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Heavy Metals pH-Mediated Microbial-Remediation in Septic Tank Effluents

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ABSTRACT: *This paper studied the role of pH in the microbial remediation of heavy metals in septic tank effluents from three sample locations in the south-south region of Nigeria. The region is famous for agriculture, industrial and auto-mechanic activities leading to the uptake and bioaccumulation of heavy metal contaminants through the food chain. Heavy metals concentrations, pH, and microbial analysis in effluent samples, were determined using standard methods. The material balance approach (see Supplementary Information) was adopted to account for analytes remediation and speciation in the system according to the Law of Conservation of Mass (Matter). The pH of the study area ranged from 6.5 to 7.7. The pH conditions in location A was alkaline, while locations B and C were slightly acidic. Iron was the most abundant metal with a concentration range of 0.01 to 0 9.41 mg/L. The order of magnitude of heavy metals in the septic tanks in the study area is Fe > Pb > Cr > Zn > Cd > Mn > Cu > Ni > V. Metal removal mechanism followed the metal-microbes adsorption and precipitation processes dictated by the* in situ *pH of the system. Total heterotrophic bacteria were the most dominant in the septic tanks. A removal coefficient of* ≤ 0.33 *(i.e,* $0 < x \leq 0.33$ *(where* $x \neq 0$*)) was considered optimum. The percentage metal ion removal was inversely proportional to the removal coefficient and vice versa. Acidic conditions favoured the sequestration of more heavy metals from the effluent and the attainment of the 67.0 % and 0.33 removal efficiency thresholds. Finally, the heavy metals concentrations were indicative of bioaccumulation. Secondly, the pH conditions are not ideal enough to cause the efficient remediation of heavy metals from effluents. Further treatment of the sludge component before disposal is seriously advocated.*

KEYWORDS: Heavy metals, pH, microorganisms, effluents, and sludge.

INTRODUCTION

Heavy metals are elements whose densities are high, typically above 5 g/cm^3 (Ali & Khan, 2019; Briffa *et al.*, 2020; Chu, 2018; Hawkes, 1997; Sayo *et al.*, 2020). Examples of heavy metal elements include lead, zinc, chromium, cadmium, arsenic, mercury, vanadium, manganese, and iron. They are found in the environment from geogenic and anthropogenic sources (Ibrahim & Ibrahim, 2016; Tchounwou *et al.*, 2012). They exist in the air, surface and groundwater, and soil. Thus, they can be transported through soil to plants and up the food chain to man (Nkwunonwo *et al*., 2020), as well as through air and water pollution.

Some of these metals are required only in trace quantities in the body where they are necessary for certain physiological processes (Sobolev & Begonia, 2008). However, most of these heavy metals are deleterious to health even in trace proportions (Kinuthia *et al*., 2020). They are known to be associated with various forms of cancers and the breakdown of the central nervous system (Karimi 2017; Oyem *et al*., 2015).Man is at the receiving end of these metals in the environment from uptake by plants from the soil, irrigation water, surface and groundwater and through airborne pollutants (He *et al*., 2005; Zango *et al*., 2020). In effect, man becomes a veritable bio-accumulator of these dense metals. By the process of excretion, the body attempts to remove pollutants through urine and faeces; this way, these contaminants get into the septic tank system.

Septic tanks are anaerobic digesters where organic matter from human domestic activities is remediated by microorganisms in a two-step process initiated by acidophiles which convert organic matter to fatty acids before they are subsequently converted to methane by methane-forming microorganisms. Conventional septic tank systems typically have two chambers: the raw (inlet) chamber and the semi-treated (outlet) chamber. Components of the septic tank can generally be classified into suspended solids, effluent, and sludge.

Heavy metals are not biodegradable but can be remediated (Delangiz *et al*, 2020; Karimi, 2017; Osman *et al*., 2019). However, the extent of the remediation process which takes place in the septic tank is not immediately known. Although several papers have been published on the workings of septic tank digester systems, most of these have centred essentially on methanogenesis (methane generation) and the role of microbes.

In this exercise therefore, we study the efficiency of the bioremediation of heavy metals in a septic tank system viz-a-vis the *in situ* pH dynamism of the system by comparing the concentrations of these metal ions in both the inlet and outlet chambers and obtaining the coefficient of metal removal, while also accounting for the heavy metal (material) balance of the system.

MATERIALS AND METHODS

Sample area

Sewage effluent samples were collected from different septic tanks within the three sample locations in Edo and Delta States represented by: Agbor, Benin, and Sapele in the south-south region of Nigeria (Fig. 1) and designated as locations A, B, and C respectively. The climate of the study area is characteristics of a subequatorial climate with an annual average air temperature of between 27 – 29 ^{o}C (Odjugo, 2008; Oyem *et al*., 2014). The rainfall pattern is that of double peaks (maxima) with a mean annual rainfall of 2,255 mm, and an annual humidity of 81 per cent (Avwunudiogba, 2000; Oyem *et al*., 2014; Oyem *et al*., 2015). Soil type is mostly reddish-yellow ferralsols (Avwunudiogba, 2000; Oyem *et al*., 2015; Oyem *et al*., 2014), apart from Sapele which has the yellow-white soil characteristic of the Delta region being further southwards of the other two sample areas into the Niger-Delta region. Of the three sample locations, Benin City is the most metropolitan. It has a population of 1.496 m (2015) and a projected current population of 1.8 m having an area of 1,204 sq. kilometres, with a population density of about 1495.0/ km^2 (Britannica, 2019). Agbor has a population of 162,594, with an area covering 436 sq. kilometres. The projected population of Sapele is 240,000 with a land area of 580 sq. kilometres and a population density of 413.6/km^2 (National Bureau of Statistics, 1977; Ugbomeh, 2011).

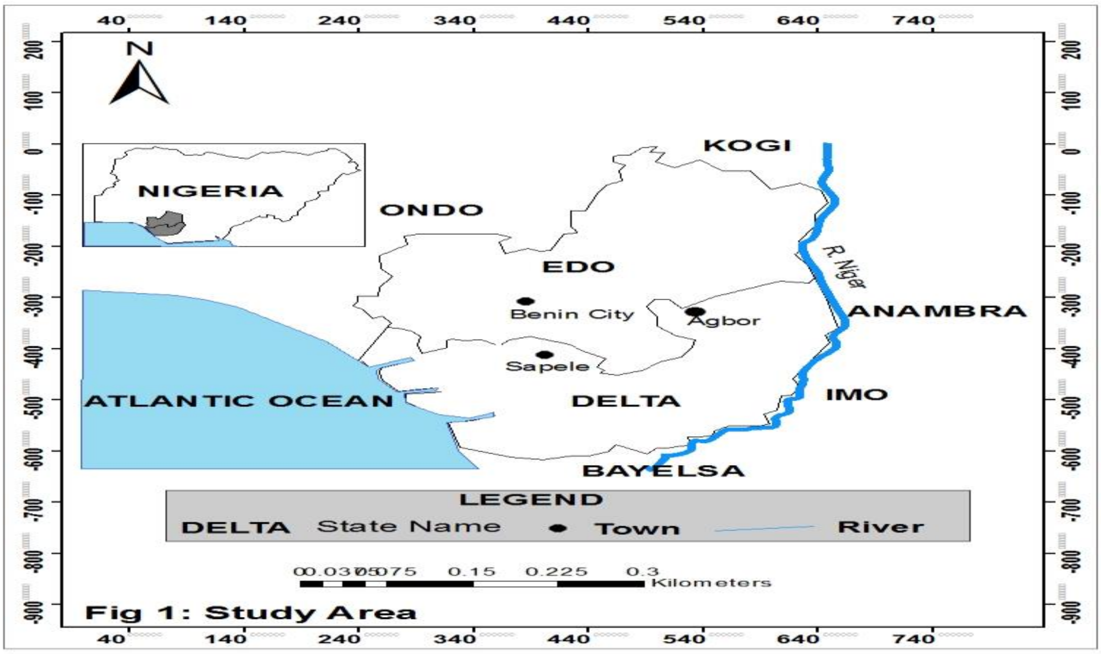

Figure 1: Study area showing locations A, B and C representing Agbor, Benin City and Sapele in Edo and Delta States of Nigeria.

Sample collection

Sewage effluent samples were aseptically collected from septic tanks within three locations in Edo and Delta States (Agbor, Benin and Sapele) in the south-south region of Nigeria for physicochemical and microbial characterization using 1 L plastic bottles and 200 mL sterile glass bottles respectively attached to a 1 m length stick just below the sewage surface during sample collection from both chambers of the septic tank. Samples were collected and placed in ice packs (4 ± 2 ˚C) and then transferred to the laboratory where a composite sample was made according to each location. Since homeowners are not often favourably disposed to having their septic tanks broken open, we were only able to sample five sites per location.

These septic tanks from each of the various sample locations (A, B, and C) were randomly chosen according to size. The only difference between the tanks was the number of persons served and the lifestyle (socio-cultural differences) of the users in each these communities. An average of 5 to 8 persons made up a household, comprising parents and between 4 to 6 children including relatives in some cases.

In general, the socio-cultural characteristics of the sample areas were approximately similar in many respect. Each household was almost evacuated by 9.00 – 10.00 in the mornings as both children and parents would have left home for school and work or sundry businesses respectively. In effect, each septic tank had a period of lag (retention time) of approximately 4 – 6 hours day time with little or no perturbation during this time before members of the households returned from their daily activities.

Samples were collected in triplicates according to the standard methods for the examination of water and wastewater (APHA, 1995).

pH determination

1 mL aliquot portions of the raw and semi-treated sewage samples were taken from the composite samples from each of the study locations in a 10 mL measuring cylinder previously sterilized. They were then serially diluted to a final volume of 1000 mL with distilled-deionized water (Ademoroti 1996a). Hydrogen ion $[H^+]$ concentration for the samples was determined using Hanna multi-parameter Metre type HI 9828 manufactured by Keison Products, United Kingdom, previously standardized.

Heavy metal analysis

Heavy metals ions in the sewage samples were extracted by acidifying with 1 mL concentrated trioxonitrate (IV) acid per 100 mL of sample. Samples were autoclaved for 1 hr to solubilize the content. The analyses were done in triplicate alongside a blank distilled-deionized water and methods were consistent with the standard methods for the Examination of Water and Wastewater by APHA (2005) and UNICEF (2008). Reagents used were of analytical grade and produced by BDH Chemicals Limited, England. Heavy metal ion concentration in these samples was determined using Solar Unicam atomic absorption spectrophotometer (AAS) model 969 AA manufactured by Vitech International BV, Netherlands). Three replicate readings were obtained for each of the metal ions, from which their means and standard deviations were then determined.

Microbial Analysis

Microbial analysis of the septic tank samples was done in the laboratory. Isolation, enumeration, identification and characterization of isolates were accomplished using standard microbiological techniques. Denaturing gradient gel electrophoresis (DGGE) was also used to fingerprint microorganisms in the septic tanks and results have already been published in a separate paper (Oyem *et al*., 2020).

Total heterotrophic bacteria (THB)

This was determined using nutrient media. It was performed in triplicates by plating out 1 mL aliquot of sewage sample with dilution factors of 10^{-4}, 10^{-5}, and 10^{-6} into already sterilized plates. Molten nutrient agar was poured into plates containing the samples and shaken properly for adequate mixing of the sample and the agar medium. Plates were then incubated at 37 ^{0}C for 24 hrs (ASTM, 1995; Pepper and Gerba, 2004). After this period, the bacterial colonies on each of the plates were counted and recorded. Identification of isolates was based on cultural characteristics and biochemical testing.

Total coliform bacteria (TCB)

The total coliform bacteria counts were determined using the Most Probable Number (MPN) technique with Mac Conkey broth containing bromocresol purple indicator (ASTM, 1995). The tubes were incubated at 37 ^{0}C for 48 hr for subsequent isolation and identification of coliform.

Total fungal count (TFC)

This was performed in triplicates by plating out 0.1 ml aliquots of sewage sample dilutions: 10^{-4}, 10^{-5} and 10^{-6} on potato dextrose agar plates containing *streptomycin*. The culture plates were incubated at room temperature for 72 hr. Distinct hyphae were transferred to sterile plates of malt extract agar and incubated at room temperature for 72 hr. The pure culture was transferred to sterile malt extract slants in McCartney bottles and stored in the refrigerator at 4 ^{o}C. Identification was based on microscopic and macroscopic examinations.

RESULTS AND DISCUSSION

Heavy Metals in Effluent Samples

In this study, the following heavy metals were analysed Fe, Mn, Zn, Cu, Cr, Ni, Pb, and V in the effluent sewage samples of the study area. This heavy metal profile was selected based on a previous similarly related study by Salem *et al*., 2000.

The pH of Septic tank Effluent

Table 1: Effluent pH of the inlet and outlet septic tanks chambers in locations A, B, and C.

	Location					
	A		B		C	
Septic tank Chamber	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet
pH	7.7	7.3	6.9	7.3	6.5	6.7

Table 1 contains pH values of the inlet and outlet chambers’ composite effluent samples in the study locations. From the values displayed, pH ranged from 6.5 – 7.7 in the study area, tending from being slightly acidic to slightly alkaline, thus appearing favourable to the methane-forming bacteria community in the septic tank system which are known to thrive in these pH conditions. Generally, according to the figures in table 1, the most acidic pH conditions were recorded in Sapele (Location C). This implies that pH-conditions in this location were likely favourable to acidophiles.

Heavy metals in effluent samples

Table 2: Heavy metals effluent concentrations in both chambers of the septic tanks in the study locations.

		Septic Tank Effluent Samples					
		Location A		Location B		Location C	
S/No.	Metal ion (Analyte)	Inlet (mg/L)	Outlet (mg/L)	Inlet (mg/L)	Outlet (mg/L)	Inlet (mg/L)	Outlet (mg/L)
1.	Fe	2.03 ± 0.2	0.87 ±0.02	9.41 ± 0.01	7.52 ± 0.02	8.77 ± 0.02	6.04 ± 0.02
2.	Mn	0.42 ± 0.02	0.33 ± 0.02	0.14 ± 0.02	0.10 ± 0.10	0.034 ± 0.002	0.05 ±0.02
3.	Zn	0.50 ±0.1	0.28 ±0.04	0.37 ±0.02	0.18 ±0.02	0.15 ±0.02	0.47 ±0.02
4.	Cu	0.20 ±0.1	0.16 ±0.02	0.26 ±0.02	0.17 ±0.02	0.072 ±0.002	0.063 ±0.002
5.	Cr	0.64 ±0.02	0.51 ±0.01	0.08 ±0.02	0.047 ±0.002	0.045 ±0.002	0.019 ±0.001
6.	Cd	0.44 ±0.02	0.35 ±0.04	BDL	BDL	0.031 ±0.001	BDL
7.	Ni	BDL	BDL	0.056 ±0.002	0.031 ±0.002	0.027 ±0.002	0.011 ±0.001
8.	Pb	0.83 ±0.02	0.75 ±0.02	0.062 ±0.002	0.015 ±0.002	0.093 ±0.002	0.058 ±0.001
9.	V	BDL	BDL	0.044 ±0.002	0.029 ±0.002	0.02 ±0.01	0.006 ±0.005

*BDL = Below detection limit.

Iron

Table 2 is a summary of the heavy metals content in the effluent samples of the septic tanks in the study locations. The results from Table 2 show that iron (Fe) is the most abundant dense metallic element present in the effluent sewage samples in the study area, with a mean concentration level of 6.74 mg/L. Its concentration ranged from (2.03 ± 0.20 – 9.41 ± 0.01 mg/L) (Table 2). Mean values of iron (Fe) concentration were significantly higher at $p>0.01$ in sludge samples than in effluent samples (data not presented). These results were found to be consistent with those reported by Lundy *et al.*, (2017) who noted that aqueous metals concentrations provide short term indications of contamination, whereas, sediments concentrations are representative of contaminant trends over a long period.

Location B had the highest Fe value (7.52 – 9.41 mg/L) for the raw effluent chamber (inlet) and semi-treated effluent chamber (outlet) respectively. The range was 0.87 – 7.52 mg/L for the semi-treated effluent samples in the study area with an average value of 4.81 mg/L for Fe. Comparatively, a difference of 1.89 mg/L was observed between the values of Fe in the inlet and outlet chambers in location B. However, the highest remediation of Fe in the study area took place in the septic tanks in location C. Indicating that slightly alkaline conditions may have favoured the removal of Fe from the effluent component.

The percentage removal of Fe for all three locations were 57.1 for location A, 20.1 % for location B, and 31.1 % for location C. Thus implying that there was appreciable treatment of Fe in the effluent samples of the septic tank in locations A which had the highest pH values comparatively. This infers that the sludge accumulating at the bottom of the septic tank was apparently rich in toxic metal deposits, considering the alkaline conditions (Agoro *et al.*, 2020, Duan *et al.*, 2017, and Steinhardt & Egler 2018). Meanwhile, it is not surprising to observe the significantly high values of Fe obtained in this study, Avwunudiogba (200) had earlier noted that the study area is rich in ferrosol. Again, in another study Onyedikachi and co-workers (2018) reported that Fe was the most abundant element.

Lead

Following Fe in the abundance series was Pb in location A of the study area. The values of Pb were significantly lower in locations B and C respectively. Lead ranged from 0.062 – 0.830 mg/L for the inlet chamber and 0.015 – 0.750 mg/L for the outlet chamber in all of the effluents samples in this study. An average value of 0.33 mg/L was obtained for Pb in the effluent samples of the inlet chambers of the septic tanks in the study area; whereas, 0.28 mg/L was the value obtained for the outlet chamber samples (Table 2). Invariably, a net value of 0.05 mg/L amounting to 18.2 per cent of the total Pb in the effluent sample (inlet chamber) was removed. From data in table 2, we can conclude that slightly neutral/near alkaline pH conditions as was the case of Fe, appeared to favour the removal of Pb from the inlet effluent samples in location B than was recorded in the other two locations.

Chromium

Cadmium followed Lead in the abundance series of heavy metals in this study. In the effluent sample of the raw chamber from location A, Chromium (Cr) concentration was found to be significant at 0.64 mg/L followed closely by 0.084, and 0.045 mg/L in locations B and C respectively. Further analysis of the data showed that approximately 20, 46, and 52 % in that order, of the Cr (total chromium) in the effluent samples of the inlet chamber were remediated from locations A, B, and C. These accounts for 0.130, 0.037, 0.026 mg/L respectively, being the differences in the values of Cr in the inlet and outlet chambers. Again, these values are believed to be deposited in the sludge at the bottom of the inlet chamber in the septic tank. An average of 0.07 mg/L of Cr was precipitated out of the effluent in the inlet chamber of the study area, translating to 9.10 per cent removal of total Cr in the septic tank effluent. By inference, data for chromium in table 2 portend that slightly acidic pH conditions positively affected the release of chromium from the effluent component of the septic tank system like was the case with iron.

Cadmium

Cadmium was next to zinc in the order of heavy metals abundance removed from the effluent samples even though it was below the detection limit in several instances in both locations B and C with values of 0.44 mg/L, below the detection limit (BDL) in location B, and 0.03 mg/L in the effluent samples of the inlet chamber in the various sample areas. This translates to a mean value of 0.16 mg/L for Cd of the inlet chamber and 0.13 mg/L in the outlet chamber. Similarly, 0.03 mg/L Cd was removed from the effluent samples in the study area, representing 18.8 % removal. The effect of pH on cadmium is not certain at this moment since data for locations B and C were not available.

Manganese

Values for Mn in the effluent samples of the inlet chamber for the three location sites are 0.42, 0.14, and 0.03 mg/L respectively (Table 2) with location A having the highest Mn concentration. The average Mn value in the inlet chamber is 0.20 mg/L. Data for Mn in the effluent samples of the outlet chamber were 0.33, 0.10, and 0.05 mg/L, with an average value of 0.16 mg/L. Thus, 0.04 mg/L accounts for the concentration of Mn removed during treatment in the inlet chamber. This translating to 20 per cent of Mn remediated from the effluent in the inlet chambers.

Zinc

Also, next on the abundance scale in the study area is Zinc after chromium, according to the results in table 2. Its values ranged from 0.15 – 0.50 mg/L in the inlet chamber effluent samples having a mean value of 0.34 mg/L. Values of Zn obtained ranged from 0.18 – 0.47 mg/L, with an average of 0.31 mg/L with a difference of 0.03 mg/L being the difference from both chambers after treatment. Again, this translates to the removal of heavy metals from effluent samples by precipitation (Qodah, 2006). Indications are that acidic conditions appear to favour the removal of Zn from effluents in the septic tanks going by a higher percentage of 51.4 % in location B than the 44.0 % in location A..

Copper

Copper was the 7th on the abundance scale of heavy metals analysed. Copper had the highest concentration in the effluent samples in location B with a value of 0.26 mg/L, just slightly above the 0.20, and 0.07 mg/L recorded in locations A and C. It is also present in the following concentrations 0.16, 0.17, and 0.06 mg/L in the effluent samples of the outlet chamber in Location A, B, and C. 0.18 mg/L was the mean value for Cu in the study area. Whereas, 0.13 mg/L was the mean for Cu in the effluent samples in the study. The highest removal percentage of copper was recorded in location B with a value of 35.0 % as against 20.0 % and 14.3 % in locations A and C respectively. This could be interpreted to mean that near-neutral pH conditions were favourable to the removal of Cu from effluent solutions in the septic tank system.

Nickel

Nickel (Ni) was below the detection limit in the inlet and outlet chamber effluent samples in location A. It was the 8th most abundant heavy metal analysed in this study. The values for Ni in the inlet and outlet chamber effluent samples were 0.06 and 0.03 mg/L in location B; 0.03 and 0.01 mg/L in location C. These show a difference of 0.03 and 0.02 mg/L respectively for values in the inlet and outlet chambers representing the concentrations of Ni removed from the effluent samples in the inlet chamber of these two locations. Thus, translating to approximately 50.0 and 66.7 % of Ni bio-adsorbed and precipitated out to the bottom of the inlet chamber as components of the sludge. Like Cu, nickel removal from effluent samples seem to be favoured by near-neutral pH conditions.

Vanadium

Vanadium was the least abundant heavy metal analysed in this study. Vanadium was not detected in both the effluent samples of the inlet and outlet chambers in location A, apparently being present in concentrations below the detection limit in these samples in location A. It was however present in the inlet and outlet chamber samples in concentrations of 0.04 and 0.03 mg/L for location B, and 0.02 and 0.01 mg/L for location C. This amounts to a difference of 0.01 and 0.01 mg/L for the effluents of both chambers of the septic tanks in locations B and C representing 25.0 and 50.0 per cent vanadium removal. Indications are that acidic conditions favoured vanadium removal.

Heavy metals percentage removal and concentration ratios (coefficient)

Percentage removal is the concentration of the metal removed in the inlet chamber expressed in percentile. It is obtained by calculating the difference between the concentrations in the inlet and outlet chambers divided by the metal's concentration in the inlet chamber. See equation 4 below:

$$Percentage\ Removal = \frac{Concentration\ (Inlet\ chamber) - Concentration\ (Outlet\ chamber)}{Concentration\ (Inlet\ chamber)}\ X\ 100 \quad (4)$$

$$Coefficient\ of\ Removal = \frac{Concentration\ (Outlet\ Chamber)}{Concentration\ (Inlet\ Chamber)} \quad (5)$$

Table 3: Analysis of heavy metals remediation showing percentage and coefficient removal from effluent samples in location A.

Location A									
Analytes (Metal ions)	Fe	Mn	Zn	Cu	Cr	Cd	Ni	Pb	V
Concentration (mg/L)									
Inlet Chamber effluent	2.03	0.42	0.50	0.20	0.64	0.44	BDL	0.83	BDL
Outlet Chamber effluent	0.87	0.33	0.28	0.16	0.51	0.38	BDL	0.75	BDL
Difference (Inlet-Outlet) chambers effluents	1.16	0.09	0.22	0.04	0.13	0.06	NA	0.08	NA
Percentage Removal (%)	57.0	21.4	44.0	20.0	20.3	13.6	NA	9.64	NA
Removal Coefficient (Inlet/Outlet)	0.43	0.79	0.56	0.80	0.80	0.86	NA	0.90	NA

*BDL = Below detection limit
*NA = Not Available

The array of heavy metal ions analysed from the septic tanks in the study area is presented in table 3 for study location A. It shows the concentrations of each of these metal analytes in the two chambers of the septic tank system. The difference in the concentrations of the inlet and outlet chambers were calculated, and the percentages of these differences as well as the ratios of the concentrations of each metal ion in the chambers of the septic tank were obtained. The difference between the concentrations of the analytes in the inlet and outlet chambers represents the concentration of metal analyte remediated by bioadsorption/precipitation and sedimentation. From table 3, we can see that Fe is the most remediated of all the heavy metals studied in location A, with a removal percentage of 57.0 % and a removal coefficient value of 0.43. This coefficient value is the closest to the optimum value of 0.33 of all the heavy metal types in location A However, this implies that conditions in the septic tank for this location is still far from the optimum for the effective remediation of heavy metals.

Table 4: Analysis of heavy metals remediation showing percentage and coefficient removal from effluent samples in location B.

Location B									
Analytes (Metal ions)	Fe	Mn	Zn	Cu	Cr	Cd	Ni	Pb	V
Concentration (mg/L)									
Inlet Chamber effluent	9.41	0.14	0.37	0.26	0.08	BDL	0.06	0.06	0.04
Outlet Chamber effluent	7.52	0.10	0.18	0.17	0.05	BDL	0.03	0.02	0.03
Difference (Inlet-Outlet) chambers effluents	1.89	0.04	0.19	0.09	0.03	NA	0.03	0.04	0.01
Percentage Removal (%)	20.1	28.6	51.4	34.6	37.5	NA	50.0	66.7	25.0
Removal Coefficient (Inlet/Outlet)	0.80	0.71	0.49	0.65	0.63	NA	0.50	0.33	0.75

*BDL = Below detection limit
*NA = Not available

The number of metals removed as insoluble salts is converted to percentiles, together with the concentration quotients obtained are used for analytical purposes. In table 3, Fe was the most precipitated out of solution onto the sludge at the bottom of the septic tank. This is evident by the concentration obtained as the difference value of Fe (1.16 mg/L) in both the inlet and outlet chambers as shown in table 3. Accounting for 57.0 per cent of the total Fe initially in the effluent of the inlet chamber. Apart from Fe, the efficiencies of removing other metal ions (analytes) in the inlet chambers of the septic tank systems were generally poor in location A.

Table 5: Analysis of heavy metals remediation showing percentage and coefficient removal from effluent samples in location C.

Location C									
Analytes (Metal ions)	Fe	Mn	Zn	Cu	Cr	Cd	Ni	Pb	V
Concentration (mg/L)									
Inlet Chamber effluent	8.77	0.05	0.47	0.07	0.05	0.03	0.03	0.09	0.02
Outlet Chamber effluent	6.04	0.03	0.15	0.06	0.02	BDL	0.01	0.06	0.01
Difference (Inlet-Outlet) chambers effluents	2.73	0.02	0.32	0.01	0.03	NA	0.02	0.03	0.01
Percentage Removal (%)	31.1	40.0	68.1	14.3	60.0	NA	66.7	33.3	50.0
Removal Coefficient (Inlet/Outlet)	0.69	0.60	0.32	0.86	0.40	NA	0.33	0.67	0.50

*BDL = Below detection limit
*NA = Not available

Usually, a high percentile value typifies a high efficiency of metal analyte remediation from the effluent in the inlet chamber. This is confirmed by a corresponding low ratio value obtained for the concentrations of the metal ion in the outlet chamber against its concentration in the inlet chamber. Whereas, a low percentile value for a metal ion translates to poor removal as demonstrated by a correspondingly high concentration ratio obtained on evaluation. A high percentile value indicates a high rate of metal ion precipitation from the effluent and translates to a low concentration ratio obtained. This explanation applies to all the metal analytes in the study area as indicated in Tables 3, 4, and 5 for all the sample locations A, B, and C.

Apart from Fe at 57.0 % (0.43 removal coefficient), the efficiency of removing other heavy metal ions (analytes) was generally poor in location A (Table 3). Lead had the highest removal efficiency at approximately 66.7 per cent (0.33 removal coefficient), all other metal analytes were also poorly removed from the effluent component of the inlet chamber in location B. Inferring that the conditions in the septic tanks in location B were most ideal for the precipitation of Pb. Apart from Pb, these neutral/near alkaline conditions were close to the optimum for Zn and Ni. Only Pb achieved the optimum precipitation value of 67.0% and 0.33 removal coefficient.

In location C, Cr, Ni, and Zn were significantly precipitated from the effluents in the inlet chambers of the septic tanks, with percentage removal values of 60.0, 66.7, and 68.0 corresponding to 0.40, 0.33, and 0.32 respectively (Karimi, 2017). Generally, a removal coefficient of less than or equal to 0.33, that is, $0 < x \leq 0.33$ (where $x \neq 0$) is considered optimum for any metal ion remediated from the effluent phase onto the sludge component of the septic tank in this study.

Invariably, almost three heavy metal types had pH conditions optimum for their precipitation from the effluent in the inlet chamber. This goes to confirm that acidic conditions were more ideal for the precipitation of heavy metals as demonstrated by data from location C (table 5).

Microbial counts analysis

Table 6: Total heterotrophic bacteria (THB), total coliform bacteria (TCB), and total fungal count (TFC) in both chambers of the septic tanks in the study area.

Locations						
	A		B		C	
	RS	SS	RS	SS	RS	SS
THB (10^5 cfu/mL)	3.4 ± 0.2	3.0 ± 0.2	4.5 ± 0.2	3.8 ± 0.1	3.8 ± 0.1	4.0 ± 0.2
TCB (10^4 cfu/mL)	4.0 ± 0.2	4.0 ± 0.2	5.1 ± 0.1	4.4 ± 0.2	3.6 ± 0.2	3.5 ± 0.2
TFC (10^4 cfu/mL)	2.8 ± 0.1	2.1 ± 0.1	3.2 ± 0.2	3.3 ± 0.2	2.5 ± 0.4	2.7 ± 0.2

The results of the microbial count analysis are displayed in table 6 above. This shows three groups of microbes: total fungal, total coliform bacteria, and total heterotrophic bacteria. Data from the analyses of these three microbes demonstrates that total heterotrophic bacteria had the highest count and dominance in the septic tanks of the study area.

Role of pH and microorganisms in remediation of heavy metals in effluent samples of septic tank

The pH for biochemical oxygen demand (BOD) is 7.2. This implies that a pH of 7.2 is ideal for the degradation of organic matter by microbial activities. Hence, the standard BOD test specifies a pH of 7.2. Therefore, pH-adjustment is necessary where sample pH for BOD determination falls below or above this standard (Ademoroti, 1996). Microorganisms performing biodegradation of organic matter are expected to be pH-sensitive (Abatenh *et al.*, 2017). They therefor require optimum pH for their physiological processes. However, in water and wastewater systems *in situ* pH conditions are hardly ideal and difficult to manipulate. In the treatment of sewage containing metal ions, the pH of the system similarly affects the dissolution, adsorption and precipitation of the metal ions (Karimi, 2017). The pH range reported in this study aligned with those obtained by (Masindi *et al*., 2018) for the adsorption/precipitation of the metal analytes in wastewater. Meanwhile, results from the previous sections of this study, show that the pH conditions which produced the heaviest metal precipitation were found in location C with a pH range of 6.5 – 6.7. This gives an average pH of 6.6; and 0.6 places short of the optimum BOD pH value of 7.2. Indications are that the pH range in location C favours the acid-forming bacteria, leading to the conclusion that there exist more than one mechanism by which heavy metals are removed from effluents – precipitation and bio-adsorption.

Heavy metal cations each have their respective pH ranges at which they precipitate out of solution. Iron precipitates from an aqueous solution where it is present in its most dissolved form as Fe (II) at a pH range of 5.5 – 8.2 (Ademoroti, 1996; Ibrahim & Ibrahim, 2016; Shan *et al*., 2019), Ni is pH of 10.0 – 10.5, Zn is pH of 9.0 – 9.5, Cu is at pH of 8.5 – 9.5 (Ain Zainuddin *et al*., 2019), Chromium is at 7.5 – 8.5 (Baig *et al*., 2003), Cadmium is at pH 11 (Shan *et al*., 2019), the pH of Lead is between 9 – 11 (Karimi, 2017). All metal hydroxides do not precipitate at a single pH (Ain Zainuddin *et al*., 2019). Therefore, the pH of the septic tanks in the study area ranged between slightly acidic and slightly basic at 6.5 to 7.2, except for the pH of 7.3 and 7.7 obtained in location A. It would appear that the pH of the septic tanks in the study area do not possess the required basic conditions which are ideal for metal ion hydroxyl precipitation. By implication, little metal hydroxide precipitation would be achieved for some metal ions in these septic tanks at the prevailing the pH-ranges. The pH conditions in the septic tanks in location A is the nearest to the optimum for significant heavy metal ion hydroxide precipitation (pH 6 – 12).

The percentage removal of heavy metals in the septic tank between the inlet and outlet chambers is given in Tables 3, 4, &5 for the three study locations. The values recorded for each of the heavy metals varied widely from location to location without any clear pattern. The pH of the effluent sample in the study area ranged from 6.5 – 7.7, with an average pH of 7.1. In location A, the pH of the inlet and outlet chambers were 7.7 and 7.3 respectively. In location B, it was 6.9 and 7.2 for the inlet and 6.5 and 6.7 respectively for outlet chambers in location C. Of these three study locations, the environment in the septic tank in location C had the least pH values. That is, acidity followed this order: location C > location B > location A. In actual terms, location A is already in the alkaline region of the pH scale.

Anaerobic digestion of hypothetical organic matter

Generally, in septic tank systems, anaerobic digestion is the prevailing means of bioremediation, where bacteria convert organic matter principally to fatty acids and methane; thereby, releasing CO_2, NH_3, H_2S, PH_3, H_2O, and H^+ in a two-stage process summarized below for a hypothetical organic matter:

$$n(CHONSP) + nH_2O \longrightarrow nCH_3COOH + nCO_2 + nNH_3 + nH_2S + nPH_3 + nH^+ \qquad (3)$$

$$4CH_3 \ \text{Hypothetical organic matter} \longrightarrow 5CH_4 + 3CO_2 + 2H_2O \qquad (4)$$

The first stage of this conversion process is effected by a group of bacteria known as acid-forming bacteria, and the second stage by another group of bacteria called methane-forming bacteria. Noteworthy, is that both groups of bacteria have their activities controlled essentially by pH of the medium. The first of these types of bacteria are favoured by pH conditions less than 6; the second group – the methane formers favour more alkaline pH conditions.

Bacteria and other microorganisms constitute part of the hydrophilic colloids in septic tanks systems. They have polar-end molecules and carry charges which cause the colloidal particles to repel each other electrostatically. Thereby preventing them from coalescing and dropping onto the bottom under the effect of gravity but remaining suspended in solution. The functionality of certain chemical groups inherent in the bacteria cell wall is thought to be responsible for the colloidal properties exhibited by bacteria. Theoretically, the polar characteristics of bacteria cells are understood to be derived from the ionic character of amino and fatty acids components of the cell wall. All viruses and bacteria are thus considered bio-colloids (Bestawy *et al*., 2013; Val & Lorenzo, 2002). Like amino acids, microbial cells can similarly be considered as charged or neutral (zwitterions) species depending on the pH of the environment (Ademoroti, 1996; Tang *et al*., 2003). Reports have it that bio-colloids, proteins, and products of protein degradation form salts (chelates) with heavy metals (Ademoroti, 1996; Jin *et al*., 2018; Sarret *et al*., 1998; Tang *et al*., 2003). Bio-precipitation (insolubility) best occurs within the range of pH 4.0 – 6.5 (Ademoroti, 1996), pH 5.5 – 6.5 (Jin *et al*., 2018), and pH 5.0 – 7.0 (Shan *et al*., 2019). That is, a pH range of 4.0 – 7.0 is suitable for bio-colloids precipitation (bio-sorption). Bio-sorption is the adsorption of dissolved species (including metal ions) to a biological matrix (cell wall) through electrostatic interaction (physically) or chemically by ion or proton exchange, complexation, or chelation (Diep *et al*., 2009). Data from all the study locations demonstrate that as the pH reduces, the mechanism of metal precipitation tilted towards bio-sorption. That is, the interaction between heavy metals and microbes increased.

The rather acidic conditions of the septic tanks in location C should favoured acid-forming bacteria and to a rather lesser extent, those in location B (being lower in acidity). Location A is already alkaline and would not be considered favourable to the functions of acidophiles. The activities of methane-forming bacteria, on the other hand, would therefore be hampered as they are notably pH-sensitive (Abatenh *et al*., 2017). Heavy metals binding sites on microbes are saturated with H^+ in strongly acidic pH regions of the pH scale, thereby limiting metal ions binding interactions with microbial cell surfaces (Delangiz *et al*., 2020; Rose *et al*., 2016).
This means that the balance of bacteria activities in the septic tank environment would be tilted in favour of the reaction represented by Eqn. 3 for fatty acid formation. Hence, pH values would be expected to drop (become more acidic), and less heavy metal bio-colloid salts/complexes are formed. This would inhibit the removal of dense metal ions from the effluent. Hence, the effluent which leaves the inlet to the outlet chamber would be less effectively clarified in terms of metal ion removal. The drop in metal ion concentration is the difference in the concentrations of both the inlet and outlet chambers, the extent of which is determined by the pH of the two chambers of the septic tank.

Microbes as bio-colloids with functionality and bio-precipitation determined by pH

If the pH is high like it is in location A (pH 7.7 and 7.3 for the inlet and outlet chambers respectively with a mean pH of 7.5), which is already above the pH range of 4.0 – 7.0 established as being optimum for bio-adsorption, it would hinder metal ion removal from the effluent as bio-chelates. This was believe to be the case in location A where Fe was the only heavy metal ion closest to the 67.0 % (and 0.33 removal coefficient) optimum at 57.0 % and 0.43 removal coefficient. This associates Fe removal mechanism in this case to hydroxyl (inorganic) precipitation rather bio-precipitation in location A. All the other dense metal ions were poorly remediated in location A (refer to Table 3 above). By contrasting the percentages values of Fe 57.0 % removed from the effluents in location A with those of location B and C (20.1 and 31.1 %) as the pH of the effluent samples in these locations decreased and became acidic, we can conclude that slightly alkaline conditions favoured the removal of Fe in location A..

Besides, the influence of Fe (III) present in the effluent which can combine with hydroxyl ions obtained in the process represented in Eqn. 5 to form Fe(III)hydroxides cannot be overlooked in location A. This sort of reactions typically thrives in alkaline conditions, a situation which favours methane-forming bacteria (Eqn. 4). By contrast, as pH-conditions moved towards acidity in location B (mean pH of 7.1) and C (mean pH of 6.6), the efficiency of heavy metal removal increased. In location B, Pb attained the 67.0 % removal efficiency and 0.33 removal coefficient, while Zn and Ni were the closest with 51.4 and 50.0 percentage removal respectively (Table 4). In location C, where the pH-conditions were within the 4.0 – 7.0 range optimum for bio-sorption, several dense metal ions (Zn and Ni particularly) finally attained the 67.0 and 0.33 removal efficiency and coefficients threshold respectively; with Cr and V coming close at 60.0 and 50.0 per cent and 0.40 and 0.50 coefficients respectively. These results confirm the effect of pH on the heavy metal remediation in the septic tank system.

Effluent clarification and heavy metal removal would therefore be expected to improve as pH drops further until the pH of 4.8 which Ademoroti (1980) affirmed the purest effluent can be obtained. However, this is rather unlikely in septic tank systems as the pH range is somewhere in between weakly acidic and slightly above neutral conditions.

In equations 3 and 4 above, the common end-product is CO_2, a major end-product of bacteria metabolism (Ademoroti, 1996). This CO_2 dissolves in the effluent to form a hydrogen carbonate ion (HCO_3^-), which then undergoes dissociation to form CO_2 yet again and hydroxyl ion (OH^-) is released into the solution. Consequently, increasing the pH (more alkaline) of the medium. See Eqn. 5 below:

$$HCO_3^-{}_{(aq.)} \qquad CO_{2\,(g)} \rightleftharpoons OH^-{}_{(aq.)} \qquad (5)$$

$$Fe(OH)_3 + \text{organic colloid} \longrightarrow \text{dense floc.} \qquad (6)$$

Equation 5 is believed to account for why in certain cases as observed in table 1, pH in some of the chambers (especially in location A) of the septic tanks recorded values above 7.0. Therefore, when microbial activities are at their optimum (BOD pH of 7.2) the pH of the medium should rise above neutrality and become more alkaline. Implications are that, metal remediation mechanism switches from bio-adsorption to hydroxyl precipitation.

Similarly, other metal ions inherent in the effluent under these increasing pH-conditions may combine with negatively charged groups to form metal salts (Ademoroti, 1996; Bestawy *et al*., 2013; Kelly *et al*., 2004; Yuncu *et al*., 2006). Bio-precipitation is the adsorption of metal ions onto cell surfaces through microbial mechanisms (Gupta *et al*., 2016; Hashim *et al*., 2011; Igiri *et al*., 2018; Osman *et al*., 2019). Interactions between metals and microbes principally result in the removal of metals from sewage (Igiri *et al*., 2018). The surface charge of the microorganisms is affected by pH, thus influencing the adsorption of heavy metal ions (Jin *et al*., 2018). Extracellular polymers with their negative charges provide binding sites for these heavy metals. The efficiency of this metal-exopolymer interaction is pH-dependent (Canovas *et al*., 2003; Delangiz *et al*., 2020; Igiri *et al*., 2018; Osman *et al*., 2019; Tytła, 2019). When the pH gradually increases on the acidity scale, the cell surface gradually becomes more negatively charged and metal bio-adsorption rises until it reaches a maximum around pH of 6.0 (Ademoroti, 1996; Hassan *et al*., 2009). Above this point hydroxyl precipitation rather predominates.

The majority of Gram-positive bacteria have the capacity for metal adsorption (Ojuederie and Bablola, 2017; Osman *et al*., 2019; Song *et al*., 2004; Yilmaz, 2003), several of these types of bacteria were discovered in the effluent samples in this study area (Oyem *et al*., 2020). Natural bacteria communities have shown great potential as tools for bioremediation of heavy metal ions (Gupta *et al*., 2016; Hashim *et al*., 2011; Ojuederie and Bablola, 2017; Osman *et al*., 2019; Wood *et al*., 2016). This may involve the transformation from more toxic to less toxic forms (Delangiz *et al*., 2020; Guatem *et al*., 2015).

The precipitation process can be divided into three types: hydroxide, carbonate, and sulphide precipitation (Karimi, 2017; Lumen Microbiology, 2020). Ionizable groups like amino, carboxyl, phosphate, and hydroxyl, on the surface of microbes, are such binding sites for metal bio-removal (El-Helow *et al*., 2000; Igiri *et al*., 2018; Usman *et al*., 2020).

$$M^{n+}{}_{(aq.)} + (\text{Colloids})^{n-}{}_{(aq.)} \longrightarrow [\text{M (Colloid)}] \qquad (7)$$

Metal hydroxide ($M(OH)_n$) are basic; their formation and presence require alkaline conditions. Even though hydroxyl ions were produced as depicted in Eqn. 5 above, they were not enough to drive up the pH dramatically in the septic tank system because the hydroxyl ions are consumed in the competing reaction in equation 6. The process represented by equation 5 is believed to be accountable for the alkalinity observed in location A, leading to the precipitation of Fe (III) as hydroxides. More so, Fe being the most abundant heavy metal in the system, means there is enough to deplete the stock of the hydroxyl ions present. This is considered to be the reason why there was as much as 57.0 % Fe removal in location A as compared to other locations where the pH was slightly acidic and the Fe removal efficiency was rather poor.

For this to be ameliorated in locations B and C where the pH is acidic, more hydroxyl ions need to be produced in Eqn. 5. The results from this study with respect to Fe leads to the conclusion that metal remediation from effluent samples in the septic tank system was occasioned by the combined processes of bio-sorption and metal inorganic precipitation all of which are pH controlled.

The balance of play between the acidophiles and methanogens should assumeably, be balanced (equilibrium) in some sense or tilted in favour of the latter (Eqn. 4) with regards to methane formation. This would lead to the production of more OH^- ions in Eqn. 5, thereby increasing the pH. Alkalinity favours the bioremediation process in the septic tank system because it drives the entire remediation process towards a conclusion in methane formation (Angeli *et al*., 2018) where energy generation is the focus. However, for heavy metal bio-removal, slightly acidic conditions were crucial for the efficient removal of toxic metal pollutants like Zn, Ni, Cr, Pb, and V; which is the focus of this study.

CONCLUSION

Effluent in the inlet and outlet chambers of septic tank sewage in three states in the south-south geo-political region of Nigeria were studied for their heavy metal contents and pH-mediated bioremediation. A pH range of 6.4 to 7.7 was recorded in the study area. While effluent samples generally tended towards being basic, slightly acidic pH conditions favoured the removal of a number toxic metals in the study. Lead, Zn and Ni are the most effectively remediated heavy metals having attained above the 66.7 per cent removal threshold. Although more data from similar studies would be required to authenticate this, however, we could conclude tentatively that the removal efficiency seems to favour heavy metals with the most stable oxidation state of 2^+ with specific reference to Pb, Zn and Ni.

Generally, heavy metal contents of effluent samples were high, which confirms that the septic tank – especially the inlet chamber – is a huge repository of these dense metal contaminants. The concentrations of these toxic metals were always lower in the effluent samples of the outlet (semi-treated) chambers. Even as the pH of these outlet chambers were always higher than the inlet chambers of the septic tank.

Metal toxicity ostensibly affected the microbial population even though it appeared that they may have evolved strategies to acclimatize as microbial counts were generally low. Microbial counts were found to be lower in the outlet chamber than they were in the inlet chamber, with total heterotrophic bacteria being the most dominant species in the septic tank.

Finally, the heavy metal removal mechanism in this study essentially followed the metal bio-sorption pathway. Inside the septic tank system, the binding of metal ions to bio-colloids was determined by pH trends, with acidic conditions apparently resulting in the attainment of the 67.0 % removal efficiency and the 0.33 removal coefficient thresholds in the effluent samples for some heavy metals.

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