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Evaluation of Water Quality of Ogbujilekwe Stream in Njikoka L.G.A of Anambra State.

Abstract

Water pollution is the contamination of waters bodies by nutrients. These nutrients may be organic or inorganic nutrients. Dissolved organic nutrient may serve as a source of living for organisms living in water, and also show some detrimental effect on aquatic ecosystem since itfavours bacterial slimes and cause eutrophication. This research work evaluated the water quality of Ogbujilekwe Stream in Njikoka L.G.A. of Anambra State. The parameters used for this evaluation include water color, odour, taste, pH, Oxygen Demand (OD), Biochemical Oxygen Demand (BOD), total dissolved solid, (T.D.S.), turbidity and microbial test.

The result obtained from this research showed that the B.O.D., was high. All the water samples used for the analysis yielded positive result indicating various forms of coliform

The detection of these coliform in the water samples indicates that stream is seriously being polluted by the animal faeces used for planting crops on the watershed. Although the concentrations were within the permissible, range for drinking water, further, inputs accumulation, may result in serious health hazard to the neighbouring communities in Anaocha Local Government Area of Anambra state

Introduction

Pollution of water bodies have been attributed to anthropogenic influence from both far and around water bodies. Watersheds are mostly the major sources of water bodies. A watershed is an area of land which collects run-off from precipitation and primarily drains large amounts of underground water, nutrients, sediments, toxins, from the soil into the nearby water bodies. The determination of water quality can be evaluated by checking water parameters which include water color, odour, taste, pH, Oxygen Demand (OD), Biochemical Oxygen Demand (BOD), total dissolved solid, (T.D.S.), turbidity and microbial test.

BOD is the measure of the amount of dissolved oxygen needed by micro-organisms in the water (demanded by aerobic biological organisms) to break down organic materials present in a given water sample at certain temperature over a specific time period. When sewage is discharged into freshwater stream, the stream becomes polluted. This does not mean that the oxygen content drops instantaneously,but the potentially for Oxygen, depletion exists whenever there is sewage. The measure of this potential is Biochemical oxygen demand (BOD). The index of pollution by nutrients in water bodies, is Biochemical oxygen demand

(BOD). It is often to check the degree of organic pollution of the water by measuring the amount of dissolved oxygen. It is expressed in milligrams per liter. Biochemical oxygen demand (BOD) depends on temperature, environment and organisms present. If the first two factors are controlled, then the rate of oxidation depends on nutrients (Sulivan, 2014).

. BOD is usually calculated by measuring the amount of dissolved oxygen in water. During the day when photosynthesis is high, rapid aeration and photosynthesis can contribute to supersaturation of oxygen since it is produced as waste product of photosynthesis. At this level dissolved oxygen is usually more than 100% air saturation. Fish in water containing excessive dissolved oxygen may suffer from gas bubble disease, which block the flow of blood and may cause death. BOD can be used as a gauge for determining the effectiveness of waste water treatment of plant dissolved oxygen (DO), which is the amount of oxygen in water that is available for micro-organisms and other aquatic organisms. DO is also affected by diffusion, photosynthesis, respiration and decomposition. Dissolved oxygen (DO) decreases with depth and reduction in dissolved oxygen results in increased mortality of fish. The permissible limit for DO in water is 9 - 20 mg/l. Concentration above this can be harmful to aquatic lives. The presence of fecal coliform in aquatic environment indicates that the water has been contaminated with pathogens or disease producing bacteria and viruses, which are found in the fecal materials of human or other animals. Fecal coliform bacteria enter water bodies through direct discharge of waste from mammals or birds from runoff and from agricultural soil. The presence of fecal contamination is an indication that potential risks exist for individuals that are exposed to the water. Fecal coliforms are relatively harmless but increased input of fecal materials will result in water borne pathogenic diseases such as typhoid fever, bacterial gastroenteritis and hepatitis

Materials and methods

Water Parameters

Water samples were collected at three different points and at three different depths of Ogbujilekwe Stream in Njikoka L.G.A. of Anambra state. A control was also collected from another point far from the area of collection of the initial samples. The water samples were carried to the laboratory, where the following water parameters are investigated: water color, odour, taste, pH, Oxygen Demand (OD), Biochemical Oxygen Demand (BOD), total dissolved solid, (T.D.S.), turbidity and microbial test. The GPS readings at the points of collection of the samples were also recorded. Table 3.6 below, showed the GPS coordinate of the points where the water samples were collected.

Table 3.6: The GPS coordinate at the point where water samples were collected

Water Samples	GPS Coordinate
Sample A	N06˚07.114′
	E006˚59.682′
Sample B	N06˚07.111′
	E006˚59.685′
Sample C	N06˚07.109′
	E006˚59.683′
Control	N06˚07.126′
	E006˚59.696′

Determination of pH of water samples

The pH of the water was determined using an electric pH meter.

Biochemical Oxygen Demand (BOD) and Dissolved Oxygen

The method of Winkler was adopted for BOD_5 and DO determination. 100 mls of each of the water samples collected were placed in a conical flask. 0.5 ml of magnesium sulphate and alkaline iodide azide were dropped in each of the samples. The alkylineiodide azide then reacted with $MgSO_4$ to give a precipitate. The samples were then mixed thoroughly by inversion and rotation until it forms two layers, the upper transparent layer and the dark supernatant at the lower layer.

The supernatant is then dissolved by addition of 1ml of concentrated sulphuric acid to form a pale yellow solution. Then a 25 ml of the solution was pipetted into a 250 cm^3 conical flask and immediately titrated against 0.025 mol/dm^3 sodium thiosulphate using freshly prepared starch solution as an indicator. The titration was carried out in duplicate.

$$D.O = \frac{\text{Mole of titrant x normality of titrant x 800}}{\text{M1 of sample used}}$$

The general equation for determination of BOD value is

$BOD\ (mg/l) = D_1 - D_2$

Where D_1 = Initial Do of the sample and D_2 = Final Do of the sample after 5 days and P = decimal volumetric fraction of sample used.

Note: If 100 ml of sample are diluted to 300 ml, then P = 0.33, but if dilution was not necessary, P = 1.0 and the BOD is determined by $D_1 - D_2$

Determination of turbidity of the water samples

Select Epa 180 ^oC as a measurement mode. Place the sample in a clean dry turbidity vial and cap securely. Wipe off excess liquid or fingerprint with a soft cloth. The result will be displayed on the measurement.

Determination of total dissolved solids of the water samples (T.D.S).

This was carried out using the procedure by APHA (Green, 2000). The fiber filter disc was prepared, by placing it wrinkle side up in the filtration apparatus. Continuous suction was applied to remove all the traces of water. A clean evaporating dish was heated to dryness on a steam bath. The evaporating dish was finally dried for 180 ^{o}C ± 2^{o}C in an oven for 1hour. It was then cooled and stored in a dessicator to balance the temperature, before weighing. Then result is calculated as follows;

$$\text{T.D.S.} = \frac{(A - B)}{Sample\ volume\ in\ ml} \times 10^5$$

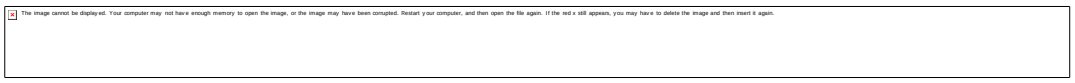
where A= Weight of dish + solid

and B = Weight of dish before use.

Microbial analysis

Determination of total coliform

The method used here is the membrane filter technique.Exactly 100 ml of the water samples were filtered through a membrane filter which helps to retain the bacteria in the samples. In the two step enrichment procedure, the filters containing the bacteria were placed on an absorbent pad saturated with lactose broth and incubated at 35 ^{o}C ± 0.5 ^{o}C for 2 hr. The filters were then transferred to an absorbent pad saturated with Eosine-methylated blue media and incubated for another 24 hr at 37 ± 0.5 .Developing colonies were then counted under magnification and reported per 100 mL of the original sample. The calculations were thus:



Determination of the total fungal count

Tenfold serial dilutions of the water samples were done and appropriate dilutions were plated on Sabouraud Dextrose Agar (SDA) plates. The cultured plates were incubated at room temperature for 48 hr for the fungal count. Thereafter the developing colonies were counted and the total fungal count was calculated as follows;

Where: TFC = Total fungal count

V = Volume of inocula plated

N = Dilution factor

CFU/ml = Colony forming unit per

Characterization and identification of bacteria

Identification of bacterial isolates was accomplished by the observation of colony characteristic. Gram reaction biochemical test (Amita, 2020) The characterization of isolates were performed, by employing Gram staining reaction, catalase test, citrate test, sugar fermentation test, coagulase test, indole test, methyl red test, motility test and vogesprausker test as described by Bergeys Manual of Determinative Bacteriology, 9^{th} edition (1994).

Gram reaction

Thin smear of the isolate was made on clean, non-greasy, dust free slides, air dried and heat fixed. The smear was flooded with crystal violate and allowed to remain on the slide for 60 sec. Thereafter, the crystal violate was washed off with gentle running water. Again, the slide was flooded with Gram's iodine, allowed to remain for 60 secs and then washed off. The slide was decolorized with acetone – alcohol mixture. The slide was then viewed under oil immersion lens microscope (× 100). Purple color indicated Gram- positive organisms while red or pink color indicated Gram - negative organisms.

Catalase test

Exactly 3 ml of 3 % solution of hydrogen peroxide (H_2O_2) was transferred into a sterile test tube. Then, 3 loopful of a 24hrs pure culture of the test bacteria were inoculated into the test tube. The tube was observed for immediate bubbling indicative of a positive, while no bubbling indicated a negative reaction.

Motility test (hanging drop method)

A loopful of 18 - 24 hr broth culture of the test bacteria was placed at the centre of a clean grease- free cover slip. Carefully, the cover slip was inverted and placed over the concave portion of a hanging drop slide. The cover-slip/slide arrangement was observed for motility at ×100 magnification on a compound microscope. Care was taken not to interrupt "drift" or a "Brownian motion" as motility. Results were recorded as motile or non- motile.

Indole Test

A loopful of an 18 - 24 hr culture was used to inoculate the test tube containing 3ml of sterile tryphone water. Incubation was done at 35-37 first for 24 hr and further for up to 48 hr. Test for indole was done by adding 0.5 ml of Kovac's reagent, shaken gently and then examined for a ring of red color in the surface layer within 10 min, indicative of a positive reaction. Absence of a red color indicated a negative reaction.

Methyl red test

Exactly 5 ml of methyl red were added to an equal volume of a 48 hr culture of the isolate in Methyl red – Voges Proskauer (MR- VP) broth. The production of a bright red color indicates a positive test while yellow color indicates a negative test after vigorous shaking.

Voges - Prausker test

Exactly 2 mL of the 18 – 24hrs culture of the test organism growing on MR – VP broth was aseptically transferred into a sterile tube. Then 0.6 mL of 5 % α-naphthol was added, followed by 0.2 mL of 40 % KOH. NB: It is essential that this reagent was added in this order. The tube was shaken gently to expose the medium to atmospheric oxygen and then allowed to stand undisturbed for 15-30 min. A positive test was indicative by the presence of a red colour, lack of pink red color denoted negative reaction.

Citrate test

A 24 hr old culture was inoculated in a test tube containing sterile Simmons Citrate agar slant and then incubated for 24 hr. A positive test was indicated by a change from green to blue color on the surface of the Simmons Citrate agar slant. No color change indicated a negative reaction.

Sugar fermentation test

Each of the isolate was tested for its ability to ferment a specific sugar. One gram (1 g) of the sugar and 1 g of the peptones were dissolved in 100 mL of water. Five milliliter (5 mL) of the solution was transferred into clean test tube using sterile pipettes. The test tubes containing peptone water and sugar were added into a Durhams's tube which were placed inversely and bromothymol blue as an indicator. These were sterilized for 10 min and allowed to cool before inoculating the innocula. The test tubes were incubated for 3 days. The production of the acid or acid only indicated utilization of sugars. Acid production was indicated by change in color of the medium from green to yellow while gas production was observed by the presence of gas in the Durhams's tubes.

Characterization and identification of fungi

The isolated fungi were identified based on the gross morphological appearance of the fungal colonies on SDA medium. The microscopic features were studied by slide culture technique with reference to the Manual of Atlas of fungi (Watanabe, 2005).

Result

Table4.21 Mean values for the physicochemical properties of water, which include: pH, B.O.D, O.D, T.D.S, and turbidity is presented in Table 4.21

Microbial Profile of Water Samples

Tables 4.30 and 4.31 showed the total coliform count and the identification of the various isolates of the water samples.

Table 4.32 and 4.33 also showed the fungal count for the samples.

Table 4.21: pH, OD, BOD, T.D.S, and turbidity of water samples

S/N	Samples	pH	OD	BOD	T.D.S	Turbidity
1	A	6.62	14.0	576*	30.0	11.6
2	B	6.54	17.5	626*	27.0	11.5
3	C	7.02	10.6*	1224*	32.0*	11.2
4	Control	6.04	7.2*	420*	19.0*	10.0*

*Mean is significant level is at $p<0.005$.

Table 4.30: Fecal coliform count of the water sample

S/N	Water samples	Number of colonies on plate	Nature of colonies on EMB Agar	Isolates
1	Water sample A	35 (25) (10)	Colourless to pale pink Colourless looking colonies	W1 W2
2	Water sample B	TFTC (1)	Pink to Red	W3
3	Water sample C	TFTC (2)	Pink to Red	W3

4	Control	(12)	Colourless to pale pink	W1

Table 4.31: Morphological and biochemical identification of the various isolates of the water samples

S/N	Isolate	Form	Surface	Colour	Margin	Elevation	Opacity	Gram	Cat	Mot	Ind	MR	VP	Cit	Lac	Glu	Suc	Fru	Mal	Identify
1	W1	Circular	Smooth	Cream	Convex	Raise	Opaque	+	-	-	-	+	+	+	+	+	+	+	+	*Enterococcus fecalis*
2	W2	Circular	Glistening	White	Entire	Raised	Opaque	+	+	+	+	+	+	+	+	+	+	(-)	+	*Proteus mirabilis*
3	W3	Circular	Glistening	White	Entire	Raised	Transparent	+	-	-	-	+	+	+	+	+	+	+	+	*Enterococcus fecalis*
Control	W4	Circular	Shiny	White	Entire	Convex	Moist	-	+	+	-	-	+	+	+	+	+	+	+	*Enterobacter aerogenes*

Key:
Gram: Gram reaction
Cat: Catalase test
Mot: Motility test
Ind: Indole test
MR: Mwthyl red test
VP: Voges-prauskauer test
Cit: Citrate utilization test
Sugar fermentation tests:
Lac: Lactose fermentation
Glu: Glucose fermentation
Suc: Sucrose fermentation
Fru: Fructose fermentation
Mal: Maltose fermentation
+: Positive
- : Negative

Table 4.32: Total fungal count

S/N	Sample	A	B	Mean (TFC)	Suspected Isolates
1	Water sample A	1(TFTC)	3 (TFTC)	2 (TFTC)	D
2	Water sample B	Nil	Nil	Nil	Nil
3	Water sample C	Nil	Nil	Nil	Nil
4	Control	Nil	Nil	Nil	Nil

Table 4.33: Cultural and microscopic features of fungal isolates

S/N	Isolate	Cultural features	Microscopic features	Organism identified
1	D	Dark to brown colonies with grey underside	Septate hyphae with erect simple and thick walled conidiophores bearing conidial heads split into over 4 loose conidial colums with 4 fragments apically.	*Aspergillus niger*

Discussion and conclusion

The result obtained from this research showed that the B.O.D., was high. All the water samples used for the analysis yielded positive result indicating various forms of coliform as showed in table 4.30. Microbiologically, the safety of drinking water is monitored by bacterial parameters which revealed coliform contamination. Sample A Contained *Enterococcus fecalis*and *Proteus mirabilis.* Sample B contained *Enterobacter aerogenes,* Specimen C contained *Enterobacter arogenes* while control contained *Enterobacter fecalis.* Fecal coliform bacteria are a collection of relatively harmless micro-organisms that live in large number in the intestine of warm- and cold-blooded animals. The detection of these coliform in the water samples indicates that stream is seriously being polluted by the animal faces used for planting crops on the watershed. Although the concentrations were within the permissible, range for drinking water, further, inputs accumulation, may result in serious health hazard to the neighbouring communities in Anaocha Local Government Area of Anambra state. This influx of animal feaces and other organic materials into the waters are the major contributors to the high BOD of the water samples.

Furthermore, for all the water samples analysed as presented in table 4.32, only sample A yielded a positive result for the fungal count, and the organism found was *Aspergillus niger*. *Aspergillus niger* is a heterotrophic organism that occurs naturally in oceans and fresh water. It is a saprophytic fungus that helps in degrading organic matter. *Aspergillus niger* is less likely to cause human diseases, although according to Paulose, K.O. (1989), it is the most common causes of a fungal ear infection called otomycosis, which results in pains, temporary hearing loss, and in severe cases damage to the ear canal and tymphatic membrane. According to him also, fungi in water affects taste and odour.

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