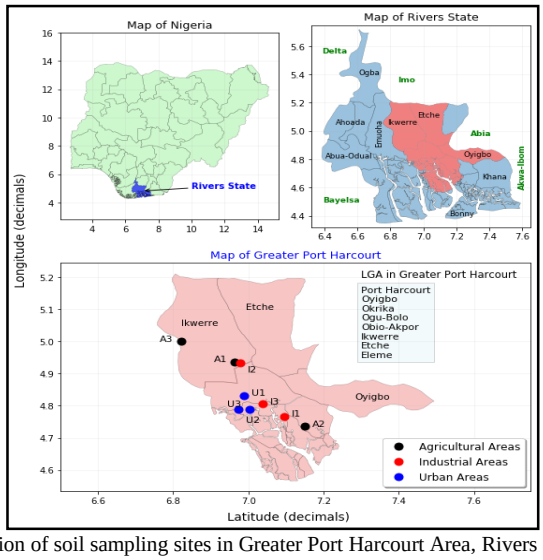

Fig 1: Map of Nigeria showing Location of soil sampling sites in Greater Port Harcourt Area, Rivers State, Nigeria. Different colours show different economic activities (black is agricultural, red is industrial, and blue is urban).

Table 1: Study sites, their GPS coordinates and the main economic activities

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

Sampling: Composite samples were collected by simple random sampling from each of the three study areas: urban, industrial and agricultural during the wet season (April to October 2018). Five (5) individual samples were collected following a random pattern around each study 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. For microbial analysis, the soil samples were collected using a stainless-steel autoclaved shovel then placed in sterile plastic sample bags (3 M, USA). The soil samples were then transported to the laboratory within 4 hours and preserved at 4 °C until microbiological analysis had been completed. Locations of the sampling sites were identified using a GPS and coordinate values were recorded. Samples were transported to the laboratory for analysis.

Determination of the levels Nickel: Levels of Ni were determined using Atomic Absorption Spectrum [AAS] (APHA, 2005 {APHA, 301A}). Two grams of soil samples were separately weighed for each site and transferred into separate Kjeldahl flasks; 20 ml of concentrated nitric acid (HNO_3) was added, and the samples pre-digested by heating gently for 20 mins. Afterwards, 10 ml nitric acid was added, and digestion continued for 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 Ni using the Atomic Absorption Spectrophotometer (AAS). The heavy metals were then analyzed at the wavelengths 341.5.

Preparation of Mineral Salt Agar and Potato Dextrose Agar: Mannitol Salt Agar (MSA) (11.10 g) was weighed and dissolved in 100 ml distilled water (as per manufacturer's instructions). The mixture was then sterilized the by autoclaving at 121°C for 15 min then cooled to 50 to 55°C. The mixture was then well mixed and dispensed aseptically in sterile petri dishes.

Potato Dextrose Agar (PDA) (39 g) powder was weighed and suspend in 1L of distilled water. The mixture was heated and stirred to dissolve all its

components. It was then sterilized by autoclaving at 121°C for 15 min then cooled to 50 to 55°C. The mixture was then well mixed and dispensed aseptically in sterile petri dishes. The plates were stored at 2 to 8°C in plastic bags to prevent loss of moisture.

Enumeration of Ni tolerant bacteria: Nickel tolerant bacteria was enumerated by pour plate method (APHA *et al*., 1998). In this method 1 g 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) mixed with antifungal reagent (fungin^{TM}) to inhibit the growth of metal tolerant fungi and hydrocarbon utilizing fungi. Fixed dose procedure was used, where microorganisms were dosed in a stepwise procedure using the fixed doses from a range of; 10 ppm, 25 ppm, 50 ppm, 75 ppm, 100 ppm, 150 ppm of Nickel (Ni). The initial dose levels were selected on basis of preliminary study as the dose expected to produce toxicity causing mortality. Plates were then incubated in an inverted position at room temperature (23 to 28°C) for 5 to 7 days. Colonies were counted in order to obtain colony forming units per gram of soil.

Enumeration of Ni tolerant fungi: Nickel tolerant fungi was enumerated by pour plate method (APHA *et al*., 1998). In this method, 1 g 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 Potato Dextrose Agar (PDA) mixed with an antibacterial reagent (Normocure™) which is a broad-spectrum antibacterial agent highly effective against Gram+ and Gram- bacteria, in order to inhibit the growth of bacteria and allow for only growth of fungi. Fixed dose procedure was used to dose microorganisms in a stepwise procedure using fixed doses of; 10 ppm, 25 ppm, 50 ppm, 75 ppm, 100 ppm and 150 ppm of Nickel (Ni). The initial dose levels were selected on basis of sighting study as the dose expected to produce toxicity effects. Plates were then incubated in an inverted position at room temperature for 5 to 7 days. Colonies were counted to obtain colony forming units per gram of soil (CFUs/g).

Determination of LC_{50} for bacteria and fungi: The percentages of dead organisms in each study concentration were determined and converted to probits. The regression analysis was then carried out and the values obtained from the probit analysis were used to compare the amount of chemical needed to generate responses between microorganisms in different study areas to different concentrations of Ni in the culture medium (Vincent, 1980). Areas that showed low values of LC_{50} were considered most toxic as compared to areas that recorded high values of LC_{50} (Vincent, 1980). Microorganisms in areas with high values of LC_{50} were considered more tolerant to exposure to the heavy metals. The 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) as shown in the formula 1.

$$y=ax+c \qquad (1)$$

Determination of total petroleum hydrocarbon content of soil: The Hewlett Packard 5890 Series II Gas Chromatograph FID method was used. In this method, 1 g of well-mixed sample was weighed into Acetone rinsed beaker. Then, 1 g of anhydrous sodium sulphate was added to the soil sample and 5 ml of solvent (1:1 of dichloromethane and acetone) was added and stirred for 15 min using a magnetic stirrer and the ensuing mixture was poured into a round bottom flask. This was repeated once more by adding 5 ml of mixed solvent. It was stirred and permitted to stand/settle and then decanted into another round bottom flask. The solvent was concentrated with 1 ml hexane to exchange it and it was re-concentrated to 2 ml. The columns were eluted (washed off) with 10 ml n-hexane. One ml (1 ml) of the extract was pipetted into the column and 10 ml of n-hexane was used to collect the aliphatic components. The extract was concentrated to 1 ml and poured into a glass vial for Gas Chromatography.

Data Analysis: Data was analyzed using IBM SPSS (Version 25.0) software. One Way analysis of variance (ANOVA) was used to evaluate the difference between the LC_{50} of the contaminated and control sites. Pearson's correlation analysis was used to determine the relationship between the LC_{50} values and the values of metal concentration and (Total Petroleum Hydrocarbon) TPH in the study areas, and $p \leq 0.05$ was considered to be the level of confidence.

RESULTS AND DISCUSSION

Microbial count for the soil and levels of LC_{50} Table 2 shows the observations of mean fungal colony forming units per gram (CFUs/g) of soil in the study sites. The industrial control site (CI) showed growth of 4.00×10^2 CFUs/g of soil at a maximum of 100 ppm. Industrial site 1 (I1) showed growth of 6.67×10^1 CFUs/g of soil at a maximum concentration of 75 ppm. Industrial site 2 (I2) showed growth of 1.80×10^2 CFUs/g of soil at a maximum concentration of 150 ppm. Industrial site 1 (I3) showed a maximum growth of 1.33×10^3 CFUs/g

of soil at concentration of 100 ppm (Table 2). In the agricultural area, the control sample (CA) showed population of 4.30×10^2 CFUs/g of soil at a concentration of 150 ppm. Aluu (A1) showed growth of 1.00×10^2 CFUs/g of soil at a concentration of 150 ppm. A2 had growth of 6.67×10^1 CFUs/g of soil at concentration of 100 ppm. Agricultural site 3 (A3) showed a population of 6.67×10^2 CFUs/g of soil (Table 3). In urban control area (CU) showed a maximum population of 1.60×10^3 CFUs/g of soil at a concentration of 50 ppm. Urban site 1 (U2) had a maximum population of 1.00×10^2 CFUs/g of soil at 75 ppm. Urban site 2 (U2) had growth of 6.67×10^1 CFUs/g of soil at 150 ppm and Urban site 3 (U3) had a maximum fungal population of 3.03×10^2 CFUs/g of soil at 100 ppm. Table 4 shows the different mean bacterial colony forming units per gram (CFUs/g) of soil as observed in different concentrations of Ni. In the industrial area, site CI had population of 7.73×10^3 CFUs/g of soil at a maximum concentration of 75 ppm. Site I1 had a population of 1.48×10^4 CFUs/g of soil. Site I2 had a population of 1.41×10^2 CFUs/g of soil at a maximum concentration of 150 ppm. Site I3 had a maximum population of 5.84×10^3 CFUs/g of soil at a maximum concentration of 100 ppm. In the agricultural areas, control site CA had population of 1.05×10^3 CFUs/g of soil at 100 ppm. Site A1 had a population of 6.67×10^3 CFUs/g of soil at a maximum concentration of 75 ppm. Site A2 had population of 1.00×10^3 CFUs/g of soil at 100 ppm, while site A3 had growth of 6.67×10^2 CFUs/g of soil at 100 ppm. In the urban area, control site CU had a population of 4.07×10^3 CFUs/g of soil at a concentration of 75 ppm. Site U1 had a population of 3.85×10^4 CFUs/g of soil at a concentration of 75 ppm. Site U2 had a population of 5.00×10^1 CFUs/g of soil at a concentration of 150 ppm and site U3 had population of 4.47×10^3 CFUs/g of soil at a concentration of 75 ppm. The highest populations of CFUs/g of soil were recorded at a maximum concentration of 150 ppm and was 5.00×10^1 and 1.41×10^2 CFUs/g of soil observed in sites I2 and U2 respectively.

Mean Lethal concentration 50 (LC_{50}) of Ni in fungi varied between 0.43±0.05 ppm to 15.05±5.34 ppm. Mean Lethal concentration 50 (LC_{50}) in bacteria varied between 0.73±0.54 ppm to 1.36±0.29 ppm. There was significant difference ($p \leq 0.000$) in mean levels of LC_{50} of fungi between the study sites (Table 3). There was no significant difference ($p = 0.918$) in mean values of LC_{50} of bacteria between the study sites (Table 4).

Table 2: Mean CFUs/g of soil Nickel tolerant fungi in different concentrations in Greater Port Harcourt Area, Nigeria

Study site	**0 ppm**	**10 ppm**	**25 ppm**	**50 ppm**	**75 ppm**	**100 ppm**	**150 ppm**	**LC_{50}**
CI	5.21×10^4	1.37×10^4	2.40×10^3	2.30×10^2	1.97×10^2	4.00×10^2	0	0.81±0.50
I1	6.17×10^5	2.53×10^4	1.80×10^3	1.77×10^3	6.67×10^1	0	0	0.43±0.05
I2	1.32×10^4	4.97×10^3	2.40×10^3	1.33×10^3	1.07×10^3	8.00×10^2	1.80×10^2	2.44±1.02
I3	7.50×10^4	4.90×10^4	3.33×10^3	2.30×10^3	1.93×10^3	1.33×10^3	0	1.67±0.37
CA	6.18×10^3	4.07×10^3	2.70×10^3	2.40×10^3	2.13×10^3	1.80×10^3	4.30×10^2	15.05±5.34
A1	1.45×10^5	1.23×10^4	2.40×10^3	8.03×10^2	3.63×10^2	1.77×10^2	1.00×10^2	0.60±0.16
A2	3.27×10^4	3.40×10^3	1.83×10^3	1.13×10^3	7.33×10^2	6.67×10^1	0	0.92±0.26
A3	8.23×10^3	4.07×10^3	2.77×10^3	1.97×10^3	1.27×10^3	1.10×10^3	6.67×10^2	6.53±3.66
CU	7.70×10^4	3.93×10^3	2.47×10^3	1.60×10^3	0	0	0	0.65±0.07
U1	1.44×10^4	4.23×10^3	2.07×10^3	1.80×10^2	1.00×10^2	0	0	1.06±0.29
U2	6.60×10^4	5.43×10^3	3.23×10^3	1.70×10^3	1.40×10^3	5.60×10^2	6.67×10^1	1.54±0.93
U3	6.23×10^4	4.23×10^4	2.50×10^3	1.80×10^3	1.13×10^3	3.03×10^2	0	1.40±0.62
p =								0.000

Table 3: Mean CFUs/g for soil Nickel tolerant bacteria in different concentrations in Greater Port Harcourt Area, Nigeria

Study site	**0 ppm**	**10 ppm**	**25 ppm**	**50 ppm**	**75 ppm**	**100 ppm**	**150 ppm**	**LC_{50}**
CI	7.17×10^7	3.82×10^7	2.12×10^5	1.99×10^4	7.73×10^3	0	0	1.18±0.72
I1	1.37×10^7	7.43×10^6	2.56×10^5	3.10×10^4	1.48×10^4	0	0	0.98±0.15
I2	7.43×10^6	3.73×10^6	3.81×10^5	2.01×10^5	7.51×10^3	2.83×10^3	1.41×10^2	1.25±0.24
I3	3.37×10^7	4.80×10^6	4.04×10^5	2.27×10^5	5.84×10^3	0	0	1.01±0.84
CA	6.17×107	1.48×10^7	2.80×10^6	3.93×10^5	2.24×10^5	1.05×10^3	0	0.81±0.29
A1	1.30×10^6	6.77×10^5	8.53×10^4	7.07×10^3	6.67×10^3	0	0	1.06±0.15
A2	7.07×10^7	3.37×10^7	1.68×10^6	2.75×10^5	5.37×10^4	1.00×10^3	0	1.11±0.39
A3	5.73×10^7	1.26×10^7	1.70×10^6	1.57×10^5	2.57×10^4	6.67×10^2	0	0.74±0.42
CU	3.33×10^6	5.53×10^5	3.53×10^4	1.95×10^4	4.07×10^3	0	0	1.00±0.57
U1	4.57×10^7	2.12×10^7	1.51×10^6	6.17×10^4	3.85×10^4	0	0	1.13±0.42
U2	1.88×10^7	6.43×10^6	1.42×10^6	6.63×10^5	5.19×10^4	1.63×10^3	5.00×10^1	1.36±0.29
U3	1.96×10^7	5.77×10^6	1.96×10^5	1.94×10^4	4.47×10^3	0	0	0.73±0.54
p =								0.918

Mean soil TPH, Ni and LC_{50} of contaminated and control sites: Table 4 shows the mean values of the Ni, TPH and LC_{50} for fungi and bacteria in the contaminated and control areas. The mean values for Ni were 1.38±0.23 in industrial area, 1.41±0.36 ppm in agricultural area and 1.02±0.64 in urban area. There was no significant difference ($p = 0.222$) between the study and control areas. All the values were within

permissible limits of WHO of ≤50 (ppm). The mean values for TPH were 4,405.46 ppm in industrial area, 55.65 ppm in agricultural area and 1,304.53 ppm in urban area. The mean values of TPH varied and were oscillating above or within the EPA recommended standard level of ≤500 ppm. All the mean values of TPH in the contaminated areas were higher than the TPH values in the control areas. There was no significant correlation between the concentration of TPH in soil and LC_{50} for fungi (r = -0.169) and bacteria (r = 0.042) (Table 4). Further, there was no significant correlation between concentrations of Ni in soil with LC_{50} of fungi (r = 0.175) and bacteria (r = 0.079) (Table 4).

Table 4: Comparison of mean levels of Ni and TPH in agricultural, industrial and urban soils of Greater Port Harcourt Area, Nigeria

Study area	LC_{50} NiTF	LC_{50} NiTB	TPH (ppm)	Ni (ppm)
Industrial	1.51±1.03	1.10±0.46	4,405.46	1.38±0.23
Industrial Control	0.81±0.49	1.18±0.72	174.16	1.31±0.32
Agricultural	2.68±0.34	0.97±0.34	55.65	1.41±0.36
Agricultural Control	15.05±5.34	0.81±0.29	16.18	1.58±0.06
Urbanized	1.33±0.61	1.07±0.46	1,304.53	1.02±0.64
Urban Control	0.65±0.07	1.00±0.56	236.71	1.52±0.27
p =	0.000	0.918	0.003	0.222
LC_{50}/TPH r =	-0.169	0.042	1.00	
LC_{50}/Ni r =	0.175	0.079		

Comparison of mean levels of LC_{50} of fungi and bacteria in agricultural, industrial and urban areas: Figure 2 compares the mean values of LC_{50} for fungi and bacteria between the contaminated and control sites. The Mean levels of LC_{50} for fungi and bacteria in agricultural area was 2.68±3.42 and 0.97±0.34 ppm respectively. The Mean levels of LC_{50} for fungi and bacteria in industrial area was 1.51±1.03 and 1.08±0.46 ppm respectively. The Mean levels of LC_{50} for fungi and bacteria in urban area was 1.33±0.61 and 1.07±0.46 ppm respectively. The highest values of tolerance were observed in fungi in agricultural area which was 2.68±0.34 ppm. Further, the agricultural control area showed higher value (15.05±5.34 ppm) as compared to the agricultural study site (2.68±0.34 ppm**).**

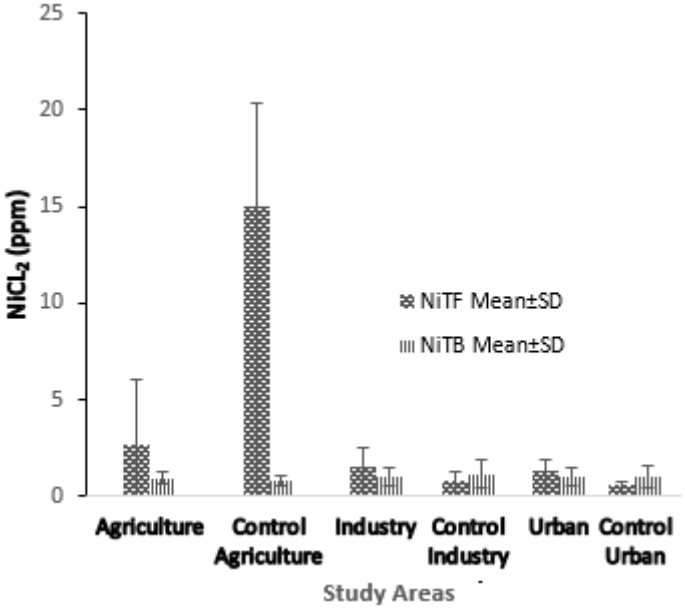

Fig 2: Mean levels of LC_{50} for Ni on fungi and bacteria in agricultural, industrial and urban areas of Greater Port Harcourt Area, Nigeria. (NiTF = Nickel Tolerant Fungi, NiTB = Nickel Tolerant Bacteria)

Sources of oil contamination in the environment can be grouped as; natural, during exploration/extraction/production and spills in the course of transportation (Hester & States, 2018). Therefore, anthropogenic activities are a large source of oil to the environment (Ramseur, 2012; Jernelöv, 2018; Wang *et al*., 2018). Nickel is one of the metals that has been linked to oil sources. Nickel is added in gasoline and has been associated with soils on the roadside (Arslan, 2006). Nickel properties and concentrations in soil are also dependent on soil properties and can also be influenced by anthropogenic activities (Elbana & Selim, 2019) including mining, fertilizers, sewage, combustion of fossil fuels and

metal smelting (Gonnelli and Renella, 2013; Wang *et al*., 2018). In this study, the mean concentrations of Ni varied among the study areas which agrees with findings of Kabata-Pendias, (2011). Levels of Nickel in soil range between 0 to 80 ppm and vary depending on geology and anthropogenic inputs in an area (Kabata-Pendias, 2011). The variation in values of Ni in the current study can be linked to the magnitude of pollution by human activities (Mohamed, Shaala, Zahmir, & Ismail, 2015), as well as retention capacity of the soils (Elbana & Selim, 2019). Retention of nickel in soil is dependent on a number of factors; pH, soil carbon, clay, silt and $CaCO_3$ content (Elbana & Selim, 2019). Total Petroleum Hydrocarbon (TPH) were varied among the study areas but did not show any significant relationship to the values of nickel. This implies that the values of Nickel can be linked to other sources including mining, fertilizers, sewage, combustion of fossil fuels and metal smelting (Gonnelli and Renella, 2013; Wang *et al*., 2018), rather than contamination with oil. Not all crude oils are characterized with high levels of Nickel but rather is varied. The findings of this study agree with those of (John, 2001) which showed varied amounts of contamination of Ni in crude oil. Crude oil has a concentration of 8 ppm in Kuwait, 9 ppm in Prudhoe Bay in Alaska and 10 ppm in Arab light oil in the Middle East. Contrarily to the low concentration of Ni in crude oil in some countries, there are other places where crude oil is produced with high concentrations of Ni. These areas include; Beta (Los Angeles Beach) which ranges between 104 -166 ppm, Cerro negro (Venezuela) at 118 ppm and California offshore at 166 ppm. This is an indication that crude oil in the study areas had low concentration of Ni, as the tainted soils showed low concentrations that are close to control samples. Therefore, Ni plays a vital role in classification of petroleum according to the relationship between oil properties and source rock properties (Barwise, 1990).

Highest mean CFUs/g of soil Nickel tolerant fungi was recorded in Agricultural sites at 150 ppm. The findings of this study are in agreement with those of Afzal, Rasool, Waseem, & Aslam in 2017, on the study of tolerance bacterial and fungal isolates to Nickel (Ni) to a maximum concentration of 50% (Afzal *et al*., 2017). Tolerance of microorganisms to Ni varies according to levels of pre-exposure to varied concentrations. Further, the findings agree with those of Renella, (2017), who linked distribution of soil microorganisms to levels of contamination of soil. Microbial populations vary among different locations and 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; Gonnelli and Renella, 2013; Renella, 2017). In the current study there was no significant correlation between LC_{50} of nickel tolerant bacteria and levels of nickel in the soils of the study sites. Also, there was no significant relationship between LC_{50} of nickel tolerant fungi and bacteria to levels of TPH among the study sites. Other sources of Ni in soil include; windblown dust, volcanoes, combustion of oil, incineration of domestic wastes, Ni refining, production of steel, natural occurrence, electroplating, zinc base casting and battery industry (Gonnelli and Renella, 2013; Paul, 2017). These could be the sources of Ni in the industrial and urban areas in the current study. However, the levels are within the WHO permissible limits of ≤ 50 ppm. Washing off of bacteria and fungi from adjacent ecosystems to the study areas could also be a contributing factor to the varied distribution of Ni tolerant microorganisms among the study areas (McKinney, 2004). Underground soils, act as reservoirs of varied soil microorganisms which are resilient and emerge when survival conditions are optimal (McKinney, 2004). There is variation in availability and abundance of basic requirements for flourishment of microorganisms in various microhabitats and is a major reason for varying nitches with diverse taxa. The type and quantity of organic substrates available in soil directly translates to abundance of soil microorganisms as well as their diversity in soil (Grayston, 2001). Spore forming bacteria are more resilient in harsh conditions as they exist in form of spores and can undergo dormancy. When conditions are normal, they propagate once again (McKinney, 2004).

Conclusion: The findings in this study reveal the area which harbours Ni tolerant Fungi and bacteria can be useful for selection of microorganisms for bioremediation of Ni contaminated areas. Ni was within the recommended limits of ≤ 50 ppm in all study sites. However, the highest values of LC_{50} for Ni were observed in the agricultural areas which harbours petroleum activities an indication that farm produce may be contaminated. Also, the study does not associate the levels of Ni in the soils only to TPH, but other probable sources like natural sources and other human activities like agricultural inputs in the area.

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