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Bacteriological Analysis of Leachate at Dumping Sites of Selected Areas in Damaturu Yobe State, Nigeria

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ABSTRACT
Assessing surface and groundwater quality and developing strategies to protect aquifers from contamination are necessary for proper planning and designing water resources. Therefore, this study aimed to assess the bacterial load of leachate in surface water and soil sediment at dumping sites of Nayinawa and Babban-Tsangaya, Damaturu, Yobe State. Simple random sampling was used for sample collection. Bacteriological determination was done through aerobic mesophilic bacterial count, bacteria gram staining most probable number (MPN) counting and biochemical test. Bacteriological quality determination revealed high contamination level of the samples for bacterial colony count (6.14×10^6 cfu), MPN (387.6 ml) and Gram staining (all negative). Biochemical confirmatory test revealed that for water and soil sediment in both months, catalase and coagulase were positive, urease, citrate and oxidase are negative. The development and application of integrated leachate treatment process of different physical, biological and chemical technologies could be a suitable option to reduce the contamination levels of the leachate. This study revealed that water from the study areas (Nayinawa and Babban-Tsangaya) has significant levels of bacterial load which poses a serious health risk to the people in the area.

Keywords: Leachate; Bacteria; Dumping site; Groundwater; Contamination; Damaturu

INTRODUCTION
Waste dumping has been identified as one of the major threats to surface and groundwater sources; it is the most common method of waste disposal in Nigeria (Ezeuo *et al*., 2016) Open dump area (site) is thought to be active sources for the gradual release of harmful compounds mixed with nontoxic precursors into the environment (Sozan *et al.,* 2022). Leachate and gases are produced in the open dumpsite as a result of biological, chemical, and physical processes that promote waste disintegration (Papadopoulou *et al.,* 2007). As water percolates through the waste, it picks up a variety of organic and inorganic compounds flowing out of the waste and accumulates at the bottom of the dumpsite (Abudu *et al*., 2023). The resulting contaminated water termed leachate can percolate through the soil and eventually contaminate the groundwater if not adequately managed (Ejikeme and Kewve, 2015; Janaina *et al.,* 2023). The discharge of landfill leachate can lead to serious environmental problems, since leachate contains different types of contaminants: dissolved organic matters; inorganic compounds such as ammonium, calcium, magnesium, sodium, potassium, iron, sulphate, chlorides; heavy metals such as cadmium, chromium, copper, lead, zinc, arsenic and xenophobic organic substances (Abdelwaheb *et al.,* 2012). The rate and characteristics of leachate produced depends on many factors such as solid waste composition, particle size, degree of compaction, hydrology of site, age of landfill, moisture and temperature conditions, and available oxygen (Irma *et al*., 2016).Soil, groundwater acidification and nitrification have been linked to waste dumps as well as microbial contamination of soil and groundwater system (Amadi *et al*., 2012). Good public health requires regular water quality monitoring to prevent people from contracting diseases (Carlos *et al.,* 2023).

Most environmental bacteria are not harmful to healthy individuals, they can be hazardous once they concentrated in colonies (Hussaien *et al.* 2023). Fecal pollution is the main source for disease causing agents in water (Kumar *et al.*, 2019), including bacteria present in excreta from humans and warmed-blooded animals. *E. coli* is a type of bacteria that normally live in the intestines of warm-blooded animals, though some toxic strains (e.g., *E. coli* O157:H7) can cause abdominal cramps, vomiting, and diarrhea (Proce and Wildeboer, 2017). In landfills and open dumpsites, leachate generation is unavoidable, resulting in environmental pollution, especially where there are no plans or schemes for onsite leachates treatment or discharge of leachates from leachate dumps for co-treatment with domestic wastewater. Leachates are hazardous and can cause serious harm to the body (Abudu *et al.*, 2023). Therefore, this study will inform the community on the health risk associated with leacheates and its associated pollution potentials in Nayinawa and Babban-Tsangaya selected study areas.

MATERIAL AND METHODS

Study Area

Yobe is a state located in northeastern Nigeria, Yobe state is located within latitude 11 North and longitude 13.5 East. The capital of the State is Damaturu, and its largest and most populated city is Potiskum. Situated in the North Eastern flank of Nigeria, Yobe State occupies 45,502 square kilometres. The population of the State according to the National Head Count conducted in 2006 is about 2.6 million (NPC, 2006).

The climate condition of Yobe is warm with daily temperature of 37 °C (98.6 °F). November being the sunniest month and rainy month is between August and December (World Data, 2022). Nayinawa and Babban-Tsangaya were selected Sampling sites where people around Gashua and its environs dump their generated wastes and the water from the pit is used for domestic and irrigation purposes. The waste dumped in the water generated chemical reaction and may resulted in leacheate which may percolates through and cause contaminations of the water (Fig. 1).

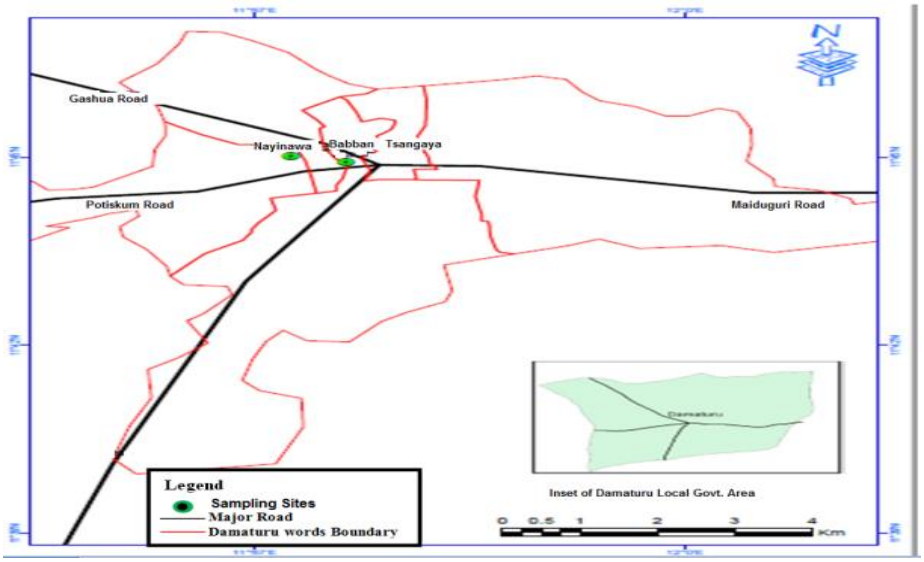

Figure 1: Map of the Study Area Showing Sampling Sites (Source: Ministry of Land and Survey Damaturu, 2018)

Preparation of Soil Sample for a Coliform Count (serial dilution)

One gram (1 g) of the sample was weighed using weighing balance and put into the test tube containing 10 ml of sterile water. The samples were serially diluted using 10-folds dilution method. In each sample, 10^{-1} to 10^{-5} were recorded and aseptically inoculated into the appropriate media for bacterial growth. It is not a measure of the entire bacterial population; it is a generic test for organisms that grow aerobically at mesophilic temperatures (25 to 40°C; 77 to 104 °F) (Moses *et al.*, 2016). Water from the two designated (Nayinawa and Babban-Tsangaya) sampling area were collected and examined following the method of Rice *et al*., (2012).

Isolation of Bacterial Colony on Solid Media

Twenty eight gram (28 g) of nutrient agar was weighed on analytical weighing balance and dissolved in 1000 ml conical flak containing distilled water.

The mouth opening of the conical flask was covered with cotton wool wrapped in aluminum foil. It was sterilized in an autoclave at 121° C for 15 minutes. The petri dishes were washed with detergent and sterilized in a hot air oven at 160 °C for 1 h. The sterilized media was removed from the autoclave and allowed to cool; the plates were appropriately labeled (Cheesbrough, 2000).

Inoculation, Incubation and Gram Staining

A sample of 0.5 ml of the leachate was poured into the plate, and then the media was poured in each plate containing the water and soil sample for colony forming (Cheesbrough, 2000). The plates were incubated for 24 hours at 37˚C. A heat fixed bacterial smear was taken. The smear was flooded with crystal violet for 1 minute, and then washed with water (Primary Stain). The smear was flooded with Iodine for 1 minute, and then washed with water. The smear was flooded with Ethanol-Acetone, quickly, and then washed with water decolorizer. The smear was flooded with safranin for 1 minute, and then washed with water (Counterstain). The smear was blotted, air dry and observed under microscope (Cheesbrough, 2004).

Microscopic Examination

The smear was examined under microscope (desktop/optical microscope nikonalphaphot), violet or purple color indicates gram positive bacteria while pink or red color indicates gram negative bacteria. The bacteria displayed rod or bacilli, round or cocci and spiral shape (Cheesbrough, 2004).

MPN Determination

The MacConkey broth was prepared in single and double strength concentration. The double strength medium was dispensed in 5 tubes (10 ml in each tube) and another 5 tubes containing double strength medium (1 ml in each tube) , lastly 5 tubes containing single strength (0.1 ml in each) and then Durham tube was added in inverted position (Wehr and Frank, 2004).

Inoculation of the Sample

Using a sterile pipette 10 ml of water was added into 5 tubes containing 10 ml double strength medium. Similarly 1 ml of water was added to 5 tubes containing 9 ml double strength medium and 0.1 ml water to remaining 5 tubes containing 10 ml single strength. The tubes were washed with soap and rinsed with ionized water and later put them into oven for sterilization at 160˚C. The samples were diluted serially and inoculated in MacConkey broth, coliforms if present in water utilize the MacConkey broth present in the medium to produce acid and gas. The presence of acid was indicated by color changed of the medium and the presence of gas was detected as gas bubbles collected in the inverted Durham tube present in the medium. The number of total coliforms was determined by counting the number of tubes giving positive reaction that is both color change and gas production (Wehr and Frank, 2004). **Statistical Analysis**

Simple descriptive statistics means and standard deviations were used to analyse the raw data. Analysis of Variance (ANOVA)

was used to analyzed the difference between the means obtained and Fisher`s Least Significant Difference (LSD) at ($P<0.05$) for multiple comparison with the aid of DSAASTAT (Ver. 1. 101, 2014) excel add-in software. Index MPN Generator 1.44 software was used to obtained MPN value per 100 ml at lower and upper limit.

RESULTS AND DISCUSSION

Coliform bacteria are pollution indicators, the total coliforms and faecal coliforms are bio-indicator of underground water pollution. High level of bacteriological contamination of water may pose health problem. Table 1 in the month of August revealed that mean bacterial colony count for Babban-Tsangaya has the highest colony contamination in July and August for water and soil (6.14×10^4; 4.75×10^6) and (6.11×10^4; 4.92×10^6), respectively. Nayinawa has the highest contamination in March (3.89×10^3; 3.09×10^6) and April (3.56×10^3; 3.33×10^6). A wide array of small molecules of both synthetic and natural origin have been tested against ureases of different species of bacteria some revealed positive while others were negative (Natalia *et al*., 2023), this corroborates our work were Ureases were tested negative in Babban-Tsagaya and Nayinawa water and soil sample. Further analysis of the soil and water samples revealed also negative citrates (Hassaeinin *et al*., 2023) and oxidases from the Two sampling sites (Table 2). The bacteriological pollution, may not be limited to human sources (Chenghuan, 2023) and coming perhaps from the remains of dead bodies of animals or even a grave yard nearby. It was observed that the dumping of feces from the public disposal systems due to the lack of functional sewage systems in some parts of the two dumping sites of Nayinawa and Babban-Tsangaya. Furthermore, two study area revealed an unacceptable level of bacteria above the accepted limits (WHO, 2004; NSDWQ, 2007; FAO, 2006) as set out by standards of reputable bodies.

Table 1: Bacterial colony count for Nayinawa and Babban- Tsangaya (March- August, 2018)

Sites Sample	Numbers of Colony/Month (cfu /ml)			
	March	April	July	August
Nayinawa				
Water	$3.89\times10^3\pm 0.29$	$3.56\times10^3\pm 0.04$	$2.97\times10^3 \pm 0.14$	$2.98\times10^3 \pm 0.14$
Sediment	$3.09\times10^6 \pm 0.30$	$3.33\times10^6 \pm 0.10$	$4.12\times10^6 \pm 0.23$	$4.02\times10^6 \pm 0.14$
Babban- Tsangaya				
Water	$4.19\times10^4\pm0.5$	$3.30\times10^4 \pm 2.6$	$6.14\times10^4 \pm 1.54$	$6.11\times10^4 \pm 1.46$
Sediment	$3.69\times10^6 \pm 0.26$	$3.58\times10^6 \pm 0.38$	$4.75\times10^6 \pm 0.30$	$4.92\times10^6 \pm 0.52$

Table 2: Biochemical test for sample obtained from Nayinawa and Babban-Tsangaya (March – August, 2018)

		Biochemical test									
		Catalase		Coagulase		Urease		Citrate		Oxidase	
Sample	**Months**	B	N	B	N	B	N	B	N	B	N
Water	March	+	+	+	+	-	-	-	-	-	-
	April	+	+	+	+	-	-	-	-	-	-
	July	+	+	+	+	-	-	-	-	-	-
	August	+	+	+	+	-	-	-	-	-	-
Soil	March	+	+	+	+	-	-	-	-	-	-
	April	+	+	+	+	-	-	-	-	-	-
	July	+	+	+	+	-	-	-	-	-	-
	August	+	+	+	+	-	-	-	-	-	-

The biochemical test for the months and for water and soil sediment, the catalase, coagulase were positive while urease, citrate and oxidase were negative. The gram staining was negative throughout the month for both Nayinawa and BabbanTsangaya which indicates the presence of coliforms bacteria. The MPN values at upper limits were obtained from both Nayinawa and Babban-Tsangaya (Figure 1 and 2). In July and August the water sample from Nayinawa revealed higher MPN (387.6) and the lowest was recorded in April (7.3), from the soil sample July has the highest value (158.7) and the least was in April (18.8) (Table 3). Sample of water from Babban-Tsangaya has high MPN in July (387.6) and low at April (13.9), high MPN in soil sample was recorded in July (51.2) and low in March (5.6) (Table 4). The result revealed contamination in Nayinawa and Babban-Tsangaya which exceed the threshold for irrigation and human consumption (Irma *et al*. 2016; Badmus*et al*., 2014; Akinbile, 2006; SDWQ, 2007; FAO, 2006). The microbial analysis from Table 1 and Table 2, indicated very poor sanitation practice and poor human waste management system, which have damaging effects on the health of inhabitants within the vicinity of dump sites this corroborates with the work of Badmus et al. (2014). The presence of few fecal coliform bacteria indicate that water probably contains no disease causing organisms. However, the higher number of fecal coliform bacteria would indicate a very high probability that the water could contain disease producing organisms making the water unsafe for consumption as indicated by Wehr and Frank, in 2004. The occurrence of organisms *E. coli*, *Salmonella sp*. and *Shigella sp*. investigated in the study, the site Babban- Tsangaya shows *E. coli* to be much higher in soil sample than water sample (Figure 2), *Salmonella sp*. was only found in soil while *Shigella sp*. was found higher in water than soil sample in the month of March. In July (Figure 3) *E. coli* was found to be higher in water sample than soil sample, *Salmonella sp*. was found in soil sample only while *Shigella sp*. was higher in water sample than soil sample (Figure 2 and 3), for August *E.coli* was observed to be higher in water sample, this corroborates with the work of Carlos et al. (2023), that the bacteria (*E. coli*) can found themselves in the body of human through direct drinking and other domestic usage, however, soil sample recorded lower *E. coli* counts., *Salmonella* sp. was found in water sample only while *Shigella sp*. was found higher in soil than water it has an inhibition zone of 5 mm to 11mm (Jehan, 2023). From the result for April. *E. coli* was higher in soil than water, *Salmonella sp*. was absent in both soil and water samples while *Shigella sp*. was high in water sample than soil sample. Genarally, in Babban- Tsangaya *E.coli* was found to be much in water sample, salmonella in soil sample and *Shigellasp.* in water. The occurrence of *E. coli*, *Salmonella sp*. and *Shigella sp*. in Nayinawa shows *E. coli* to be much higher in water sample than soil sample, *Salmonella sp*. was not found in soil and water samples while *Shigella sp*. was found higher in soil than water samples in the month of March. In April *E. coli* was found to be higher in water sample than soil sample, *Salmonella sp*. was absent in both soil and water samples while *Shigella sp*. was higher in soil than in water sample. For July *E.coli* was observed to be higher in water sample than soil sample, *Salmonella sp*. was not in water and soil samples while *Shigella sp.* was found higher in soil than water sample. From the result for August, *E.coli* was higher in water than soil; *Salmonella sp*. was absent in both soil and water samples while *Shigella sp.* was found in soil sample only. In Nayinawa *E. coli* was found to be much higher in water sample, *Salmonella sp*. was not found in both soil and water sample and *Shigellasp.* in soil sample. The presence of very few fecal coliform bacteria would indicate that water probably contains no disease-causing organisms (Chenghuan, 2023), while the presence of large number of fecal coliform bacteria indicates a very high probability of the water contain disease causing bacteria which renders the water unsafe for consumption (Wehr and Frank, 2004).

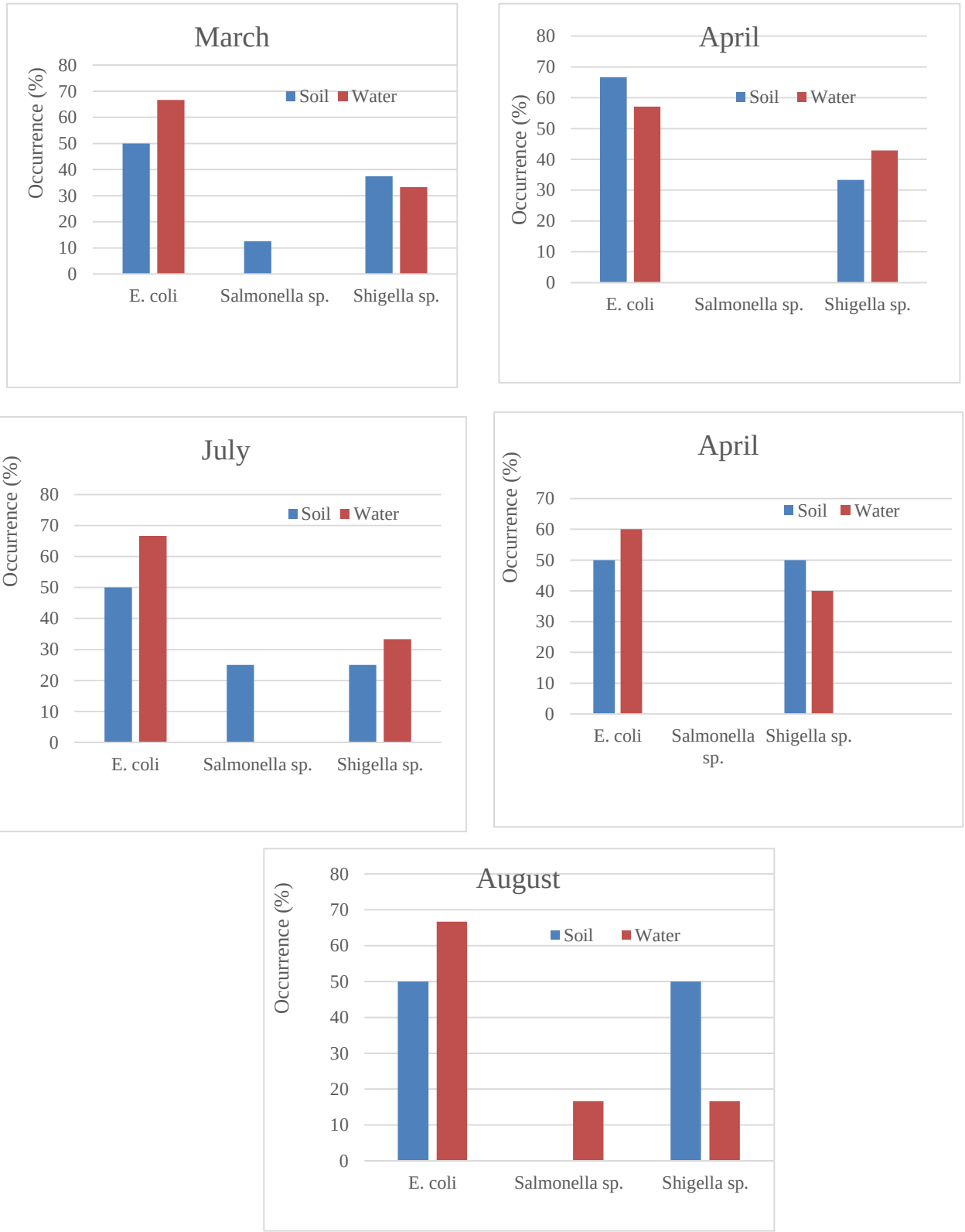

Figure 2: Percentage occurrence of some suspected organisms, Babban-Tsangaya, March, April, July and August, 2018 B= Babban-Tsangaya, N= Nayinawa.

Table 4: MPN Value of Babban-T sangaya from March - August, 2018

	Months											
Samples	**March MPN/ml**			**April MPN/ml**			**July MPN/ml**			**August MPN/ml**		
	(95%CL)	**L**	**U**	**(95%CL)**	**L**	**U**	**(95%CL)**	**L**	**U**	**(95%CL)**	**L**	**U**
BW1	17.8	10.8	29.4	45.3	31.5	65.6	>200.5	151.4	inf.	>200.5	>200	inf.
BW2	22.0	14.1	35.2	62.4	44.6	88.8	>200.5	167.9	inf.	>200.5	167.9	inf.
BW3	34.4	23.3	51.2	17.8	10.8	29.4	200.5	135.8	387.6	25.4	16.5	39.4
BW4	13.7	7.9	23.9	6.4	3.0	13.9	34.4	23.3	51.2	17.8	10.8	29.4
Mean	21.9	14.0	34.9	32	22.5	49.4	159	120	219.2	111	99	34.4
BS1	9.9	5.3	18.8	25.4	16.5	39.4	25.4	16.5	39.4	>200.5	>200	inf.
BS2	25.4	16.5	39.4	8.7	4.5	17.1	>200.5	>200	inf.	>200.5	>200	inf.
BS3	19.2	11.9	31.3	13.7	7.9	23.9	34.4	23.3	51.2	>200.5	>200	inf.
BS4	1.0	0.3	5.6	22.2	14.1	35.2	>200.5	>200	inf.	32.4	21.8	48.7
Standards												
WHO	1											
FAO	10											
NSDWQ	1											

Key: BW1-BW4= Babban-Tsangya Water Sample 1-4, BS1-BS4= Babban-Tsangaya soil sample 1-4, NW1-NW4= Nayinawa Water Sample 1-4, NS1-NS4= Nayinawa Soil Sample 1- 4.

Table 3: MPN Value of Nayinawa from March - August, 2018

Sample	Month											
	April			May			June			July		
	MPN ml (95%CL)	L	U	MPN ml (95%CL)	L	U	MPN ml (95%CL)	L	U	MPN ml (95%CL)	L	U
NWI	32.4	21.8	48.7	2.0	0.6	7.3	200.5	135.8	38.6	144.5	102.3	22.4
NW2	13.7	7.9	23.9	8.7	4.5	17.3	25.4	16.4	39.4	>200,5	135.8	387.6
NW3	6.4	3.0	13.9	13.7	7.9	23.9	>200.5	>200	Inf.	62.4	44.6	88.8
NS1	>200.5	>200	Inf.	9.9	5.3	18.8	109.1	78.6	158.7	>200.5	156.0	Inf.
NS2	34.4	23.3	51.2	13.7	7.9	23.9	>200.5	>200	Inf.	>200.5	>200	Inf.
NS3	25.4	16.5	39.4	19.2	11.9	31.3	25.4	16.5	39.4	>200.5	>200	Inf.
STANDARDS												
WHO	1											
FAO	10											
NSDQW	1											

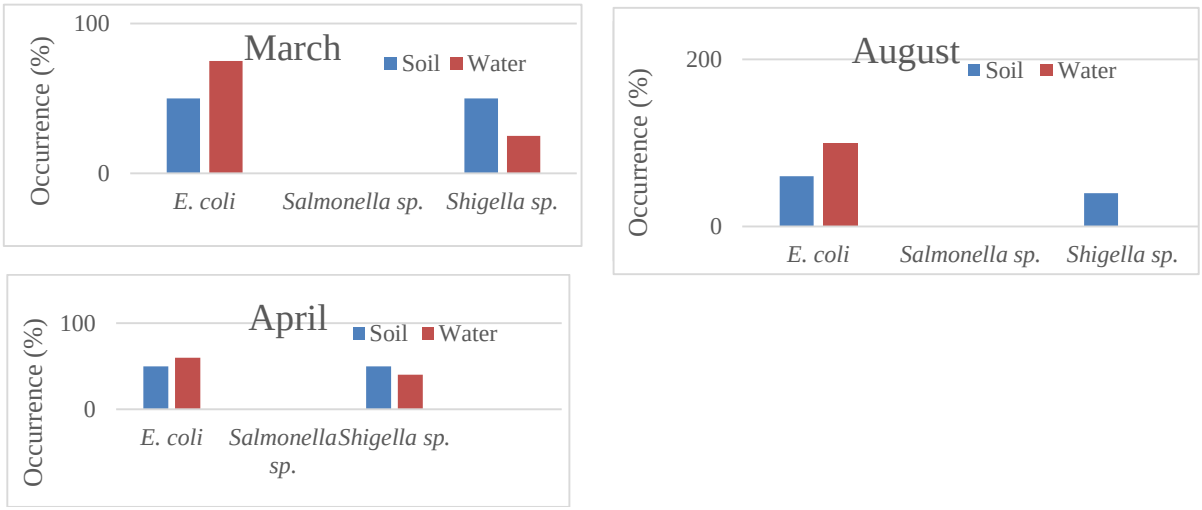

Figure 3: Percentage Occurrence of Some Suspected Organisms, Nayinnawa, March, April, July and August, 2018

CONCLUSION

The bacteriological analysis for water and soil sediment in both studies revealed that the waste water used to irrigate crop and domestic purposes by the settlement poses a serious health risk to the people around the area and immediate intervention from the government responsible agency for control of use should be monitored.

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