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INCIDENCE AND SURVIVAL OF LIPOLYTIC BACTERIA MONITORED FOR TWELVE MONTHS IN DOMESTIC WASTEWATER AND RECEIVING STREAM

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ABSTRACT

The incidence and survival of lipolytic organisms in domestic wastewater and receiving stream were monitored over 12 months. The average total bacterial count in the wastewater samples reduced in April and November by 24.2% and 41.6% respectively. There was also a reduction of 42.3% and 60.1% in the load in the receiving stream in August and July. Subsequently, at 5m downstream from the entry of the wastewater the microbial load reduced in March (19.2%) and June (19.2%). However, the occurrence of coliforms was more affected in the months of May (53%) to July (87.2%). At 5m and 10m downstream the coliform population reduced by 27.9% and 30.1% respectively. Of the twelve (12) bacterial isolates recovered at the exit of the wastewater into the receiving stream, only four (4) possessed lipolytic activity. These include the species of *Enterococcus*, *Klebsiella*, *Pseudomonas* and *Staphylococcus*. There was no significant difference in the amount of nutrients found in the domestic wastewater and receiving stream during the months. This paper also discusses the implication of disposing large amounts of wastewater effluents into the receiving water and the need to remedy and minimize the overall impact of such pollution on the environment.

Keywords: Lipolytic organisms, coliform, wastewater, receiving stream, environmental pollution

INTRODUCTION

The effluents generated from domestic and industrial activities constitute a great burden to wastewater management. The wastes can consequently lead to a point-source pollution which introduces a wide range of chemical pollutants and microbial contaminants to water sources and increases treatment cost considerably **(Eikelboom and Draaijer, 1999; Amir *et al.*, 2004; Akpor and Muchie, 2011)**. The quality of wastewater effluents determines the rate of degradation of the receiving water bodies, such as lakes, rivers and streams. The potential deleterious effects of polluted wastewater effluents on the quality of receiving water bodies are manifold and depend on the volume of the discharge, the chemical and microbiological concentration/composition of the effluents **(Odeyemi *et al.*, 2011)**.

Bacteria in water can originate from intestinal tracts of both humans and other warm-blooded animals such as pets, livestock and wildlife. Human sources include failing septic tanks, leaking sewer lines, wastewater treatment plants, combined sewer overflow (CSOs), boat discharges, swimming "accidents" and urban storm water runoff. In urban watersheds, faecal indicator bacteria are significantly correlated with human density **(Frenzel and Couvillion, 2002)**. Lands used for the breeding of domesticated animals such as cattle, hogs, or horses also serve as sources of bacterial contamination, particularly if the animals have assess to the water utilized for drinking or if heavy rains wash manure from the land into the receiving waters.

Microbial degradation of oil wastewater has become a concern in recent years. A variety of microorganisms such as bacteria, molds, and yeasts, have been shown to be capable of completely degrading oil wastewater either aerobically or anaerobically **(Masse *et al.*, 2001; Ettayebi *et al.*, 2003; Ammar *et al.*, 2005; Dhouib *et al.*, 2006)**. For instance, *Pseudomonas aeruginosa* LP_{602}, (a lipolytic strain isolated from restaurant wastewater) showed good potential for use in the treatment of wastewater which contained high lipid content **(Dharmsthiti and Kuhasuntisook, 1998)**. It was reported that waste decomposition by natural microorganisms already present in the wastewater did occur, but only at very low efficiency. To increase the efficiency of decomposition, some mineral salts required for the growth of the lipolytic organisms must be added **(Dharmsthitia and Mongkolthanaruka, 2002)**.

The number of bacterial colonies in a given environment can be influenced by weather and seasonal effects **(Jamieson *et al.*, 2002)**. This variability makes the bacterial concentrations in natural water difficult to predict at any one time. Bacteria populations often increase following a heavy rain storm, snow melt or other excessive runoff **(Borchardt and Statzner, 1990)**. This paper examines the incidence and survival of lipolytic bacteria in wastewater effluents and the effect of seasonal variation on microbial populations in the receiving stream.

MATERIAL AND METHODS

Source and collection of wastewater samples

The study site is a popular restaurant (Falegan) located within Ado-Ekiti, the capital of Ekiti State. There is a stream that flows beside the restaurant where the wastewater is usually discharged. The wastewater comprised mainly food remnants (rice, cowpea, root tuber products - pounded yam locally called *iyan* and *amala,* fermented stem products- *eba* and *fufu,* stew – mixed leafy vegetables, ground melon, palm oil in different proportions; **Aderiye and Laleye, 2003**) and soap solution (detergent/water).

The wastewater and water samples were obtained from the wash basin, and different sampling points along the waste drainage and the receiving stream. These samples were collected in sterile 250ml sampling bottles twice weekly for a period of one year (September to August). Also two hundred gram of soil sample was collected along the drainage using sterile spatula to scoop the soil into sterile polythene bags. All samples were labeled and transported on ice to the Microbiology Laboratory of Ekiti State University, Ado-Ekiti for analyses.

Bacteriological analysis

One milliliter each of domestic wastewater, stream and soil solution was inoculated on Plate count and MacConkey agar media and incubated at 37°C for 24h. Pure colonies of bacterial isolates were later inoculated on sterile Tributyrin agar (TBA) and incubated at 37°C for 24h. The zone of clearance around distinct microbial colonies indicated the lipolytic activity of the bacteria. The isolates were identified on the basis of their biochemical, physiological and morphological characteristics as described by **Olutiola *et al*.** (**1991**) and **Cheesebrough** (**2006**) and matched against standard microbial cultures to the genera level as in previous work **(Odeyemi *et al.*, 2013)**.

Physicochemical analyses

The temperature of the water and wastewater samples was taken at the site of collection using temperature meters (Wagtech International, UK), electrical conductivity was measured with a CDM 83 conductivity meter (Radio Meter A/S Copenhagen, Denmark) while the pH was determined using Water Proof Scan 3+ Double Junction (Wagtech International, UK) and HI 98311-HI 98312 (Hanna) Water Proof EC/TDS. Other physicochemical characteristics determined were the total dissolved solid and total suspended solid using the gravimetric method while the acidity, alkalinity and sulphate were determined by titrimetry. Both nitrate and phosphate contents were determined colorimetrically using a Spectronic -20 photometer (Gallenkamp, UK) **(AOAC, 2005)**. While the concentrations of Pb^{2+}, Zn^{2+}, Fe^{3+}, Mg^{2+}, Mn^{2+} and Ca^{2+} were determined with an Atomic Absorption Spectrophotometer (AAS) (model Perkin Elmer 2380), Na^{+} and K^{+} contents were determined using a Flame photometer **(AOAC, 2005)**. Also the speed of the receiving stream was measured using the Moving Boat Method (Chow *et al*., 1988), while the amount of rainfall (precipitation) was determined according to the WMO guide to Meteorological Instruments and Methods of Observation (WMO-8, 2008).

Determination of metal concentration in soil samples

One gram of soil sample was weighed into 250 ml beaker and digested with a mixture of HNO_3 and HCl (3:1 v/v) on the hot plate until a clear solution was obtained and cooled **(Kisku *et al*., 2000)**. It was then filtered through a Whatman No. 40 filter paper into a 100ml volumetric flask. The residue was washed with warm deionised water and the solution made to mark with distilled water, and transferred into 100ml clean plastic container for the metal analysis using UNICAM 969 Atomic Absorption Spectrophotometer (AAS) **(AOAC, 2005)**.

RESULTS AND DISCUSSION

The speed of the stream flowing beside the restaurant was found to be 0.04m/s at the upper stream and 0.03m/s downstream during the raining season but with a lesser speed during dry season. The estimation of precipitation of rain and microbiological analyses of the receiving stream and the wastewater drainage was carried out twice weekly for one year. There were no precipitations from January to early April (0.00mm) while the amount of rainfall increased from 4.00mm mid-April to 10.63mm in July (Tables 1). There was very low precipitation in the month of August and later heavy rains were recorded between September and mid-November (23.6mm-19.22mm respectively). This trend actually confirms the seasonal period in Nigeria (i.e. harmattan (or dry) and rainy seasons) **(Rowell *et al*., 1995)**.

The initial total bacterial count (TBC) ranged from 0.26 CFU x 10^5/ml in September and increased to 8.48 CFU x 10^5/ml in August (Table 2). Meanwhile the bacterial population decreased in April and November (24.2% and 41.6% respectively), in July (60.1%), and downstream where the wastewater is emptied into the stream. There was also a reduction in the microbial load at 5m downstream after the entry of the wastewater (ranging between 19.2% in March and 68.9% in June).

The coliform population was more affected during the months of May (53.0%) to July (87.2%) (Table 2). Similarly, the coliforms reduced downstream at 5m and 10m (27.9% and 30.1% respectively). The reduction in the total number of bacterial colonies may have been influenced by the seasonal changes, water flow and the volume of receiving stream **(George *et al.,* 1995)**.

The antibacterial activity of long-chain unsaturated fatty acids has been well known for many years **(Hou, 1995)**. Fatty acids function as the key ingredients of antimicrobial food additives which inhibit the growth of unwanted microorganisms **(Chang *et al*., 2005)**. Linoleic and oleic acids are major antibacterial components found in melon, *Citrullus vulgaris* and 'iru', *Parkia biglobosa* respectively **(Paster *et al*., 1990; Karaman *et al*., 2003)**. These fermented legumes are commonly used as condiments in soup preparation in most restaurants and homes in West Africa **(Aderiye and Laleye, 2003)**. Besides the normal fatty acids, fatty acid derivatives show potential antimicrobial activities that exist in nature. These are mainly found in microorganisms, algae, or plants, which may mediate chemical defense against microorganisms **(Pfefferle *et al*., 1996)**. Additionally, long-chain unsaturated fatty acids are bactericidal to some important pathogenic microorganisms, including the Methicillin-resistant *Staphylococcus aureus* **(Farrington *et al*., 1992)**.

The reduction in microbial population along the drainage and in the receiving stream may therefore be due in part, to the antimicrobial effect of some of these fatty acids obtained from the biodegraded dietary oil discharged from the restaurant. The antibacterial activity of these acids are usually attributed to long-chain unsaturated fatty acids such as oleic acid, linoleic acid, and linolenic acid, while the long-chain saturated fatty acids, including palmitic acid and stearic acid, are less active **(Sun *et al*., 2003; Seidel and Taylor, 2004)**.

Interestingly, the average numbers of consumers patronizing the restaurant per day ranged between 30 in May and 74 in September. Meanwhile higher microbial counts (TBC: 60.1%; TCC: 74.4%) were recorded in May whereas in September with higher average number of consumers, lower microbial counts were obtained (TBC: 54.0%; TCC: 38.3%). There is no correlation between the number of consumers that patronized the restaurant and the incidence/presence of total bacteria and coliforms in the wastewater but may be ascribed to the amount of rainfall in each month.

The physicochemical property of the wastewater at the onset of rain (March) and during intense rainfall (July) was determined (Figs. 1 and 2). There was no significant difference in the amount of nutrients recorded during the months.

Table 1 Average amount of rainfall (mm) and number of consumers

	Months											
	January	February	March	April	May	June	July	August	September	October	November	December
*Ave amt. of rainfall (mm)	0.00	0.00	0.00	4.00	10.79	10.10	10.63	9.24	23.60	19.78	19.22	0.00
**Ave no. of consumers	36.5	44.0	51.0	39.0	30.0	34.5	41.5	46.0	73.5	45.5	46.5	50.0

*Average readings for the week were estimated every Friday.
**Average number of consumers per day.

Table 2 Average microbial counts (CFU x10^5/ml) of wastewater in drainage and water samples of adjoining stream

Sampling point	Months											
	January		February		March		April		May		June	
	TBC	TCC	TBC	TCC	TBC	TCC	TBC	TCC	TBC	TCC	TBC	TCC
U_1	7.25	0.78	5.23	1.00	7.60	0.53	11.5	2.53	3.63	0.53	1.83	ND
U_2	2.58	0.78	3.40	1.06	5.20	0.75	5.75	0.90	1.80	0.25	0.78	0.28
D_1	103	32.1	97.8	33.6	87.0	38.3	103	70.4	100	68.5	105	73.7
D_2	95.3	21.8	52.3	25.1	98.4	42.9	97.6	53.9	91.8	56.3	95.7	57.9
D_3	74.4	19.7	87.8	22.8	84.3	31.5	88.4	40.6	64.9	30.2	88.2	43.4
S_1	39.3	14.5	45.3	10.9	68.1	27.9	67.0	19.1	25.9	7.63	27.4	8.48
$(D_3\text{-}S_1/ D_3)\%$	47.2	26.4	48.4	52.2	19.2	11.4	24.2	53.0	60.1	74.7	68.9	80.5
S_2	27.2	6.28	43.1	12.3	55.2	21.8	52.9	17.6	10.6	2.88	18.2	5.70
Sampling point	**July**		**August**		**September**		**October**		**November**		**December**	
	TBC	TCC	TBC	TCC	TBC	TCC	TBC	TCC	TBC	TCC	TBC	TCC
U_1	5.48	0.50	8.48	0.78	0.26	ND	0.89	ND	4.08	0.03	5.58	0.78
U_2	3.35	ND	4.98	0.53	0.26	0.5	2.53	0.75	1.58	0.28	1.33	ND
D_1	94.5	50.8	101	54.6	35.3	17.5	53.2	16.0	63.3	14.8	88.1	38.1
D_2	99.0	40.4	107	57.3	28.9	12.7	41.7	14.4	68.8	19.8	92.3	27.4
D_3	66.6	32.4	86.5	47.8	21.4	6.95	30.9	10.8	34.4	7.75	64.1	14.7
S_1	26.6	4.15	49.9	16.1	9.85	4.29	20.7	6.86	20.1	5.00	30.3	6.45
$(D_3\text{-}S_1/ D_3)\%$	60.1	87.2	42.3	66.3	54.0	38.3	33.0	36.5	41.6	35.5	52.7	56.1
S_2	14.9	2.05	28.0	9.25	13.0	1.93	16.7	5.64	18.8	5.10	23.3	6.48

Key: D_1- Basin outlet exit; D_2- 5m to exit; D_3- 10m from exit of wastewater in to stream, S_1- 5m downstream from exit of wastewater into stream; S_2- 10m downstream from exit of wastewater into stream, U_1- 5m upper stream from exit of wastewater into stream; U_2- 10m upper stream from exit of wastewater into stream, (D3-S1/D3)%- Percentage average of microbes into the receiving stream

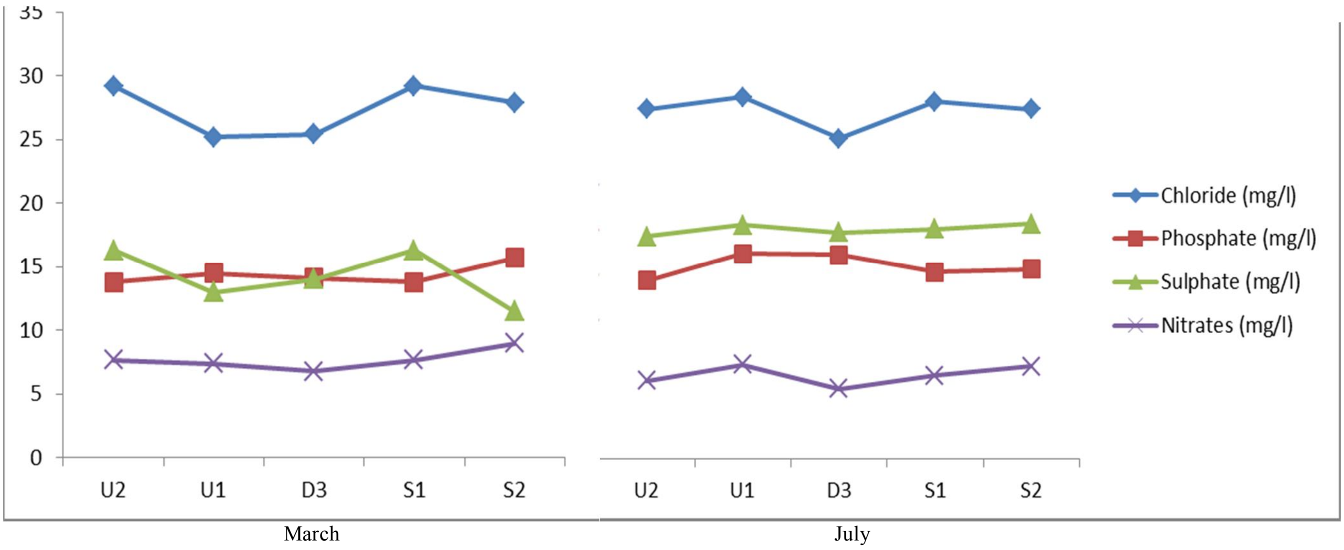

Figure 1 Chloride, nitrate, phosphate and sulphate contents of wastewater in March and July

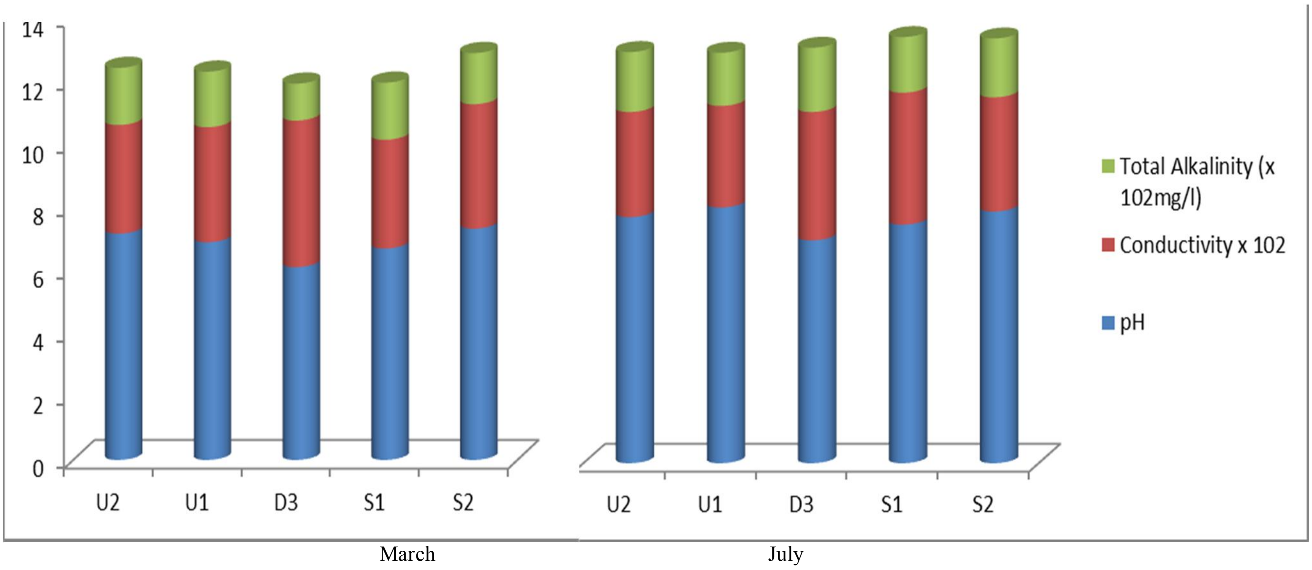

Figure 2 Conductivity, pH and total alkalinity of wastewater in March and July

The average number of bacterial isolates and probable lipolytic strains obtained from the wastewater and receiving stream are shown in Table 3. The coliforms constituted about 12.03% and 18.21% of the total average bacterial population upstream (U_2 and U_1 respectively), while the percentage coliforms population decreased from 49.4, 44.4 to 39.2% in the drainage (D_1, D_2 and D_3 respectively); a further reduction of the load to 30.4% and 30.15% was recorded at 5m and 10m from the exit of the wastewater downstream.

Most intestinal bacteria that contaminate environmental waters were not detected as they are not able to survive and multiply in this environment **(Ferreira, 2005)**. Survival rates vary widely among faecal bacteria introduced into environmental waters. The ability of faecal bacteria to survive in environmental waters generally increases as the temperature decreases. Other factors that influence survival include dissolved organic carbon concentration, sunlight intensity (which were not determined) and the ability to enter the viable but non-culturable state **(Medema *et al*., 2003)**.

Of the twelve (12) bacterial isolates that were recovered at the point of entry of the wastewater into the receiving stream, only four (4) were possessed lipolytic activity (growth on Tributyrin agar). These include the species of *Enterococcus*, *Klebsiella*, *Pseudomonas* and *Staphylococcus*. More bacterial isolates were recovered/isolated at 5m downstream, following the deposition of the wastewater, where only *Staphylococcus* and *Enterococcus* spp. were prevalent.

Similarly, at about 10m downstream, the species of *Pseudomonas, Serratia* and *Staphylococcus* were isolated (Table 3). The presence of *Serratia* spp. may have been from the upper segment of the stream while *Pseudomonas* and *Staphylococcus* spp. were earlier associated with the wastewater. *Raoultella planticola*, *R. terrigena, Enterobacter amnigenus* and *Kluyvera intermedia* (*Enterobacter intermedius*), *Serratia fonticola*, and the genera *Budvicia*, *Buttiauxella*, *Leclercia*, *Rahnella*, *Yersinia*, and most species of *Erwinia* and *Pantoea* have been reported to live in fresh waters, on plants and small animals **(Leclerc *et al*., 2001)**. Contaminants commonly found in storm water runoffs include faecal and pathogenic bacteria whereas storm water transports pollutants to water bodies such as streams and lakes **(EPA, 2009)**.

The average number of bacteria and the likely lipolytic strains obtained from the soil samples along the drainage are shown in Table 4. There was reduction in the bacterial counts from the point of exit of the drained pipe (2.63×10^8 CFU/ml) to the point of entry of wastewater to the receiving stream (1.38×10^8CFU/ml). The percentage population of coliforms of the soil samples ranged between 0% and 5.94%. Of the bacterial isolates recovered from the soil samples, 31.8% were lipolytic in their activities.

Interestingly, the extracted oil from the soil samples along the drainage (quantity ranged A>B>C>D>E) revealed the activity of the lipolytic isolates and the reason (microbial utilization) for the drastic reduction/absence of oil from the downstream. The colour of the soil samples obtained at 2m (Wet- Mars black) and 4m (Dry- Vandbrown) in the drains indicated that there was higher microbial activity among the bacterial isolates. Microbiological activity has been defined by **Nannipieri *et al*. (1990)** to include all the metabolic reactions and interactions carried out by the microflora and microfauna found in soil. High microbial activity in the soil therefore indicates high oxidative metabolism in soils **(Xiao *et al*., 2008)**, because, being exclusively intracellular, it is linked to viable cells of lipolytic organisms. The various colour appearances might be due to low oxygen content that may permit mostly the activity of coliform as denitrifier **(Madigan *et al*., 2000)**.

Odeyemi *et al*. (2011) reported that in Nigeria wastewater was usually collected in open landfills or allowed to drain into open land and uncovered drainages. A specific example of what happens is the logging of the contaminated water in the soil. In such situations, oxygen becomes less available as an electron acceptor, prompting denitrifying bacteria to reduce available nitrate into gaseous nitrogen (perceived by foul odour generated from the soil sample) which enters the atmosphere with resultant negative effects **(Madigan *et al*., 2000)**.

Table 3 Average number of bacteria isolates and probable lipolytic strains from the wastewater and receiving stream

Sampling Site	Average microbial load CFU x 10^5/ml			No. of isolates	Number of isolates growing on TBA	Strain (s) identified
	Total Bacteria	Total Coliform	Percentage of coliform			
U_2	5.15	0.62	12.03%	6	1	*Serratia* sp.
U_1	2.80	0.51	18.21%	3	0	-
D_1	85.9	42.4	49.4%	32	7	*E.coli*
						Pseudomonas spp.
						Staphylococcus spp.
D_2	80.7	35.8	44.4%	21	6	*Enterococcus* sp.
						E.coli
						Pseudomonas sp.
						Klebsiella sp.
						Staphylococcus spp.
D_3	66.0	25.7	39.2%	12	4	*Pseudomonas* sp.
						Enterococcus sp.
						Klebsiella sp.
						Staphylococcus sp.
S_1	35.9	10.9	30.4%	15	2	*Staphylococcus* sp.
						Enterococcus sp.
S_2	26.8	8.08	30.15%	12	3	*Pseudomonas* sp.
						Serratia sp.
						Staphylococcus sp.

TBA: Tributyrin Agar; U_1, U_2, D_1-D_3, S_1 and S_2: as in Table 2

Table 4 Average number of bacteria isolates, Optical density (470nm) and Colour appearance of soil samples along the drainage

Soil samples	Bacterial counts (x 10^6 CFU/ml)			No. of isolates	Number of isolates growing on TBA	Optical density		*Colour appearance	
	Total Bacteria	Total Count	Percentage of coliform			Wet	Dry	Wet	Dry
A	263	2	0.76%	7	4	0.14	0.21	Peryl violet	Raw Umber
B	219	13	5.94%	5	0	0.22	0.22	Mars black	Vand brown
C	220	9	4.09%	4	1	0.17	0.26	Mars black	Vand brown
D	150	ND	0%	3	2	0.20	0.25	Vandbrown	Vand brown
E	138	ND	0%	3	0	0.44	0.11	Vandbrown	Capmorviolet

Key: A- Soil sample collected at the exit of drain pipe; B- Soil sample obtained 2m from the exit of drain pipe;
C- Soil sample obtained 4m from the exit of drain pipe; D- Soil sample obtained 6m from the exit of drain pipe;
E- Soil sample obtained from the point of exit into the receiving stream.

Table 5 Mineral components of wastewater from the wash hand basin, drainage and exit point into the receiving stream

Samples	Mineral (mg/l)							
	K	Na	Mg	Mn	Pb	Zn	Fe	Ca
Exit of Basin	44.1	26.2	39.4	0.17	ND	0.13	16.6	11.6
Mid-drainage	19.3	15.8	22.7	0.11	ND	0.06	18.9	12.5
Exit point to stream	10.6	23.6	19.3	0.10	ND	0.10	20.5	11.4

Water pollutants mainly consist of heavy metals, microorganisms, fertilizer and thousands of toxic organic compounds **(APHA, 1998)**. Heavy metals which consist of Cd, Cr, Cu, Pb, Ni, Fe, Mn, Hg, Zn, Al, Se as well as metals of group III and IV, have toxic effect on microbial physiology **(WHO, 1999; Bruins *et al*., 2000)**. In addition to carbon, nitrogen, and phosphorus, microorganisms also need trace amounts of iron, sulfur, potassium, magnesium, manganese and other substances as nutrients for bacterial growth **(Doelman *et al*., 1994)**. Generally the concentration of all the mineral nutrients except Na and Fe reduced as the wastewater flowed out from the wash basin through the drains to the receiving stream (Table 5). The wastewater contained high potassium (44mg/ml), magnesium (39.4mg/ml) and sodium (26.2mg/ml). At the exit point of the wastewater into the receiving stream, the concentrations of Mg and K had reduced drastically by 51% and 76% respectively. The reduction in the concentration of these metallic ions and other nutrients along the drainage might be due to microbial utilization and leaching away of the minerals into the soil. Introduction of metals in various forms into the environment can produce numerous modifications of microbial populations and communities and affect their activities **(Hiroki, 1994)**.
Few traces of Mn (0.17mg/ml) and Zn (0.13mg/ml) were detected in the wastewater samples while Pb was not detected. At relatively low concentrations, some heavy metal ions (e.g. Co^{2+}, Cu^{2+}, Zn^{2+} and Ni^{2+}) are essential for microorganisms since they provide vital co-factors for metallo-proteins and enzymes for various activities **(Doelman *et al*., 1994; Bruins *et al*., 2000)**.

CONCLUSION

Conclusively, in the polluted soil along the drainage and the receiving stream, the occurrence of pollutants (detergent and oil) has greatly led to the reduction of the microbial population. This study reveals the deletritious effects the activities in restraurants may have on the environment. Therefore, conscious and concerted efforts should be employed to minimize/prevent the indiscriminate disposal of this untreated wastewater from the restaurant to reduce the rate of environmental pollution.

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