Research article

Interactions of trace metals with bacteria and fungi in selected agricultural soils of Egbema Kingdom, Warri North, Delta state, Nigeria

Alex A. Enuneku [a], Peter I. Abhulimen [b], Patrick Omoregie Isibor [c,*], Charming Osaro Asemota [b], Benjamin Okpara [b], Tunde O. Imoobe [b], Lawrence I. Ezemonye [a]

[a] Department of Environmental Management and Toxicology, Faculty of Life Sciences, University of Benin, PMB 1154, Benin City, Nigeria
[b] Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin, PMB 1154, Benin City, Nigeria
[c] Department of Biological Science, Covenant University, PMB 1023, Ota, Ogun State, Nigeria

ABSTRACT

This study was aimed at investigating the relationship of trace metals with prevalence of bacteria and fungi in polluted farmland of Egbema Kingdom, Delta state. This may contribute to the knowledge on the influence of the microorganism to the arability of agricultural soil from an ecotoxicological viewpoint. Soil samples were analyzed for physico-chemical parameters and trace metals using atomic absorption spectrophotometer (Buck Scientific, 210VGP). The samples were also screened for fungi and bacteria isolates. Trace metals (Fe, Zn, Cd, Ni and Cu) determined in the agricultural soils of Egbema kingdom were below WHO limits. The fungi species with the highest number of occurrence was *Aspergillus niger,* while the species with the lowest occurrence were *Fusarium oxsporium, Penicillium dirty white,* and *Penicillium sp*. The order of abundance of fungi across the stations was Station 2> Station 4> Station 3> Station 1> Station 7> Station 5> Station 6> Station 9> Station 8. The bacteria species with the highest number of occurrence was *Proteus sp I,* while the species with the lowest occurrence was *Alcaligenes sp*. which occurred only once at Station 5. The order of abundance of bacteria in the soil samples across the stations was Station 4> Station 3> Stations 5 and 9> Station 2> Station 8> Station 7> Station 6. All trace metals in the agricultural soils of Egbema kingdom were below WHO limits. Fe, Zn, Cd and Ni showed no relationship with the microbial contents of the soil. Various strains of fungi and bacteria (gram positive and gram negative) were observed in the soil samples. An antagonistic relationship was observed between bacteria and fungi. Significant positive correlation occurred between the concentration of copper and bacteria counts indicating that the concentration of copper in the soil might have enhanced the abundance of bacteria in the soil, vice versa. The bacteria might be a promising tool in regulation of soil copper concentration.

1. Introduction

Although oil exploration and exploitation has boosted many economies around the world, the impact on the environment has been devastating (Isibor et al., 2020). These anthropogenic activities have been linked to alarming concentrations of trace metals in the soil samples (Simeonov et al., 2010) from the Niger Delta areas of Nigeria (Isibor et al., 2020).

Mercury (Hg), cadmium (Cd), lead (Pb), chromium (Cr) and arsenic (As) are non-essential trace metals which are particularly responsible for biological toxicity (Sungur et al., 2014; Unsal et al., 2014). Other essential trace metals such as zinc (Zn), copper (Cu), nickel (Ni), stannum (Sn), vanadium (V), etc. may also be toxic at high concentrations beyond needed. In recent years, the advent of global economic development has been accompanied by rise in metal-mediated deterioration of the environment (Rakesh and Raju, 2013).

Trace metals enter into the environment from both natural and anthropogenic sources. Trace metal toxicity may result in compromised soil arability which in many cases has shown inhibitory effects on plants growth (Unsal et al., 2014). Exposed plants may elicit disrupted physiological activities such as photosynthesis, gaseous exchange and nutrient absorption which result in plant growth reduction and dry matter accumulation (Oketola and Fagbemigun, 2013) thus threatening national and global food security and causing land tenure problems (Imarhiagbe et al., 2017; Igiri et al., 2018).

Trace metals may contaminate food sources and accumulate in agriculture products and seafood through water, air and soil pollution (Rajaganapathy et al., 2011; Tchounwou et al., 2012; Shahid et al., 2013; Wyszhowska et al., 2013; Kabiru et al., 2015) Contamination of soil may further pose risks to humans health through direct ingestion within the food chain (soil-plant-human or soil-plant-animal-human), drinking of contaminated water, or contact with contaminated soil.

Elevated trace metal concentrations in soil may affect microbial proliferation and enzymatic activities, possibly leading to a decrease in the rates of the biochemical process in the soil environment (Filazi et al., 2003; Ubani and Onyejekwe, 2013). Unlike carbon-based molecules, trace metals are non-biodegradable rather bioaccumulative, toxic, and persistent. The soil's physicochemical properties confer immobility on trace metals, thereby promoting their accumulation and persistence in the soil system (Bhaskar et al., 2010; Daniel et al., 2016).

Organisms influencing soil development range from microscopic bacteria to large animals; including man. Microorganisms such as bacteria and fungi assist in the decomposition of plant and animal litter (Ajaz et al., 2010; Aguilar et al., 2013). This litter is mixed into the soil by macro organisms (soil animals) such as worms and beetles. Soil horizons are less distinct when there are much soil organism activities (Fashola et al., 2016). The nature of the soil humus is determined by the vegetation cover and resultant litter inputs. Roots bind up soil and conserve the inherent nutrients and trace metals, thereby impeding natural depuration processes such as erosion or leaching (Bhagure and Mirgane, 2010; Ghosal et al., 2016).

Metal distribution between soil and vegetation is a key issue in assessing environmental effects of metals in the environment (Abioye, 2011; Abdul et al., 2013).

There is need to improve on already existing remediation techniques and also develop new ones that can help to effectively checkmate the negative impacts of trace metal contamination in the soil (Ou-Yang et al., 2010; Peter and Adeniyi, 2011; Olaniran et al., 2013). Understanding the relationships between trace metals and soil microbes may give insights to more informed management options for contaminated agricultural soils,

enzymatic activity, photosynthetic activity and accumulation of other nutrients (Orimoogunje et al., 2010; Oketola et al., 2013). Soil is an ecological pivot which transports contaminants to other environmental media such as surface water, groundwater, atmosphere, food and biota (Lankinen et al., 2011; Iram et al., 2013; Ihl et al., 2015).

The activity of soil microorganisms is closely related to soil fertility and environmental quality (Wang et al., 2006). Fungi and bacteria constitute the main components of the soil microbial biomass and serve as very constructive models for studying the harmful effects of metals at the cellular level (Kouchou et al., 2017; Akinsanya et al., 2020). Isibor and Imoobe (2017) submitted that the most economical and reasonable method for monitoring trace metals in the atmosphere is using soil and plant samples, which have been widely used as cumulative matrices for long and short term exposure to pollutants (Sungur et al., 2014; Unsal et al., 2014; Ihl et al., 2015; Kouchou et al., 2017).

This study was aimed at investigating the interrelationships between trace metal levels and prevalence of bacteria and fungi in polluted farmland of Egbema Kingdom, Delta state. The findings may proffer a sustainable means of restoring soil arability.

2. Materials and methods

2.1. The study area

To the North of Egbema Kingdom are the Olodiama of Edo State and the Itsekiri of Delta State.To the West of the kingdom is the Arogbo Kingdom of Ondo State, the East is Gbaramatu Kingdom and the Itsekiri of Delta State, and to the South are the Ilaje of Ondo State and the Atlantic Ocean (Figure 1). The people are mainly fishermen, hunters, canoe builders, distillers of local gin and farmers. Nine (9) Stations were selected in different communities, which include Station1- Tsekelewu (E 004.97356°, N 05.04335°), Station 2- Kpoku-gbene (E 005.0163°, N 05.94935°), Station 3-Iralatei (E 005.02834°, N 05.94627°), Station 4- Agoduba (E 005.04122°, N 05.94014 °), Station 5- Ozuoedodo (E 005.04335°, N 05.94054°), Station 6- Weke-gbene (E 005.04945°, N
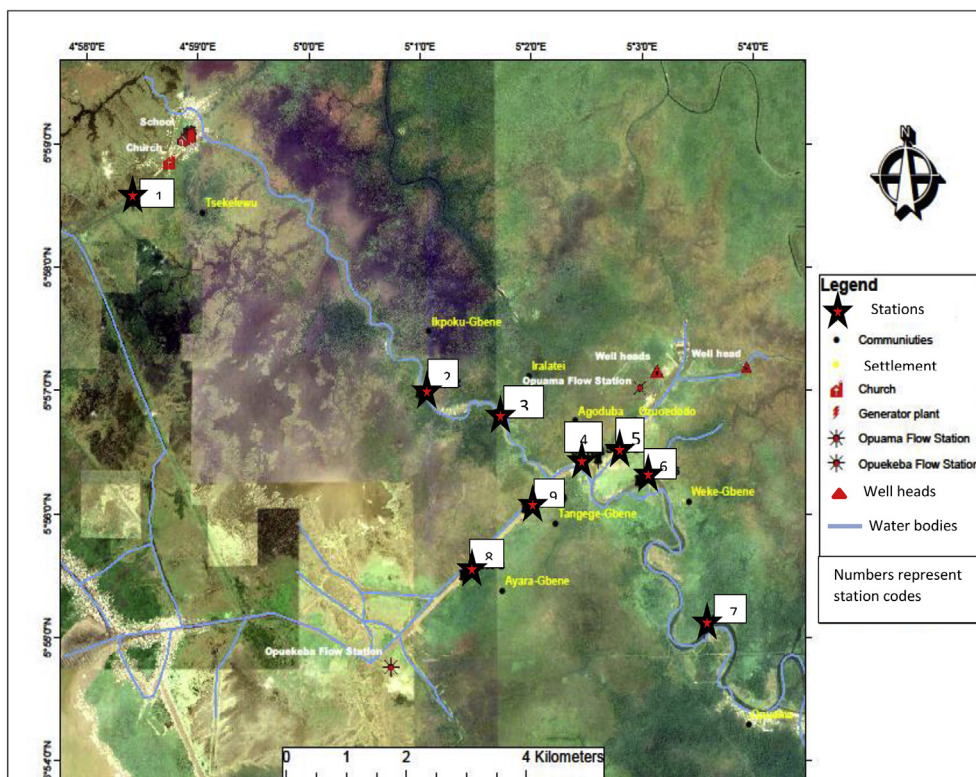

Figure 1. Map of study area showing sampling stations.

05.93746°), Station 7- Opuama (E 005.05929°, N 05.9175°), Station 8-Ayara-gbene (E 005.02323°, N 05.92470 °), and Station 9- Tangege-gbene (E 005.03247°, N 05.93379 °).

2.2. Collection of samples

Nine (9) soil samples were collected monthly from April–June, 2019 at each station. The soil sample for analyses were collected by using a sterile digger at the particular station to scoop off the amount of soil needed.

2.3. Physical and chemical properties of soil

2.3.1. Particle size distribution

This was determined by Day (1965). 51g of air-dry was collected a baffled stirring cup. The cup was half-filled with 100 mL distilled water and 50 mL of $(NaPO_3)_6$ solution was added. The solution was mixed with stirring rod and allowed to stand for 30 min. The cup of mixtures was stirred for 5–10 min until the soil aggregates were broken down. The stirred mixture was quantitatively transferred to the settling cylinder by washing the cup with distilled water. The cylinder was then filled to the mark with distilled water. The suspension was shaken vigorously to allow proper mixing. The content of the cylinder was allowed to stand for 2 h then carefully read using hydrometer.

Then the hydrometer and temperature readings were taken.

$$\% \text{ silt} + \% \text{ clay} = \frac{HR \pm 0.36 \ (T-20)°C \times 100}{\text{Weight of the sample used}}$$

$$\% \text{ clay} = \frac{HR \pm 0.36 \ (T-20)°C \times 100}{\text{Weight of the sample used}}$$

HR and T reading for above (clay) are the 2 h reading

$\%$ sand $= 100 - (\%$ silt $+ \%$ clay$)$

$\%$ silt $= (\%$ clay $+ \%$ silt$) - \%$ clay

OR $= 100 - \%$ sand $- \%$ clay

2.3.2. Soil pH

Soil pH was determined at 1:1 soil to water ratio using with an electrode pH meter in water.

2.3.3. Total nitrogen

Soil sample was digested using the micro-Kjeldahl method and determined spectrophotometrically using the Iodophenol method. Organic Carbon: Organic carbon was determined using wet oxidation method as modified by. 1.0 g of the prepared soil sample was weighed into a 250 mL conical flask and 10 mL potassium dichromate was added. 20 mL sulphuric acid was added and allowed to stay for 30 min. 100 mL of deionized water was added, afterwards 5 drops of ferroin indicator was added. The mixture was centrifuged at 3,000 rpm for 10 min. Afterwards the mixture was titrated against $0.5N$ $FeSO_4$ and reading was taken. Percentage organic carbon was also determined.

2.3.4. Available phosphorus

Available phosphorous was extracted using Bray1 method. The phosphorus concentration in the extract was determined calorimetrically by the ascorbic acid molybdenum-blue method.

2.3.5. Exchangeable cations

Exchangeable bases (Ca, K, Mg and Na) were extracted using 1N ammonium acetate (NH_4OAC) at pH 7.0. Calcium and magnesium content were determined using the Atomic Absorption Spectrophotometer while potassium and sodium were read using the Flame Photometer (Chapman, 1965).

2.3.6. Exchangeable acidity

Exchangeable acidity was determined by leaching the soil with potassium chloride (KCL solution) and the extract titrated with 0.1 N standard sodium hydroxide solution. 3 g of soil sample was digested with 60 mL was added 1N KCl. 25 mL of the filtrate was collected in a conical flask and 100 mL of deionized water was added. Three to four drops of phenolpthaline was added and titrated against 0.01 N NaOH until a permanent pink was obtained.

2.3.7. Effective cation exchange capacity (ECEC)

ECEC was determined by summation of exchangeable cations and exchangeable acidity.

2.3.8. Base saturation

Base saturation (BS) was calculated by dividing the sum of exchangeable bases (Ca, K, Mg and Na) by the ECEC and multiplying the quotient by 100.

$$BS\% = \frac{(\text{Exchangeable cation}) \times 100.}{\text{ECEC}}$$

2.4. Analysis of soil sample for trace metals

2.4.1. Chemicals and reagent

All chemicals and reagent were analytical grade. Material and reagent were used including 72% HNO_3 (BDH), 37% HCL (JHD). In order to construct the calibration curves, working standard solutions for Cd, Pb, Cu, Ni, Fe and Z were freshly prepared by diluting an appropriate aliquot of standard solution containing 1000 ppm with serial concentration for each element using 0.1% HNO_3. Glass ware and polyethylene container were cleaned and soaked in 10% HNO_3 for 48 h and then rinsed thoroughly with deionized water.

2.4.2. Sample digestion and heavy metal analysis

In the laboratory, the soil samples were air dried for 48 h and grounded with ceramic mortar and pestle. Digestion of soil samples was carried out. Then 1gram of sample was digested in 10ml freshly prepared aqua regia (3:1, HNO3: HCL) in a hot sand bath on a hot plate for 45 min. It was allowed to cool. 20 mL distilled water was then added. Then it was filtered through a Whatman filter paper 110 mm) into a 100 mL standard flask. It was made up to mark with distilled water. Samples were then analyzed for trace metals using atomic absorption spectrophotometer (Buck Scientific, 210VGP).

2.5. Isolation of bacteria and fungi

A serial dilution method was aseptically carried out in tubes. Sterile test tubes were used for the ten-fold dilution. Test tubes labeled $10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}$ were used for each of the samples and 1 g of each of the samples was mixed with 9 mL of sterile distilled water into a test tube. 1 mL of the aliquot was obtained from each of the samples and transferred into the test tube labeled 10^{-1} and mixed properly. Aliquot (1 mL) was then transferred serially from the tube labeled 10^{-1} to tubes labelled 10^{-2}, 10^{-3}, 10^{-4} respectively. This was done for each of the samples and at the end of each serial dilution. The 1 mL left in the pipette tip was discarded. Aliquots from the appropriate tubes were then used to inoculate appropriate media for isolation and/or detection of target bacteria and fungi using the pour plate method.

The pour plate technique was used for the isolation of bacteria during the study. The agar media used were prepared according to manufacturers' instruction. The plates were labeled appropriately, and with the aid of a 0.1 mL pipette aliquots of the appropriate dilutions were

Table 1. Physico-chemical characteristics of the study stations at Egbema Kingdom.

Parameters	Units	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6	Station 7	Station 8	Station 9	p-value
pH		4.8 ± 0.2 (4.6–5.0)	5.2 ± 0.2 (5.0–5.4)	5.6 ± 0.3 (5.3–6.0)	8.1 ± 0.2 (7.9–8.3)	4.2 ± 0.8 (3.5–5.0)	4.1 ± 0.2 (3.9–4.2)	5.3 ± 0.2 (5.1–5.5)	4.4 ± 0.4 (4.0–4.7)	4.4 ± 0.1 (4.3–4.6)	P > 0.05
OC	g/kg	26.2 ± 0.7 (25.4–6.7)	30.0 ± 0.8[A] (20.2–21.8)	29.2 ± 0.6[A] (28.7–29.9)	17.3 ± 0.8 (16.6–18.2)	25.5 ± 0.5 (25.0–25.9)	27.9 ± 0.6 (27.3–28.5)	29.9 ± 0.4[A] (929.6–30.3)	30.1 ± 0.2[A] (2.9–30.5)	29.7 ± 0.3[A] (29.5–30.0)	P < 0.05
TN	g/kg	56.7 ± 42.5[A] (7.6–82.0)	10.0 ± 0.3 (9.7–10.2)	9.9 ± 0.3 (9.6–10.2)	11.1 ± 0.1 (11.0–11.2)	17.0 ± 0.2 (16.9–17.2)	8.5 ± 0.5 (8.0–9.0)	16.8 ± 1.5 (15.6–18.5)	23.2 ± 0.5 (22.6–23.5)	17.1 ± 0.3 (16.9–17.5)	P < 0.05
Nitrates	mg/kg	31.9 ± 1.4[B] (30.9–33.5)	28.5 ± 1.0[B] (27.5–29.5)	79.4 ± 0.8[A] (78.8–80.2)	41.5 ± 1.3[B] (40.2–42.9)	21.2 ± 0.2[B] (21.0–21.5)	15.6 ± 0.6 (15.0–16.2)	15.8 ± 1.0 (15.0–16.9)	122.1 ± 2.9[A] (120.0–125.5)	71.2 ± 11.8[A] (60.0–83.5)	P < 0.05
EA	Cmol/kg	9.6 ± 7.3[A] (1.2–14.2)	0.7 ± 0.5 (0.3–1.2)	0.9 ± 0.6 (0.5–1.6)	0.8 ± 0.4 (0.4–1.3)	7.4 ± 1.0[A] (6.3–8.2)	3.1 ± 0.3[B] (2.7–3.3)	2.2 ± 0.5[B] (1.7–2.6)	5.2 ± 1.1[B] (4.1–6.2)	2.1 ± 0.5[B] (1.6–2.6)	P < 0.05
K	Cmol/Kg	4.2 ± 1.2[A] (3.1–5.4)	2.8 ± 0.4 (2.5–3.3)	2.9 ± 0.2 (2.6–3.0)	5.1 ± 0.3[A] (4.7–5.2)	2.0 ± 0.4 (1.6–2.4)	1.7 ± 0.5 (1.1–2.0)	2.4 ± 0.5 (1.9–2.9)	2.8 ± 0.6 (2.1–3.1)	2.3 ± 0.5 (1.8–2.7)	P < 0.05
Ca	Cmol/Kg	4.6 ± 0.4 (4.2–5.0)	5.7 ± 0.5 (5.3–6.2)	7.8 ± 0.6 (7.4–8.5)	9.7 ± 0.5 (9.2–10.2)	2.8 ± 0.6 (2.4–3.6)	5.0 ± 0.2 (4.8–5.2)	7.8 ± 0.6 (7.0–8.2)	5.1 ± 0.7 (4.4–5.8)	4.4 ± 0.9 (3.4–5.0)	P > 0.05
Mg	Cmol/Kg	3.3 ± 0.9 (2.5–4.2)	2.7 ± 0.3 (2.5–3.0)	2.8 ± 0.2 (2.7–3.0)	3.1 ± 0.4 (2.6–3.3)	2.6 ± 0.3 (2.4–2.9)	2.8 ± 0.2 (2.5–3.0)	3.1 ± 0.3 (2.7–3.3)	2.7 ± 0.3 (2.4–3.0)	2.9 ± 0.3 (2.6–3.0)	P > 0.05
Na	Cmol/Kg	1.4 ± 0.5 (1.1–2.0)	1.1 ± 0.1 (1.0–1.1)	0.9 ± 0.5 (0.2–1.2)	1.3 ± 0.2 (1.2–1.5)	1.2 ± 0.3 (1.0–1.6)	1.5 ± 0.5 (1.2–2.0)	1.6 ± 0.5 (1.1–2.1)	1.6 ± 0.4 (1.2–2.0)	1.5 ± 0.5 (1.1–2.1)	P > 0.05
ECEC		23.1 ± 9.3 (12.5–30.1)	12.9 ± 1.1 (11.8–14.0)	15.2 ± 1.0 (14.3–16.2)	19.9 ± 1.6 (18.0–20.9)	15.9 ± 2.5 (13.7–18.6)	14.1 ± 1.1 (13.2–15.3)	17.1 ± 2.3 (14.5–18.8)	17.3 ± 1.9 (15.5–19.4)	13.1 ± 2.4 (10.4–14.7)	P > 0.05
BS		64.5 ± 22.5[A] (50.3–0.9)	95.2 ± 3.5 (91.2–97.7)	94.3 ± 4.0 (89.8–96.2)	96.22 ± 1.96 (94.02–97.78)	53.60 ± 2.07 (51.5–55.6)	77.9 ± 1.5[A] (76.3–79.3)	87.3 ± 1.01[A] (86.5–88.5)	70.3 ± 3.2[A] (67.8–73.8)	84.2 ± 2.6[A] (81.4–86.5)	P < 0.05
Clay	%	21.5 ± 1.3 (20.2–3.2)	23.2 ± 1.0 (22.2–24.2)	24.2 ± 1.0 (23.2–25.2)	17.0 ± 0.6 (16.3–17.3)	17.6 ± 1.2 (16.3–18.3)	18.2 ± 2.0 (16.2–20.2)	21.9 ± 0.6 (21.2–22.2)	19.9 ± 1.2 (19.2–21.2)	8.2 ± 10.4[A] (2.2–20.2)	P < 0.05
Sand	%	61.8 ± 1.0 (60.8–62.8)	64.8 ± 1.0 (63.8–65.8)	61.8 ± 1.0 (60.8–62.8)	71.3 ± 1.2 (70.6–72.6)	79.8 ± 0.6 (79.5–80.5)	69.7 ± 1.0 (68.7–70.7)	67.4 ± 0.6 (66.8–67.8)	71.3 ± 0.6 (70.7–71.7)	69.4 ± 0.6 (68.7–69.7)	P > 0.05
Silt	%	15.3 ± 1.5 (14.0–7.0)	12.1 ± 1.0 (11.0–13.1)	14.0 ± 1.0 (13.0–15.0)	11.7 ± 0.6 (11.1–12.1)	3.2 ± 1.0[A] (2.2–4.2)	12.1 ± 1.0 (11.1–13.1)	10.7 ± 1.2 (10.0–12.0)	8.8 ± 0.6 (8.1–9.1)	9.7 ± 1.2 (9.1–11.1)	P < 0.05

OC = organic carbon, TN = total nitrogen, EA = exchangeable acidity, ECEC = effective cation exchange capacity, BS = base saturation. Numbers with different superscripts are significantly different (p < 0.05), while numbers with same superscript are not significantly different (p > 0.05). Sample size (N) = 3.

Table 2. Trace metal concentrations (mg/kg) in agricultural soils of Egbema Kingdom.

Trace metals	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6	Station 7	Station 8	Station 9	WHO (1993)
Cadmium	0.01 ± 0.02	0.02 ± 0.03	0.03 ± 0.04	0.03 ± 0.05	0.01 ± 0.00	0.03 ± 0.03	0.04 ± 0.05	0.01 ± 0.01	0.02 ± 0.02	1
	(0.00–0.03)	(0.00–0.05)	(0.00–0.07)	(0.00–0.08)	(0.01–0.01)	(0.00–0.06)	(0.01–0.10)	(0.00–0.02)	(0.00–0.04)	
Lead	0.57 ± 0.74^{A}	0.17 ± 0.12	0.13 ± 0.06	0.63 ± 0.78^{A}	0.10 ± 0.17	0.24 ± 0.22	0.37 ± 0.46	0.17 ± 0.14	0.20 ± 0.10	2
	(0.00–1.40)	(0.10–0.30)	(0.10–0.20)	(0.00–1.50)	(0.00–0.30)	(0.10–0.50)	(0.10–0.90)	(0.02–0.30)	(0.10–0.30)	
Copper	0.17 ± 0.07	0.20 ± 0.06	0.20 ± 0.16	0.16 ± 0.05	0.20 ± 0.05	0.20 ± 0.04	0.19 ± 0.04	0.15 ± 0.01	0.16 ± 0.06	30
	(0.09–0.22)	(0.14–0.26)	(0.19–0.22)	(0.12–0.21)	(0.15–0.24)	(0.16–0.23)	(0.15–0.22)	(0.14–0.16)	(0.10–0.21)	
Nickel	0.15 ± 0.13	0.23 ± 0.14	0.27 ± 0.08	0.29 ± 0.10	0.19 ± 0.11	0.25 ± 0.08	0.35 ± 0.11	0.22 ± 0.15	0.26 ± 0.11	1600
	(0.03–0.29)	(0.10–0.38)	(0.21–0.36)	(0.18–0.38)	(0.07–0.28)	(0.16–0.32)	(0.24–0.45)	(0.13–0.40)	(0.13–0.33)	
Iron	38.03 ± 2.06	38.57 ± 1.95	38.47 ± 2.53	37.97 ± 2.00	25.97 ± 22.56^{A}	37.60 ± 2.00	38.07 ± 2.11	37.49 ± 1.83	37.40 ± 0.10	48
	(36.10–40.20)	(37.20–40.80)	(36.20–41.20)	(35.90–39.90)	(0.00–40.70)	(35.60–39.60)	(36.10–40.30)	(35.56–39.20)	(37.30–37.50)	
Zinc	0.50 ± 0.10	0.60 ± 0.12	0.56 ± 0.15	0.51 ± 0.10	0.46 ± 0.08	0.62 ± 0.17	0.60 ± 0.07	0.49 ± 0.11	0.61 ± 0.09	60
	(0.39–0.56)	(0.49–0.72)	(0.47–0.73)	(0.41–0.60)	(0.37–0.53)	(0.43–0.75)	(0.55–0.68)	(0.39–0.60)	(0.51–0.67)	

Numbers with different superscripts are significantly different ($p < 0.05$), while numbers with same superscript are not significantly different ($p > 0.05$). Sample size (N) = 3.

inoculated into the agar plates. The agar plates were then incubated at room temperature ($28 \pm 2\ ^{\circ}$C) for 24–48 h for the bacteria and 3- 5d for fungi.

2.5.1. Identification and characterization of isolates

The identification of bacterial isolates was based on their morphological, cultural and biochemical characteristics. Gram reaction, oxidase, catalase, sugar fermentation (glucose, maltose, sucrose, and mannose), indole, urease, citrate utilization, methyl red (MR) and Voges-Proskauer (VP) tests were carried out. The identification of the isolates was carried out using Cowan and Steel's (1974) Manual for the Identification of Medical Bacteria.

2.5.2. Morphology and cultural characteristics of isolates on media

Each isolate was cultured with agar for 48 h and cultural characteristics were thus determined. The features examined in the colonies were the edge, shape, colour, opacity and surface appearance while 3–5 d cultures of fungi plates were used to study the culture, plate culture reversal and nature of growth.

2.5.3. Gram's reaction

Gram staining reaction was carried out a smear of the organism on a clean, grease-free slide with the aid of a sterile wire loop. The smear was fixed by passing the slide through a Bunsen burner flame. Thereafter the smear was covered with crystal violet dye for about 30–60 s. Lugol's iodine (a mordant) was applied to the slide for 30 s. This was washed over the sink using distilled water. The next step involved

decolourization with ethanol. After decolourization, a counter-stain (safranin) was applied onto the slide and allowed to stay for about 30 s and washed off over the sink with slow-running distilled water. This was done for each of the isolates. The slides were allowed to air dry and then viewed with the aid of a light microscope using the oil immersion objective.

2.5.4. Sterilization of materials

All glass wares such as pipettes used in this study were thoroughly washed with detergent and rinsed in clean water to ensure that they were grease-free. They were allowed to drip-dry and arranged in canisters. The glass wares were then properly sterilized in an autoclave at 121 $^{\circ}$C for 15 min at 15 Pa/pressure. Those that had screw caps were sterilized with their caps relatively loose around the glass mouth. The inoculating loops were sterilized by the red heat method with the aid of the Bunsen burner flame before and after use. Commercial Petri dishes which had been already sterilized were used.

3. Results

3.1. Physico-chemical properties the soil

Higher organic carbon concentrations were detected at Stations 2,3,7,8, and 9 ($p < 0.05$) than the other stations (Table 1). The concentration of total nitrogen was significantly higher at Station 1 than other stations ($p < 0.05$), meanwhile the concentrations of nitrates at Station 3,
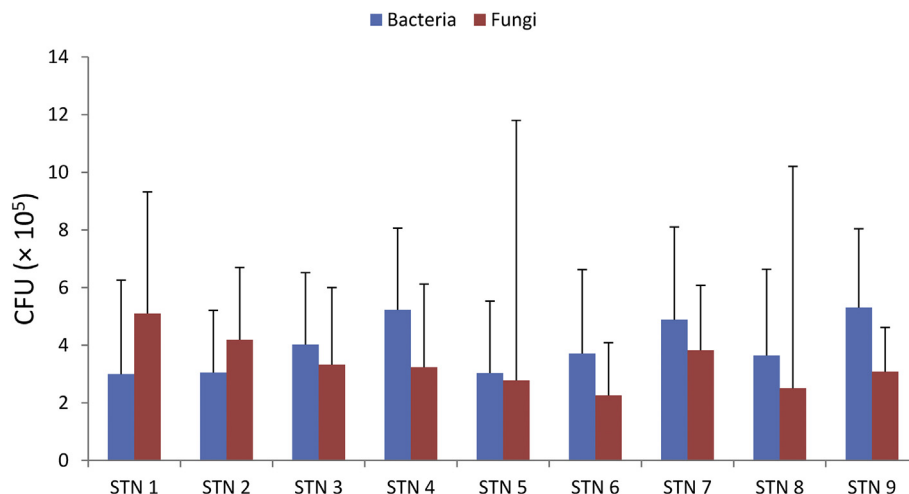

Figure 2. Mean bacteria and fungi counts in agricultural soils of Egbema Kingdom.

Table 3. Occurrence of fungi isolates in soils samples of Egbema Kingdom.

Fungi isolates	Stations									Total
	1	2	3	4	5	6	7	8	9	
Aspergillus flavus	1	1	1	1	0	1	1	0	0	6
Aspergillus fumigatus	0	1	0	0	0	0	0	0	0	1
Aspergillus nidulans	1	2	1	3	1	2	0	1	0	11
Aspergillus niger	1	3	3	3	3	3	3	2	2	23
Aspergillus sp	1	1	1	0	0	0	1	0	0	4
Aspergillus sp (dark)	0	1	1	0	0	1	0	0	0	3
Aspergillus tamari	0	1	1	2	1	0	1	0	1	7
Botrydiplodia sp	0	0	0	0	0	1	0	0	1	2
Chaonephora sp	0	0	0	1	0	0	0	0	0	1
Cladosporium sp	1	1	1	1	1	1	2	1	1	10
Cryptomonas sp yeast	0	2	1	0	0	0	2	2	0	7
Crytococcus sp	0	0	1	1	1	0	1	1	1	6
Fusarium sp	1	1	0	1	2	0	2	0	2	9
Fusarium oxsporium	0	0	0	0	0	0	0	0	0	0
Helminthosporium sp	0	1	0	0	0	0	0	0	0	1
Mucor mucedo	2	2	2	0	2	2	1	2	1	14
Mucor sp	2	2	1	1	1	1	2	1	1	12
Mycospherella fijiesis	1	2	2	2	2	3	1	2	1	16
Mycospherella musicola	1	2	2	2	2	3	1	2	1	16
Mycospherella sp	1	2	1	0	2	1	1	1	1	10
Nerospora sp	1	2	2	1	1	0	2	0	0	9
Botrydiplodia sp	1	0	0	0	0	0	1	0	0	2
Penicillium cyclopium	1	0	0	0	1	0	0	0	0	2
Penicillium italicum	2	1	2	2	1	1	1	2	0	12
Penicillium Oxalicum	1	1	1	1	0	1	0	0	0	5
Penicillium red reverse	1	1	1	2	1	0	0	1	0	7
Penicillium pink	1	0	1	1	1	1	0	0	1	6
Penicillium creamy	1	1	0	2	1	1	1	0	1	8
Penicillium dirty white	0	0	0	0	0	0	0	0	0	0
Penicillium black	1	0	1	0	0	0	1	0	0	3
Penicillium bluish	1	0	1	0	0	1	0	0	0	3
Penicillium clavate	1	0	0	1	0	0	0	0	0	2
Penicillium sp	0	0	0	0	0	0	0	0	0	0
Penicillium white	1	0	0	1	0	0	0	0	1	3
Penicillium grey	0	0	0	0	0	0	0	0	2	2
Rhodococcus sp	1	1	0	2	1	1	1	0	2	9
Saccharomyces sp	1	3	2	2	2	2	1	3	1	17
Sclerotia rubsi	0	0	0	0	0	0	1	0	1	2
Trichoderma sp	2	1	1	0	0	0	1	0	1	6
Trichoderma viridi	2	1	1	0	0	0	1	0	1	6
Yeast (lightbrown)	0	1	0	0	0	0	0	0	0	1
Yeast rhodococus sp	0	0	0	1	0	1	1	1	1	5
Yeast candida sp	1	0	2	2	2	0	0	0	0	7
Number of individuals =	33	38	34	36	29	28	31	22	25	268

8, and 9 were significantly higher than the concentrations at Stations 1,2,4, and 5, which were in turn higher than Stations 6 and 7 ($p < 0.05$).

The exchangeable acidity at Stations 1 and 5 were significantly higher than Stations 6,7,8 and 9, which were in turn higher than other stations ($p < 0.05$). The levels of potassium at Stations 1 and 4 were significantly higher than other stations ($p < 0.05$). The amount of clay at Station 9 was significantly higher than what was obtained at other stations ($p < 0.05$), while the silt at Station 5 was significantly lower than the amount at other stations ($p < 0.05$).

Other parameters tested such as the pH, Ca, Mg, Na, ECEC, and sand were not significantly different across all stations ($p > 0.05$).

3.2. Trace metal concentrations in soil

There was no significant difference ($p > 0.05$) in the concentrations of all trace metals in the soil samples among the stations (Table 2). The concentrations of metals across all stations were also below the standard regulatory limits established by the World Health Organization (WHO), and the Food and Agricultural Organization (FAO). Although the concentrations of iron across the stations were also lower than regulatory limits, they were however close to the benchmark, except for Station 5 where significantly lower concentration than other stations was recorded ($p < 0.05$).

Table 4. Occurrence of bacteria isolates in soils samples of Egbema Kingdom.

Bacteria isolates	Station									Total
	1	2	3	4	5	6	7	8	9	
Acinetobacter sp	0	1	2	3	1	2	1	0	1	11
Alcaligenes sp	0	1	0	0	1	0	0	0	0	2
Baccillus sp	3	1	2	3	2	1	3	1	3	20
Bacillus subtilis	3	2	2	2	2	2	1	2	3	20
Corynebacterium	1	0	1	2	1	1	0	0	2	6
Escherichia coli	2	3	1	3	2	1	1	1	3	17
Flavobacterium sp	2	1	1	3	2	1	1	1	1	13
Klebsiella sp	1	1	2	2	1	0	0	2	1	10
Micrococcus Sp	2	3	3	1	3	2	2	3	3	22
Micrococcus roseus	1	1	1	2	1	0	2	2	2	12
Proteus spI	3	3	3	3	3	3	3	3	3	27
Proteus spII	0	1	0	1	0	0	0	1	1	4
Proteus mirabilis	0	1	1	2	2	0	0	1	1	8
Pseudomonas aeruginosa II	0	1	1	1	0	1	1	0	0	5
Pseudomonas sp1	1	1	1	0	0	1	1	1	1	7
Pseudomonas sp2	0	0	1	0	0	1	1	1	1	5
Pseudomonasa eruginosa I	0	2	2	2	2	0	3	2	1	14
Pseudomonas flourescens sp	0	0	0	0	1	0	0	1	1	3
Staphyloccus epidermidis	3	3	3	3	3	3	3	3	1	25
Staphyloccus aureus	2	3	3	3	3	3	3	3	1	24
Staphylococcus sp	1	2	2	3	2	1	1	2	2	16
Streptococcus sp	2	1	1	2	1	0	1	1	1	10
Number of individuals =	27	32	34	41	33	23	28	31	33	181

Mean bacteria counts observed in the study were 3.00×10^5 CFU in station 1, 3.05×10^5 CFU in station 2, 4.02×10^5 CFU in station 3, 5.22×10^5 CFU in station 4, 3.03×10^5 CFU in station 5, 3.71×10^5 CFU in station 6, 4.88×10^5 CFU in station 7, 3.64×10^5 CFU in station 8 and 5.31×10^5 CFU in station 9. No significant difference was observed between the mean bacterial counts in the stations (Figure 2).

Mean fungi counts observed were 5.10×10^5 CFU in station 1, 4.19×10^5 CFU in station 2, 3.33×10^5 CFU in station 3, 3.23×10^5 CFU in station 4, 2.78×10^5 CFU in station 5, 2.26×10^5 CFU in station 6, 3.82×10^5 CFU in station 7, 2.51×10^5 CFU in station 8 and 3.08×10^5 CFU in station 9. No significant difference ($p < 0.05$) was observed between the mean fungi counts in the nine stations.

A total of 268 individual fungi isolates were identified in the soil samples across the stations. These individuals belong to 43 species, which include *Aspergillus flavus, Aspergillus fumigatus, Aspergillus ridularis, Aspergillus niger, Aspergiullus sp, Aspergillus sp (dark), Aspergillus tamarii, Botrydiplodia sp, Chaonephora sp, Cladosporium sp, Cryptomonas sp, Crytococcus sp, Fusarium sp, Fusarium Oxsporium, Helminthosporium sp, Mucor mucedo, Mucor sp, Mycospherella fijiesis, Mycospherella musicola, Mycospherella sp, nerospora sp, Botrydiplodia sp, Penicillium sp, Penicillium cyclopium, Penicillium italium, Penicillium oxalium, Penicillium red reverse,* *Penicillium pink, Penicillium creamy, Penicillium dirty white, Penicillium black, Penicillium bluish, Penicillium clavate, Penicillium sp, Penicillium white, Penicillium grey, Rhodococcus sp, Saccharomyces sp, Sclerotia rubsi, Trichoderma sp, Trichoderma viride, Yeast (lightbrown), Yeast rhodococcus sp, Yeast candida sp.* The fungi species with the highest number of occurrence was *Aspergillus niger* which dominated Stations 2–9 (Table 3). The species with the lowest occurrence were *Fusarium oxsporium, Penicillium dirty white,* and *Penicillium sp.* which were absent at all the stations. The order of abundance of fungi across the stations was Station 2> Station 4> Station 3> Station 1> Station 7> Station 5> Station 6> Station 9> Station 8.

A total of 181 individuals of bacteria isolates were identified in the soil samples collected. These individuals belong to 22 species, which include *Acinetobacter sp, Alcaligenes sp, Bacillus sp, Bacillus sutilis, Corynebacterium, Escherichia coli, Flavobacterium sp, Klebsiella sp, Micrococcus sp, Micrococcus roseus, Proteus sp, Proteus sp II, Proteus mirabilis, Pseudomonas aueruginosa II, Pseudomonas sp 1, Pseudomonas sp 2, Pseudomonas aeruginosa 1, Pseudomonas flourescens sp, Staphylococcus epidermidis, Staphylococcus aureus, Staphylococcus sp and Streptococcus sp.* The bacteria species with the highest number of occurrence was *Proteus sp I* which dominated other species across the entire stations, while the species with

Table 5. Relationship between trace metals and bacterial and fungi counts.

	Cadmium	Lead	Copper	Nickel	Iron	Zinc	Bacteria	Fungi
Cadmium	1							
Lead	.275	1						
Copper	.009	.209	1					
Nickel	.493	.395	.195	1				
Iron	-.056	-.103	-.305	-.066	1			
Zinc	.100	.258	**.642**	.403	-.067	1		
Bacteria	**.827**	-.132	**.518**	.222	-.153	-.026	1	
Fungi	-.155	-.026	-.398	-.207	.227	-.086	-.031	1

Emboldened figure represents significant correlation at $p < 0.05$.

the lowest occurrence was *Alcaligenes sp.* which occurred only once at Station 5 (Table 4). The order of abundance of bacteria in the soil samples across the stations was Station 4> Station 3> Stations 5 and 9> Station 2> Station 8> Station 7> Station 6.

The relationship between the trace metals and the microbes showed a highly significant positive correlation (0.827) occurred between cadmium and bacteria (Table 5). Copper also exhibited a significant positive correlation with bacteria (0.518) and Zinc (0.642).

4. Discussions

4.1. Physico-chemical characteristics and heavy metals

The collected soil samples in this study were acidic in all the stations except in station 4 where an alkaline pH of 8.14 was recorded. Alkaline pH can contribute to reducing the bioavailability of trace metals, hence their harmful effects on soil biota. Conversely, the acidic pH observed in the current study may have far-reaching effects on the soil organismal community and the dependent plants, owing to the strong influence of pH on solubility and and spatial variation of metals both in soil as a whole and particularly in the soil moisture (Sungur et al., 2014; Kouchou et al., 2017). The soil biota may benefit from the essential metals but not without the simultaneous toxicity of the non-essentials.

Station 6 had the lowest pH of 4.05 which is highly acidic. Studies have confirmed that near-neutral pH is the most preferable by the majority of soil microorganisms and it supports the largest and most diverse composition of bacterial populations (Lal, 2005). Microorganisms may also boost the bioavailability of the metals through localized acidification of the environment.

Although cadmium, lead and zinc have been considered strong inhibitors of soil microorganisms (Kabata-Pendias and Pendias, 1999; Unsal et al., 2014), the cadmium levels recorded in the study stations were within the safe limit (3 mg/kg) established by FAO/WHO (2001).

Lead (Pb) may damage cell membranes, and destroy the structure of DNA in microbes. This harmfulness is generated by the displacement of metals from their native binding sites or ligand interactions (Olaniran et al., 2013). Mean lead concentration of 0.57 mg/kg in station 1, 0.17 mg/kg in station 2, 0.13 mg/kg in station 3, 0.63 mg/kg in station 4, 0.10 mg/kg in station 5, 0.24 mg/kg in station 6, 0.37 mg/kg in station 7, 0.17 mg/kg in station 8 and 0.20 mg/kg in station 9 were observed during the sampling period, revealing no significant difference. The lead levels in the soils were far below 50mg/kg-the FAO/WHO (2001) permissible limits for soil. The concentration of lead in the study sites had no significant positive or negative relationship with the microbial content of the soil samples.

Mean copper concentrations of 0.17 mg/kg in station 1, 0.20 mg/kg in station 2, 0.20 mg/kg in station 3, 0.16 mg/kg in station 4, 0.20 mg/kg in station 5, 0.20 mg/kg in station 6, 0.19 mg/kg in station 7, 0.15 mg/kg in station 8 and 0.16 mg/kg in station 9 were observed during the sampling period. No significant difference was observed between the mean copper concentrations of the study stations. The FAO/WHO (2001) permissible limit for copper in soil is 100 mg/kg; hence copper concentration in this study was far below the WHO/FAO permissible limits. Significant negative correlation was observed between copper and fungi counts indicating that the concentration of copper in the soil might have hampered the prevalence of the fungi.

Mean nickel concentrations of 0.15 mg/kg in station 1, 0.23 mg/kg in station 2, 0.27 mg/kg in station 3, 0.29 mg/kg in station 4, 0.19 mg/kg in station 5, 0.25 mg/kg in station 6, 0.35 mg/kg in station 7, 0.22 mg/kg in station 8 and 0.26 mg/kg in station 9 were observed during the sampling period revealing no significant difference (p > 0.05). Station 1 and 7 had the lowest and highest nickel concentrations respectively. A nickel concentration of 20 mg/kg has been reported to be toxic to fungi and bacteria as it affected microbial functioning in the sampled soil (Lankinen et al., 2011).

As an essential mineral, iron plays an important role in fundamental biological processes such as photosynthesis, respiration, nitrogen fixation and assimilation, and DNA synthesis (Kabiru et al., 2015; Akinsanya et al., 2020). Iron is also a co-factor of many enzymes involved in the synthesis of plant hormones (Briat, 2005). However, an excess concentration of iron in soil can pose adverse effects to plants and man (Akinsanya et al., 2020). Mean iron concentrations of 38.03 mg/kg in station 1, 38.57 mg/kg in station 2, 38.47 mg/kg in station 3, 37.97 mg kg in station 4, 25.97 mg/kg in station 5, 37.60 mg/kg in station 6, 38.07 mg/kg in station 7, 37.49 mg/kg in station 8 and 37.40 mg/kg in station 9 were observed during the sampling period. The concentrations of iron were far below the FEPA maximum limits of 400 mg/kg for agricultural soil, thus indicating some level of safety from iron induced toxicities.

Excess amount of zinc disrupts the homeostasis of soil by interfering with the control mechanisms at the genetic level, thus inhibiting the activity of microbial enzymatic proteins. Mean zinc concentrations of 0.50 mg/kg in station 1, 0.60 mg/kg in station 2, 0.56 mg/kg in station 3, 0.51 mg/kg in station 4, 0.46 mg/kg in station 5, 0.62 mg/kg in station 6, 0.60 mg/kg in station 7, 0.49 mg/kg in station 8 and 0.61 mg/kg in station 9 were observed during the study period. The zinc levels in this study were below the 300 mg/kg maximum limits set by the FAO/WHO (2001), thus indicating safety from toxicity of zinc.

4.2. Bacteria and fungi content of soil

The sensitivity of fungi to trace metals differs among species and strains (Baldrian, 2003; Lankinen et al., 2011). The fungi isolates identified in the soils samples from the stations were *Aspergillus flavus, Aspergillus fumigatus, Aspergillus ridularis, Aspergillus niger, Aspergiullus sp, Aspergillus sp* (dark)*, Aspergillus tamarii, Botrydiplodia sp, chaonephora sp, Cladosporium sp, Cryptomonas sp, Crytococcus sp, fusarium sp, fusarium oxsporium, helminthosporium sp, mucor mucedo, mucor sp, mycospherella fijiesis, mycospherella musicola, mycospherella sp, nerospora sp, Botrydiplodia sp, Penicillium sp, Penicillium cyclopium, Penicillium italicum, Penicillium oxalicum, Penicillium red reverse, Penicillium pink, Penicillium creamy, Penicillium dirty white, Penicillium black, Penicillium bluish, Penicillium clavate, penicillium sp, penicillium white, Penicillium grey, Rhodococcus sp, Saccharomyces sp, Sclerotia rubsi, Trichoderma sp, Trichoderma viridi,* Yeast (light brown), Yeast *rhodococcus sp, candida sp.* From the sampling, *Aspergillus niger, mucor mucedo, Saccharomyces viridi* and *Penicillium oxallicum* had the highest mean frequency of occurrence. Fungi were less affected by the trace metals than the bacteria. This observation corroborates earlier findings as fungi and yeasts were considered to be the most tolerant and thus versatile group of soil microorganisms (Iram et al., 2013). This is attributed to the fact that fungal cell walls have shown some metal biosorbents tendencies (Gavrilescu, 2004). Owing to these properties, studies have shown that some fungal species such as *Aspergillus niger* or *Mucor rouxii* exhibits promising results in absorption of metals, hence can be useful in metal removal from the environment.

Various strains of bacteria were observed in this study which include both gram positive and gram negative bacteria. The gram positive bacteria isolated were *Staphylococcus epidermidis, Staphylococcus sp, Micrococcus sp, Micrococcus roseus, Staphylococcus epidermidis, Staphylococcus aureus, Staphylococcus sp* and *Streptococcus sp, Bacillus sp* and *Corynebacterium* while the gram negative bacteria were *Pseudomonas Pseudomonas sp 1, Pseudomonas sp 2, Pseudomonas aeruginosa 1, Pseudomonas, Escherichia coli, Proteus sp, Flavobacterium, Klebsiella sp, Acinetobacter sp* and *Alcaligens sp.* From the results, *Microccus sp, Staphylococcus epidermidis* and *Staphylococcus sp* had the highest mean level of occurrence. *Proteus sp* (a gram negative bacteria) occurred with the highest preponderance in all the stations throughout the sampling period. This corroborates several studies which have demonstrated that gram-negative bacteria tend to be more tolerant to heavy metals than the gram positive (Ferreira da Silva et al., 2007).

Further studies have shown that the ability of microbes to tolerate a definite level of trace metals under natural conditions might be different owing to the complex nature of the soil ambience. Studies in different ecosystems have indicated that fungi and bacteria can have antagonistic relationships in which an increase in the activity of a group of organisms may result in a decrease in the activity of other groups (Rajapaksha et al., 2004). This observation was corroborated by the current study as stations that had high fungi counts had lower bacteria counts and vice versa.

5. Conclusion

Trace metals (Fe, Zn, Cd, Ni and Cu) in the agricultural soils of Egbema kingdom were below WHO (1993) limits. Significant positive correlation was observed between the concentration of copper and bacteria counts indicating that the concentration of copper in the soil might have enhanced the abundance of bacteria, vice versa. Meanwhile, Fe, Zn, Cd and Ni showed no relationship with the microbial contents of the soil. An antagonistic relationship was observed between bacteria and fungi counts. The order of resilience of the soil microbes to the toxicity of the trace metals was fungi > gram negative bacteria > gram positive bacteria.

Declarations

Author contribution statement

Patrick Omoregie Isibor: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Alex A Enuneku: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data.

Peter I Abhulimen: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Charming Osaro Asemota, Benjamin Okpara, Tunde O Imoobe, Lawrence I Ezemonye: Conceived and designed the experiments.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

Abdul, H., Al Obaidy, J., Athmar, M., 2013. Heavy metal contaminations in urban soil within Baghdad city, Iraq. J. Environ. Protect. 4, 72–82.

Abioye, P.O., 2011. Biological remediation of hydrocarbon and heavy metals contaminated soil. In: Pascucci, S. (Ed.), Soil Contamination. InTech, Vienna, Austria, p. 14.

Aguilar, R.B., Bautista, F., Goguitchaichvili, A., Quintana, P., Carvallo, C., Battu, J., 2013. Rock-Magnetic properties of top soils and urban dust from Morelia, Mexico: implications for anthropogenic pollution monitoring in medium-size cities. Geofisc. Int. 52 (2), 121–133.

Ajaz, R.H., Thirumalai, A.V., Narayananb, K.R., Zahir, H., 2010. Bioremediation of heavy metal contaminated soil by the exigobacterium and accumulation of Cd, Ni, Zn and Cu from soil environment. Int. J. Biosci. Technol. 1 (2), 94–101.

Akinsanya, B., Isibor, P.O., Onadeko, B., Abe-Alimi, T., 2020. Impacts of trace metals on African common toad, *Amietophrynus regularis* (Reuss, 1833) and depuration effects of the toad's enteric parasite, *Amplicaecum africanum* (Taylor, 1924) sampled within Lagos metropolis, Nigeria. Heliyon 6 (2020), e03570.

Baldrian, P., 2003. Interactions of heavy metals with white-rot fungi. Enzym. Microb. Technol. 32, 78–91.

Bhagure, G., Mirgane, S.R., 2010. Heavy metal concentrations in groundwaters and soils of thane region of Maharashtra, India. Environ. Monit. Assess. 173 (1), 643–652.

Bhaskar, C.V., Kumar, K., Nagendrappa, G., 2010. Assessment of heavy metals in water samples of certain locations situated around Tumkur, Karnataka, India. J. Chem. 7 (2), 349–352.

Briat, J.F., 2005. Iron from soil to plant products. Bull academic Natl. Med. 189, 1609–1619.

Daniel, E.S., Musa, J.J., Akos, M.P., Yerima, I.Y., Dada, P.O., Jibril, I., Manta, I.H., 2016. Assessment of Heavy Metal Pollution in Some Nigerian Soils: A Review. The Annual Conference and Annual General Meeting–"Minna 2016".

FAO/WHO, 1976. List of maximum levels recommended for contaminants by the joint FAO/WHO codex Alimentarias Commission. 2nd series. CAC/FAL 3, 1–8.

Fashola, M.O., Ngole-Jeme, V.M., Babalola, O.O., 2016. Heavy metal pollution from gold mines: environmental effects and bacterial strategies for resistance. Int. J. Environ. Res. Publ. Health 13 (11), 1047–1052.

Ferreira da Silva, M., Vaz-Moreira, I., Gonzalez-Pajuelo, M., Nunes, O.C., Manaia, C.M., 2007. Antimicrobial resistance patterns in Enterobacteriaceae isolated from an urban wastewater treatment plant. FEMS Microbiol. Ecol. 60 (1), 166–176.

Filazi, A., Baskaya, R., Kum, C., Hismiogullari, S.E., 2003. Metal concentrations in tissues of the black sea fish *Mugil auratus* from sinop-Icliman, Turkey. Hum. Exp. Toxicol. 22 (2), 85–87.

Gavrilescu, M., 2004. Removal of heavy metals from the environment by biosorption. Eng. Life Sci. 4, 219–232.

Ghosal, D., Ghosh, S., Dutta, T.K., Ahn, Y., 2016. Current state of knowledge in microbial degradation of polycyclic aromatic hydrocarbons (PAHs): a review. Front. Microbiol. 7, 1369.

Igiri, B.E., Okoduwa, S.I.R., Idoke, G.O., Akabuogu, E.P., Adeyi, A.O., Ejiogu, I.K., 2018. Toxicity and bioremediation of heavy metals contaminated ecosystem from tannery wastewater: a Review. J. Toxicol. 38, 1–16.

Ihl, T., Bautista, F., Cejudo, R., Delgado, C., Quintana, P., Aguilar, D., Gogichaishvili, A., 2015. Concentration of toxic elements in topsoils of the metropolitan area of Mexico City: a spatial analysis using Ordinary kriging and Indicator kriging. Rev. Int. Contam. Ambient. 31 (1), 47–62.

Imarhiagbe, E.E., Osarenotor, O., Obayagbona, O.N., Eghomwanre, A.F., Nzeadibe, B.N., 2017. Evaluation of physicochemical, microbiological and polycyclic aromatic hydrocarbon content of top soils from Oka market waste collection site, Benin City, Nigeria. J. Appl. Sci. Environ. Manag. 21 (1), 112–117.

Iram, S., Zaman, A., Iqbal, Z., Shabbir, R., 2013. Heavy metal tolerance of fungus isolated from soil contaminated with sewage and industrial wastewater. Pol. J. Environ. Stud. 22, 691–697.

Isibor, P.O., Imoobe, T.O.T., 2017. Trace metals in water, bottom sediment, shrimp and dependent human blood in Ukwuani local Government area of delta state, Nigeria. Asian J. Environ. Ecol. 2 (3), 1–16.

Isibor, P., Akinsanya, B., Soyinka, O., Pentho, K., Obe, A., Saliu, K., 2020. *Raphidascaroides brasiliensis* (Nematoda: Anisakidae) infection and bioaccumulation of polycyclic aromatic hydrocarbons in *Gymnarchus niloticus* (Cuvier, 1829) in Lekki Lagoon, Nigeria. Egypt. J. Aquat. Biol. Fish. 24 (1), 99–118.

Kabata-Pendias, A., Pendias, H., 1999. Biogeochemistry of Trace Elements. PWN Scientific Publishing House, Warsaw.

Kabiru, S., Yakubu, R., Lukman, A., Akintola, T., Alegbemi, M., 2015. Heavy metal content in soil in Garki area council of federal capital territory, Abuja, Nigeria. Biochem. Anal. Biochem. 4 (44), 1.

Kouchou, A., Rais, I., Elsass, F., Duplay, J., Fahli, N., Ghactouli, E.L., 2017. Effects of long-term heavy metals contamination on soil microbial characteristics in calcareous agricultural lands (Saiss plain, North Morocco). J. Mater. Environ. Sci. 8 (2), 691–695.

Lal, R., 2005. Encyclopedia of Soil Science, second ed. CRC Press, Taylor and Francis Group, Boca Raton.

Lankinen, P., Kahkonen, M.A., Rajasarkka, J., Virta, M., Hatakka, A., 2011. The effect of nickel contamination on the growth of litter decomposing fungi, extracellular enzyme activities and toxicity in soils. Boreal Environ. Res. 16, 229–239.

Oketola, A.A., Fagbemigun, K.T., 2013. Determination of Nonylphenol, Octylphenol and Bisphenol-A in water and sediments of two major rivers in Lagos, Nigeria. J. Environ. Protect. 4 (7), 38–45.

Oketola, A., Seun, M., Adekolurejo, O.O., 2013. Water quality assessment of River Ogun using multivariate statistical techniques. J. Environ. Protect. 4, 466–479.

Olaniran, A.O., Balgobind, A., Pillay, B., 2013. Bioavailability of heavy metals in soil: impact on microbial biodegradation of organic compounds and possible improvement strategies. Int. J. Mol. Sci. 14 (5), 10197–10228.

Orimoogunje, O.O.I., Ayanlade, A., Akinkuolie, T.A., Odiong, A.U., 2010. Perception on effect of gas flaring on the environment. Res. J. Environ. Earth Sci. 2 (4), 188–193.

Ou-Yang, X., Chen, J.W., Zhang, X.G., 2010. Advance in supercritical CO_2 fluid extraction of contaminants from soil. Geol. Bull. China (11), 1655–1661.

Peter, E., Adeniyi, G., 2011. Spatial relationships of urban land use, soils and heavy metal concentrations in Lagos Mainland Area. J. Appl. Sci. Environ. Manag. 15 (2).

Rajaganapathy, V., Xavier, F., Sreekumar, D., Mandal, P.K., 2011. Heavy metal contamination in soil, water and fodder and their presence in Livestock and Products: a Review. J. Environ. Sci. Tech. 4, 234–249.

Rajapaksha, R.M., Tobor-Kaplon, M.A., Baathi, E., 2004. Metal toxicity affects fungal and bacterial activities in soil differently, 70 (5), 2966–2973.

Rakesh, M.S., Raju, N.S., 2013. Correlation of heavy metal contamination with soil properties of industrial areas of Mysore, Karnataka, India by cluster analysis. Int. Res. J. Environ. Sci. 2 (10), 22–27.

Shahid, M.A.K., Hussain, K., Raza, A., 2013. Impact of different pollutant sources on human health using solid Aerosol's elemental analysis. J. Basic Appl. Sci. 9, 91–98.

Simeonov, L., Kolhubovski, M., Simeonov, B., 2010. Environmental Heavy Metal Pollution and Effects on Child Mental Development. Springer, Dordrecht, *Netherlands*, pp. 114–115.

Sungur, A., Soylak, M., Ozcan, H., 2014. Investigation of heavy metal mobility and availability by the BCR sequential extraction procedure: relationship between soil properties and heavy metals availability. Chem. Speciat. Bioavailab. 26 (4), 219–230.

Tchounwou, P.B., Clement, G., Yedjou, A.K., Patlolla, J.S., 2012. Heavy metal toxicity and the environment. EXS 101, 133–164.

Ubani, E.C., Onyejekwe, I.M., 2013. Environmental impact analyses of gas flaring in the Niger delta region of Nigeria. Am. J. Sci. Ind. Res. 4 (2), 246–252.

Unsal, Y.E., Tuzen, M., Soylak, M., 2014. Evaluation of metal concentrations of soil samples from Yahyali-Kayseri, Turkey. Fresenius Environ. Bull. 23 (10), 2488–2491.

Wang, A.S., Angle, J.S., Rufus, L.C., Delorme, T.A., 2006. Soil pH effects on uptake of Cd and Zn by *Thlaspi caerulescens*. Plant Soil 281 (1), 325–337.

World Health Organization, 1993. Standard Maxima for Metals in Agricultural Soils.

Wyszhowska, J., Borowik, A., Kucharski, M., Kucharski, J., 2013. Effect of cadmium, copper and zinc on plants, soil microorganisms and soil enzymes. J. Elementology 18, 769–796.