Influence of Anthropogenic Activities on the Physico-chemical Characteristics of Open Drainage Channels in Port Harcourt

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Authors’ contributions

This work was carried out in collaboration between all authors. Author DNO designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors JOO and LBK managed the analyses of the study. Author LBK managed the literature searches. All authors read and approved the final manuscript.

ABSTRACT

Wastewater and sediment samples from selected locations of the Ntanwogba creek in the metropolitan city of Port Harcourt were collected. Physicochemical parameters were determined using standard analytical procedures. The results of the physicochemical analysis of the wastewater at different locations in this study was reported to have fallen short of the recommended limits in terms of the turbidity, BOD₅, nitrate, pH, TDS, temperature, DO, COD and chloride concentrations. The pH of the wastewater effluent samples ranged from 6.4–7.4, while sediments ranged from 5.3–5.7, the temperature regime for the samples at all study sites ranged 26.6-29.5°C. Similarly, TDS and EC levels from wastewater samples ranged from 375–449 mg/l and 501-812.6 µS/cm, respectively. BOD₅ ranges from 33.6 to 201.6 mg/l while chloride concentrations ranged from 34.30-61.06 mg/l. Similar trends were observed for sediments in all stations. The
physicochemical characteristics from both sources were statistically significantly different at \( p < 0.05 \), and these values were not within the recommended limits for such effluents throughout the sampling period. The conclusion is that wastewater with a high domestic load has the highest negative impact on water quality in a river which suggests that the water resources has lost it potability. This calls for urgent attention for responsible agencies to ensure proper implementation of waste management policies to sanitize our environment. This is because failure to enforce the regulations might pose severe threat to receiving water bodies/resources.

Keywords: Anthropogenic activities; wastewater; sediments; Ntanwogba creek; physico-chemical characteristics.

1. INTRODUCTION

Poor drainage systems in Port Harcourt metropolis has been associated with lack of maintenance or reinforcement of drainage systems which eventually leads to environmental hazards. Sometimes the drains become flooded, make the roads practically impassable for motorists. In many instances, torrential rainfalls literally submerged the cities, halting human and vehicular activities thereby forcing residents to stay indoors as a pre-emptive measure against human disaster. Also, lack of adequate waste collection, disposal and waste management systems cause blockages of drains. Increased population, human activities and inflow materials into the area results in the generation of larger volumes of wastes, coupled with irrepresible location of physical development of infrastructures such as offices, facilities, markets and residential structures which are located and built along natural erosion routes and drainage channels.

Drainages that were constructed a long time ago lacked proper maintenance, as debris and waste materials are indiscriminately dumped into the drains thereby inhibiting flow of water into the drains. This indiscriminate attitude occurred majorly by road users who drop waste materials into the drains. Much of these wastes are in solid and liquid forms consisting of domestic organic and inorganic wastes, spent oil or lubricants (crank case oil), agricultural pesticides and fertilizers, water from flash floods (storm water), runoff from rain water, running through cracks in the ground and into gutters), water from swimming pools, water from car garages and cleaning centres. Many people also dump their garbage into gutters, streams, lakes, rivers, and seas, thus making water bodies the final resting place of cans, bottles, plastics, and other household products. In areas where drainages and sanitation are poor, such waters run over the ground during rain storms and pick up faeces and contaminates water resources. The widespread use of such wastewater containing toxic substances contaminated by pathogenic microorganisms may likely cause increase in the incidence of wastewater borne disease which is the most common health hazards associated with untreated drinking and recreational waters. Also, their presence in wastewater can result to breakdown of organic solids which may consume much of the dissolved oxygen in the receiving water bodies [1].

After each occurrence of flooding and storm, wastes are dumped in ditches and drainage channels. Theses drainage channels remains unattended for too long and thereby get clogged. This causes blockage of channels for the subsequent runoffs and other contents. Also, as this blockage exists, the road pavement attached to these drains is also under threat. Water builds up on the pavement (flood) thereby causing a wear and tear, with washing of bitumen and other road components into drains thereby causing further damage and leading to drain failures. Sediments constitute varying physicochemical characteristics, in content and type of organic matter, particle size distribution, and pH [2] Contaminated sediment is a significant environmental problem affecting many marine, estuarine and freshwater environments throughout the world and serve as both a reservoir for contaminants and a source of contaminants to the water column and organisms that live there. Sediment contaminants in addition to water column contamination affect bottom-dwelling organisms and other sediment associated organisms, as well as both the organisms that feed on them and humans. Sediments are an integral part of the aquatic environment that provide habitat, feeding, spawning, and rearing areas for many aquatic organisms [3]. Sediment is an important compartment in the marine ecosystem. Anthropogenic compounds enter the aquatic environment through various activities in the environment such water runoff, erosion, flooding, dumping of wastes into drains which eventually
causes blockage and this results in build up of contaminants that eventually defaces the environment. Depending on their physical and chemical properties some substances remain dissolved in the water phase while others bind onto particles, sink to the ground and become part of the sediment. In this way, an accumulation of many hydrophobic (and in general strongly adsorbing compounds) takes place. Therefore sediments are assumed to represent a sink for special kinds of pollutants due to re-suspension processes because the compounds can be remobilized again, and act as a source for contaminants within sediments of ecosystems [1]. Sediment and wastewater quality assessments are useful in determining sediment quality in receiving streams of whole effluents, previously impacted sites, and other contaminated areas. Most contaminants of concern are chemically and biologically reactive and rapidly become associated with particles in freshwater systems. Consequently, uptake or sorption onto particles is the primary mechanism for removing chemically reactive contaminants from the water column, and sedimentation is the principal mechanism for the accumulation of these contaminants in off-site areas over long time periods. Therefore this study was undertaken to determine the physico-chemical characteristics of wastewater and sediments from the open drainage system to evaluate the effect of human activities and its effect on water sources and the environment because of the beehive of socioeconomic activities along the creeks.

Plate 1. Status of open drainage systems in Port Harcourt metropolis showing indiscriminate dumping of wastes materials causing blockage of drains

Plate 2. Ntanwogba creek showing discharge of wastewater into the drains

Plate 3. Blockage of water channel with assorted (especially Xenobiotic) waste products associated with anthropogenic activities around the creek in Port Harcourt
2. MATERIALS AND METHODS

2.1 Area of Study

The Ntanwogba Creek is located on the western flank of Port Harcourt city of Rivers State, Nigeria. The stream lies between latitude 4° 50' and 5° 00'N and longitude 6° 55'N and 7° 00'E. The climate of the area is that of tropical equatorial latitude with rainfall occurring almost all year round [4]. The Ntanwogba creek is a black water stream with its water source running through Orazi forest of Rumueme town across Abacha Road, Cherubim Road, Olu-Obasanjo Road, Okija Road and Afam Street (D/line), and meanders through the densely populated city of Port Harcourt into the Upper Bonny Estuary. Five sampling sites were studied. Sampling was done 500 m apart along the stream (Fig. 1).

2.2 Sediment Samples

Sediment samples were collected using a grab sampler. The grab sampler was thoroughly rinsed with wastewater along the same water course to remove any visible sediment before and after use. At each sampling point, the sampler was lowered to the water bed and the topmost layer of the sediment heaved out. The sediment sample was scooped from the grab’s cup and transferred into sterile sample container. The sample was labeled and then transported to the laboratory in a cooler packed with ice blocks for analysis.

Fig. 1. Map of Port Harcourt showing sampling stations along the Ntanwogba creek
(Source: Rivers State Ministry of Lands and Survey, Nigeria)
2.3 Waste Water Samples

Waste water samples were collected using the methods of Adesemoye et al. [5]. Sterile 2.0 litre sample containers were used aseptically to collect the waste water. The samples were collected at five different stations coded P1, P2, P3, P4 and P5 as the wastewater was running along the drainage system. To collect the wastewater, base of the sterilized sample container was held with one hand, plunged about 30 cm below the water surface with the mouth of the sample container positioned in an opposite direction to water flow (7). About 500ml of the sample collected from each station were pooled together to get a composite sample. After collection, the samples were placed in a cooler containing ice blocks and transported immediately to the laboratory for analysis.

2.4 Preparation of Samples

The sediment and wastewater samples were processed using the method of [5]. Ten grams of the sediment and 10 ml wastewater samples were weighed and added to 90ml of sterile distilled water to get an aliquot. One milliliter of the aliquots, waste water samples were then serially diluted using the ten-fold serial dilution method as described by [6].

2.5 Analysis of Wastewater Samples for Physicochemical Characteristics

Samples were analyzed for the following physico-chemical parameters: pH, temperature, turbidity, total suspended solid, total dissolved solid, biochemical oxygen demand (BOD), Chemical Oxygen demand (COD) and conductivity according to methods used by Ristola et al. [2]. The pH value of the samples were determined with a pH meter (Unicam 9450, Orion model No. 91-02). Temperature was measured with mercury thermometer immediately after sample collection. Turbidity was determined with Milton Roy (USA) Spectronic 20D meter. Gravimetric method involving filtration and evaporation were used to measure total suspended solids and total dissolved solids. The measurement of BOD and COD were followed using standard methods (7). Wastewater sample was drawn into a 250 ml bottle, incubated in the dark for five days at 20°C and at the end of five days, the final dissolved oxygen (DO) content was determined. Decrease in DO between the final DO reading and the initial DO reading was corrected for sample dilution and recorded as the BOD of the sample. The COD was estimated by determining equivalent amount of oxygen required to oxidize organic matter in the samples. Conductivity was determined using a conductivity meter (Metrohm 640, Switzerland).

Statistical analysis was carried out on the data obtained during the study using a computer based program SPSS version 20 for Analysis of Variance (ANOVA) across the five sampling stations.

3. RESULTS

3.1 Physicochemical Characteristics

The results of the physicochemical characteristics of the various sampling points are shown in Tables 1 and 2 for wastewater and sediment samples respectively. For the wastewater samples, the pH of the effluent samples ranged 6.4–7.4, while it ranged from 5.3–5.7 for sediments. The pH values from both sources were statistically significantly different at p < 0.05, and these values were not within the recommended limits for such effluents throughout the sampling period. The values recorded from the sampling sites shows that pH was slightly acidic in stations P1, P3 and P5. The temperature regime for the samples at all study sites ranged 26.6-29.5°C. The temperature profiles were also statistically significant at p < 0.05. The significant difference for these results were between stations for wastewater and sediment.

Similarly, TDS and EC levels from wastewater samples ranged as 375–449 mg/l and 501-812.6 μS/cm, respectively. The TDS concentrations from the wastewater samples were significant at p < 0.05, the turbidity profile varied significantly (p < 0.05) at all sites, and ranged from 2.35 to 6.39 NTU. The dissolved oxygen in the wastewater effluents range was 1.6 to 3.46 mg/l from all stations. The statistical significance of the DO levels in the effluents was recorded. The determination of BOD5 showed ranges from 33.6 to 201.6 mg/l. The BOD5 measurements did vary significantly (p < 0.05). The chloride concentrations of the effluents varied widely, and ranged 34.30-61.06 mg/l, the concentrations were statistically significant, similar trend was observed for salinity with 0.26 to 0.37 mg/l in the wastewater samples (Table 1). Similar trends were observed for sediments in Table 2. Total Hydrocarbon (THC) content showed remarkable difference statistically with values ranging from

Statistical analysis was carried out on the data obtained during the study using a computer based program SPSS version 20 for Analysis of Variance (ANOVA) across the five sampling stations.
Table 1. Physicochemical parameters of wastewater samples

| Stations | pH     | Temperature (°C) | Conductivity (µS/cm) | Salinity (mg/l) | Turbidity (NTU) | Total Dissolved Solid (TDS) (mg/l) | Dissolved Oxygen (DO) (mg/l) | Biological Oxygen Demand (BOD) (mg/l) | Chemical Oxygen Demand (COD) (mg/l) | Chloride (mg/l) |
|----------|--------|------------------|----------------------|-----------------|-----------------|-----------------------------------|-------------------------------|--------------------------------------|--------------------------------------|----------------|
| P1       | 6.40±0.18<sup>a</sup> | 29.36±0.29<sup>b</sup> | 677.33±57.74<sup>b</sup> | 0.26±0.10<sup>a</sup> | 4.13±0.10<sup>a</sup> | 449.00±61.51<sup>ab</sup> | 1.46±0.153<sup>a</sup> | 201.6±2.57<sup>a</sup> | 214.63±0.1<sup>a</sup> | 51.33±2.60<sup>a</sup> |
| P2       | 7.26±0.21<sup>b</sup> | 29.33±0.21<sup>b</sup> | 812.6±13.10<sup>b</sup> | 0.35±0.04<sup>a</sup> | 4.9±0.70<sup>c</sup> | 543.00±17.44<sup>b</sup> | 1.33±0.12<sup>a</sup> | 180.8±9.0<sup>c</sup> | 106.79±1.0<sup>d</sup> | 61.06±0.71<sup>d</sup> |
| P3       | 6.8±0.52<sup>b</sup> | 29.5±0.06<sup>b</sup> | 646.33±58.70<sup>b</sup> | 0.37±0.03<sup>b</sup> | 6.39±0.32<sup>d</sup> | 375.00±105.7<sup>a</sup> | 1.62±0.100<sup>a</sup> | 113.6±0.153<sup>b</sup> | 214.21±0.1<sup>a</sup> | 50.00±0.557<sup>a</sup> |
| P4       | 7.4±0.100<sup>c</sup> | 26.6±2.10<sup>a</sup> | 501.00±5.30<sup>a</sup> | 0.28±0.044<sup>a</sup> | 2.35±0.040<sup>a</sup> | 397.6±43.09<sup>ab</sup> | 3.46±0.551<sup>b</sup> | 33.6±0.38<sup>b</sup> | 212.57±0.1<sup>a</sup> | 46.5±0.306<sup>c</sup> |
| P5       | 6.6±0.2<sup>d</sup> | 29.40±0.44<sup>d</sup> | 670.6±51.7<sup>c</sup> | 0.27±0.021<sup>c</sup> | 3.33±0.50<sup>b</sup> | 414.6±37.9<sup>c</sup> | 2.9±0.513<sup>b</sup> | 140.5±55.455<sup>c</sup> | 216.54±0.1<sup>d</sup> | 34.30±0.200<sup>a</sup> |

Values are means of three replicates. Means of the same superscript are not significantly different at (p ≥ 0.05) while means in the same column not followed by the same superscript are significantly different; P1=Abacha Road; P2=Cherubim Road; P3= Kaduna Street; P4= Okija Street; P5= Olu-Obasanjo Road

Table 2. Physico chemical parameters of sediment samples

| Station | pH     | EC (µS/cm) | Avail P (mg/kg) | THC (mg/kg) | Organic C (mg/kg) | Ammonium (mg/kg) | Nitrates (mg/kg) | Sulphates (mg/kg) | Sand (%) | Silt (%) | Clay (%) |
|---------|--------|------------|-----------------|-------------|-------------------|-----------------|-----------------|-------------------|----------|----------|----------|
| P1      | 5.4±1.00<sup>a</sup> | 1235.11±0.99<sup>a</sup> | 34.99±0.17<sup>a</sup> | 3.18±0.07<sup>a</sup> | 2.76±0.05<sup>bc</sup> | 14.21±0.26<sup>a</sup> | 182.10±0.10<sup>a</sup> | 550.03±0.03<sup>a</sup> | 78.65±0.05<sup>a</sup> | 7.45±0.04<sup>a</sup> | 14.43±0.40<sup>a</sup> |
| P2      | 5.6±0.90<sup>a</sup> | 293.10±4.44<sup>a</sup> | 19.67±0.35<sup>a</sup> | 0.73±0.03<sup>a</sup> | 0.87±0.02<sup>a</sup> | 21.05±0.05<sup>a</sup> | 70.04±0.03<sup>a</sup> | 237.43±0.21<sup>c</sup> | 85.20±0.05<sup>a</sup> | 0.85±0.05<sup>a</sup> | 14.15±0.15<sup>a</sup> |
| P3      | 5.4±0.85<sup>a</sup> | 779.37±0.55<sup>bc</sup> | 17.31±0.28<sup>b</sup> | 1.14±0.40<sup>bc</sup> | 1.09±0.02<sup>a</sup> | 28.05±0.05<sup>a</sup> | 203.05±0.05<sup>a</sup> | 337.57±0.08<sup>d</sup> | 85.21±0.01<sup>a</sup> | 0.83±0.38<sup>a</sup> | 14.22±0.20<sup>a</sup> |
| P4      | 5.7±0.50<sup>a</sup> | 302.45±0.61<sup>a</sup> | 10.41±0.18<sup>a</sup> | 0.58±0.03<sup>a</sup> | 0.92±0.03<sup>a</sup> | 21.04±0.05<sup>a</sup> | 112.04±0.04<sup>a</sup> | 275.15±0.15<sup>a</sup> | 85.24±0.04<sup>a</sup> | 0.84±0.40<sup>a</sup> | 14.38±0.35<sup>a</sup> |
| P5      | 5.3±0.36<sup>a</sup> | 245.10±0.90<sup>ab</sup> | 22.80±0.02<sup>a</sup> | 0.74±0.04<sup>a</sup> | 1.11±0.03<sup>a</sup> | 28.03±0.03<sup>a</sup> | 126.04±0.03<sup>a</sup> | 250.12±0.13<sup>b</sup> | 85.23±0.04<sup>a</sup> | 0.85±0.04<sup>a</sup> | 14.15±0.18<sup>a</sup> |

Means with the same superscript are not significantly different (p<0.05); P1=Abacha Road; P2=Cherubim Road; P3= Kaduna Street; P4= Okija Street; P5= Olu-Obasanjo Road
0.58-3.18 mg/kg while organic carbon had between 0.87-2.76 mg/kg showing significant difference at p < 0.05 (Table 2). Values for Ammonium, Nitrates and Sulphates showed similar significant differences at the various study sites. The nitrate content of sediment ranged from 21.04 to 28.08 mg/kg while ammonium recorded values ranging from 14.21 to 28.05 mg/kg. These values were significantly different at p < 0.05. The textural class/particle size of sediments were analyzed for sand, silt and clay. The percentage values of sand recorded between 78.65-8.24, silt had values ranging from 0.83-7.45 while clay had 14.15-14.43. The concentrations for sand and silt were statistically significant at p < 0.05 while clay samples did not show any significant difference in the values obtained (Table 2).

4. DISCUSSION

The Ntanwogba creek is an open drainage system with socio-economic activities around it. The anthropogenic activities within the areas covered by this creek may result in pollution of water resources through improper disposal of wastes in such drains [8]. These wastes may occur in solid or liquid forms consisting of organic and inorganic wastes, spent oil or lubricants, pesticides and fertilizers, storm-water, runoffs from flash floods, erosion or water from car garages and cleaning centres. The solid wastes such as bottles, cans, plastics and other household products may result in blockage of drains [9;10]. As a result of poor network of drainages, water runs over the ground during rainstorms, picks up faeces and contaminates waste water resources.

The wastewater characteristics assessed in this study have been reported to have fell short of the recommended limits in terms of the turbidity, BOD₅, nitrate, pH, TDS, temperature, DO and chloride concentrations, thus suggesting impairment of the water quality and the alteration of the ecological dynamics of the receiving water bodies. BOD measures the amount of oxygen required by microbes to break down organic matter, while COD is a measure of the amount of oxygen required for the chemical decomposition of organic and inorganic contaminants dissolved or suspended in water [11]. The determination of BOD and COD is useful in evaluating the compliance of effluents with water quality requirements standards, and also the estimation of the potential of organics present in effluent to deplete oxygen [12]. Indiscriminate defaecation and refuse disposal was observed at all the sampling stations. The slightly high BOD values may be attributed to the discharge of organic waste into water channel resulting in the uptake of DO in the oxidative breakdown of these wastes [25,26]. The nearness of most sampling stations to dumpsites is also a factor promoting the loading of the water channel with organic matter hence, the high BOD values. The implication of high BOD in surface water could also mean that the oxygen present in the water will be used for decomposition of the pollutants, and thus, is not available for aquatic life anymore. The natural background level for freshwater ranges from 1.0 to 3.0 mg/l. The BOD of a river must generally not exceed 4.0 mg/l. This would reduce DO from saturating to 5.0 - 6.0 mg/l which is still capable of supporting aquatic life [9,26]. The COD level clearly depends on the type of wastewater, this problem is associated with poor sanitation of these drains which hinders on lack of implementation of waste management practices. The continuous discharge of effluent through various socioeconomic activities around the drainage channels in Port Harcourt with low- dissolved oxygen and high BOD into drainage paths suggest increased organic loading, and in turn potential negative impacts on such receiving water systems, which may cause harm to aquatic life [13,14]. Also, low oxygen content may cause increased toxicity of certain substances, and this may induce stress responses in the aquatic ecosystem especially when it flows into major water bodies and resources. Increased COD levels also have a similar effect on surface water [12]. The BOD and COD levels recorded in this study were similar to those reported elsewhere [13,14]. Chloride occurs in all natural waters in widely varying concentrations. Excessive chloride in potable water is not particularly harmful and the criteria set for this anion are based primarily on palatability and its potentially high corrosiveness [15] Chloride content in excess imparts a salty taste to water and people who are not accustomed to high chlorides may be subjected to laxative effects.

One important characteristic of discharged wastewater effluents that often impacts receiving waters is its nutrient content. Excessive nutrient loading, especially in regards to nitrogen and phosphorus, is a major ongoing threat to freshwater quality worldwide, particularly in water-scarce countries such as the Niger Delta and other developing countries [16]. Many aquatic systems have very low ambient nutrient
concentrations, and small shifts in the nutrient load can result in dramatic changes in the aquatic community structure. High concentrations of nitrates above the 15 mg/l limit were recorded in all stations studied along the open drainage channels. Likewise, nitrate has been reported to be toxic to humans and animals. Methemoglobinemia is usually formed during the nitrate-induced oxidation of haemoglobin, which prevents normal oxygen binding and leads to hypoxia [17]. Therefore increased nitrate contamination of water bodies may raise serious concerns as seepage of such contaminants may cause pollution of aquifers or many water resources used for both domestic and industrial uses. Increased nitrate and phosphorus levels in discharged effluents will promote excessive growths of aquatic plants and algae, thus contributing to eutrophication and resulting in undesirable ecological effects within the receiving water bodies [13]. Ammonium ions have also been reported to have toxic effects on fish [17,18]. These compounds are becoming increasingly significant in water and wastewater management because the discharge of nutrients such as nitrogen and phosphorus into rivers, lakes and stagnant drains can cause adverse influences on our environment and life. An excessive increase in the quantities of these nutrients in the aquatic surroundings disturbs the ecological balance, resulting in severe damage to the environment.

There was notably excessive turbidity in the discharged effluents in the open drainages during the sampling periods (Table 1). One major implication of the excessive turbid effluents in the open water drainage system is a reduction in light penetration, which may result in the decline of the rate of photosynthesis by the aquatic plants. This may in turn lead to less food being available for the aquatic animals [19]. Organic compounds are added by human excrement and other domestic wastes. These organic and inorganic compounds are added by industrial wastes and several anthropogenic activities around the open drainage channels such as slaughter houses, factories, paper mills, creameries, chemical and metal industries which contribute acids and salts of metals and other inorganic chemical wastes [20]. Excessive turbidity affects the effectiveness of chlorination during disinfection in wastewater treatment, resulting in the failure of removal of microorganisms [21,22]. High turbidity has been shown to hinder the effectiveness of disinfection in water, and often correlates with microbial load within water resources [22]. This result actually shows that the drains were highly contaminated with different pollutants due to human activities as well as flash floods during raining seasons [23]. Nitrate concentration in most of the sampling stations were above permissible limits due to several anthropogenic activities in close proximity to the open drainages and even direct discharge of untreated waste and human feaces into the open drainages thereby causing surface pollution (Plates1-3). Oxidation of nitrogen in water as ammonia from animal and human wastes to nitrite, nitrates and other organic molecules is a possible way of nitrate entry into the groundwater aquifer [24].

5. CONCLUSION

There have been increasing detrimental impacts on freshwater ecosystems, including the notable eutrophication and pollution of many rivers. The findings of this study revealed inadequacies in effective waste management and improvement of existing drainage systems in Port Harcourt, as continuous discharge of untreated effluents arising from various anthropogenic activities around drainage channels has caused a high concentration of organic matter in the open drains hence in this study, the noncompliance of some of the physicochemical parameters assessed including BOD, COD, nutrient concentration like nitrate, turbidity, and chloride concentrations may contribute to eutrophication in the receiving watershed and consequently alter the ecosystem balance of water resources. In the event of any chlorination of such water bodies there may be a chance of formation of carcinogens resulting from the chlorination of highly turbid effluents, which constitute a public health threat. Therefore, more effort should be invested in curbing the indiscriminate discharge of poor-quality effluent into the drainage channels.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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