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Bacteriological and Physicochemical Impact of Effluents from Ebonyi Fertilizer and Chemical Plant on Azuiyiokwu Creek

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Abstract The health and environmental hazards associated with water body contamination by industrial effluents cannot be over emphasized. This study investigated the bacteriological and physicochemical qualities of effluents from Ebonyi Fertilizer and Chemical Plant and the impact on Azuiyiokwu Creek, located in Abakaliki, Ebonyi State, Nigeria between May, 2010 and March, 2011. Standard microbiological techniques were used for the isolation and identification of microbial isolates. The physicochemical parameters detected from all the samples investigated include pH, Ca, Cu, Pb, Ni, As, Al, Hg, Co, Zn, Fe, Cr, Na, P, Mg, and Mn. The study shows that the concentrations of the metals were constantly higher in fertilizer effluents, than Azuiyiokwu Creek and the control. There was a significant difference ($P \leq 0.05$) in concentration of metals between fertilizer effluents, Azuiyiokwu and the Control Creeks. The results also show that metallic contaminations of Azuiyiokwu Creek could be as a result of the infiltration of effluents from Ebonyi Fertilizer and Chemical Plant into Azuiyiokwu Creek. The study revealed higher bacterial load in the Ebonyi Fertilizer and Chemical plant effluents than Azuiyiokwu and Control Creeks. There was a significant difference ($P \leq 0.05$) in the microbial counts between the Control and Azuiyiokwu Creek. Bacteria genera encountered in this study include: *Staphylococcus, Proteus, Klebsiella, Pseudomonas, Bacillus, Escherichia, Salmonella, Vibrio* and *Aeromonas*. This shows that the effluents received by Azuiyiokwu creek have low bacteriological and physicochemical qualities and the range of microorganisms isolated in this study raise more serious concern about the public health implications. This reflects the possible pathetic condition of most water bodies in Nigeria. Therefore, adequate measures should be put in place to help ameliorate the deplorable state of our water bodies.

Keywords: *abakaliki, azuiyiokwu, bacteria, effluents, fertilizer, physicochemical, water, nigeria*

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1. Introduction

Water is an absolute necessity if life must be sustained on earth. [1] Therefore, the importance of natural water bodies does not need elaborate emphasis; this is because, whatever that affects water bodies has direct or indirect effect on human health. Naturally, water is second only to air among the most important resources for human existence; however, it is the most threatened. The impact of industrial toxic and hazardous wastes on aquatic life including microorganisms has recently received alarming concern globally. [2] The human race is under tremendous threat due to undesired changes in the physical, chemical and biological characteristics of air, water and soil. Due to increased human population, industrialization, use of fertilizers and man-made activities, water is highly polluted with different harmful contaminants. [3] Effluent is a form of point pollution, and can be said to be liquid wastes from a sewage system, factory, nuclear power station, and other industrial plants outfalls, discharged into the environment. [4] The environment into which these effluents materials are discharged could be water bodies or farmlands. The release of wastes into the environment without any form of treatment could be as a result of improper or deliberate channeling. The discharge of industrial effluents into water bodies untreated could make the water unfit for domestic purposes such as drinking, cooking, and laundry activities. It could as well make the water unfit for irrigation purposes and studies have shown that natural water is the best for irrigation because of the hardness usually associated with underground water. [5] Studies have also shown that the release of untreated effluents into water bodies has a significant effect on the aquatic lives which on the other hand endangers the human health as the sea food consumers. [6] This could result to eutrophication, which can lead to uncontrollable activities of microorganisms. These conditions can be noticeable by the formation of algae blooms, odors and tastes which could be hazardous to the health of the users. [7] According to UNEP [8] report, over half of the world's hospitals beds are occupied with people suffering from illnesses linked with contaminated water and more people die as result of polluted water than are killed by all other forms of violence including wars. Effluents are as well sources of heavy metals which are detrimental to human and animal health. [9] When the water body receiving these effluents are used for a long time for irrigation, plants can accumulate heavy metals in their tissues in concentrations above the permitted levels which is considered to represent a threat to the life of humans, and animals feeding on these crops and may lead to contamination of food chain, as observed that soil and plants that receive irrigation water mixed with industrial effluent contain many toxic metals. [10,11] Despite the hazardous impacts of fertilizer effluents discharged into water bodies on aquatic lives, [12] reports show that in many occasions, it provides aquatic microorganisms with nutrients that enhance their multiplication. [2] A similar work was carried out on Fertilizer Manufacturing Company at Okirika Creek, Rivers State, Nigeria. The result indicated that the rapid increase of effluents discharges into Okirika Creek made the water unfit for drinking and other domestic works. [13] A good number of microbial species was isolated from the Creek to ascertain the level of contamination of the water by the Fertilizer Manufacturing Plant. The isolates included: Aerococcus viridians, Alcaligenes faecalis, Bacillus cereus, Citrobacter freundii, Escherichia coli, Klebsiella pneumonia, proteus vulgaris, Pseudomonas Aeruginosa, Serratia marcescens and staphylococcus aureus. [2,14] The present study critically analyzed, the physicochemical and bacteriological qualities of effluents from Ebonyi Fertilizer and Chemical Plant, discharged into Azuiyiokwu Creek and the Control water body, Onuebonyi Izzi all in Ebonyi State, Nigeria.

2. Materials and Methods

2.1. The Study Area

This study was carried out in Onu-ebonyi Izzi, North-eastern part of Ebonyi State, South-Eastern Nigeria. Azuiyiokwu Creek forms the major source of water to Izzi village. The Creek is about 5km from Abakaliki the Capital City of Ebonyi State. The Creek flows throughout the year. The Creek is utilized for both domestic and agricultural purposes. The part of Onu-ebonyi used as the control is located between latitude 06°4`N and longitude 08°7`E, less than a kilometer (500 m) north of the Azuiyiokwu, a tributary to Azuiyiokwu Creek while Ebonyi Fertilizer and Chemical Plant (EFCP) is located between latitude 06°4`N and longitude 08°5`E. Samples were collected from Azuiyiokwu Creek, EFCP and the control Creek during dry and rainy seasons for bacteriological analysis in order to determine the type and load of the bacterial population predominant in the two seasons and to know the effect of fertilizer effluents run-off on their population.

2.2. Sample Collection

The samples were collected with sterile, cocked conical flasks, 9 of which were aseptically half-filled and subjected to microbiological analysis within 24 hours of collection. Exactly 1 ml of each water sample was aseptically measured and homogenized with 9 ml of normal saline. Ten-fold serial dilution of the sample was prepared using normal saline as the diluents [15,16].

2.3. Microbial Analysis

Exactly 1ml each of the water samples was asceptically measured into test tubes containing 9 ml of 0.85% normal saline and homogenized. The set-up was serially diluted (ten-fold) up to the 15th tube. A 0.1 ml aliquot each of the selected dilutions was inoculated on the surface of previously dried plate of nutrient agar. [17] The inoculated plates were incubated aerobically at room temperature for 18-24 hours, after which they were observed for growth and visible colonies were counted and reported as colony forming unit (cfu) per ml of the samples [18].

2.3.1. Identification of Isolates

Pure cultures were made out of the visible growths observed after incubation using streak plate methods [19] and then identified based on their morphological characteristics, Gram reaction, motility and biochemical characteristics [15].

2.4. Physicochemical Analysis

The physicochemical parameters including Calcium, Magnesium, Nickel, Phosphorus, Manganese, Iron, Zinc, Copper, Lead, Arsenic, Aluminum, Chromium, Mercury and Cobalt were determined using standard methods as prescribed by APHA. [20] In this, the samples were acidified with HNO_3 and HCl, and heated to reduce volume to defined level. After heating, they were filtered and further analyzed using an atomic absorption spectrophotometer (AAS). The pH of all samples was determined using Systronic Digital pH meter standardized with buffer tablets and the temperature determined using thermometer.

3. Results

3.1. Total Bacterial Counts

Table 1 shows the total bacterial counts of samples from Azuiyiokwu and control Creek in the dry season and rainy season, as well as Ebonyi Fertilizer and Chemical Plant in dry and rainy seasons. There was significant difference ($P \leq 0.05$) in the number of colony forming units per ml, obtained from the Azuiyiokwu, control Creek and the Ebonyi Fertilizer and Chemical Plant (EFCP) effluents. The counts from the Creeks ranged from 1.1×10^9 – 2.9×10^9 cfu/ml in the dry season and 3.6×10^9 – 8.2×10^9 cfu/ml in the rainy season.

Table 1. Total Bacterial Counts of Samples ($\times10^6$ Cfu/ml) from the three sampling sites

Sample No.	Azuiyiokwu Creek ($\times10^6$ Cfu/ml)		EFCP Effluent ($\times10^6$ Cfu/ml)		Control Creek ($\times10^6$ Cfu/ml)	
	Dry Season	Rainy Season	Dry Season	Rainy Season	Dry Season	Rainy Season
1	**1.8**	**7.9**	**4.7**	**8.1**	**1.1**	**3.8**
2	**1.9**	**7.6**	**4.9**	**9.7**	**1.5**	**3.6**
3	**1.6**	**8.2**	**3.2**	**7.7**	**1.2**	**3.6**

3.2. Identification of the Isolated Organisms

Isolates from the three different sources were characterized. The phenotypic characteristics of these organisms and their tentative identification are presented in Tables 2 - 5. Based on these characteristics, 9 genera of bacteria were identified. Among these were members of the *Enterobacteriaceae* family, the family of *Vibronaceae*, the genera *Bacillus* and *Lactobacillus*, members of the Gram positive cocci and *Pseudomonas*. Details are shown below.

3.3. Physicochemical Analysis

A total of sixteen physicochemical elements were detected as can be seen in the following Table 6 –Table 8 below. The physicochemical elements of Ebonyi fertilizer and Chemical Plant, Azuiyiokwu Creek and Onuebonyi Creek are compared in Figure 1, Figure 2 and Figure 3 below.

Table 2. Bacteria Isolated from Azuiyiokwu Creek in Abakaliki, Ebonyi State, Nigeria

Sample Source	Gram React.	Cell Morph	Ox. Test	Cat. Test	Cit. Test	Ind. Test	Ure. Test	Met. Red	VP. Test	Mot. Test	Spore Form.	Ferm.	Glu.	Suc.	Mal.	Mann.	Tentative Identification	
																	Dry Season	Rainy Season
Azu	-	R	-	-	+	-	-	+	+	+	-	F	A/G	A/G	A/G	A	*Enterobacter* sp.	*Enterobacter* sp.
Azu	+	R	-	+	-	-	-	-	+	+	+	F	A	-	-	A	*Bacillus* sp.	*Bacillus* sp.
Azu	-	R	+	+	+	-	+	+	-	+	-	O	A	-	-	-	*Pseudomonas* sp.	*Pseudomonas* sp.
Azu	-	R	-	+	+	+	+	+	+	-	-	F	A	A	A	-	*Proteus* sp.	*Proteus* sp.
Azu	-	R	-	+	+	-	+	+	-	+	-	F	A/G	A/G	A/G	A/G	*Citrobacter* sp.	*Citrobacter* sp.
Azu	+	C	-	+	+	-	-	-	-	-	-	F	A	A	A	-	*Staphylococcus* sp.	*Staphylococcus* sp.
Azu	-	R	-	+	+	+	-	+	-	+	-	F	A/G	A/G	A/G	A/G	*Salmonella* sp.	*Salmonella* sp.
Azu	-	CR	+	-	+	+	-	+	+	+	-	F	A	A	A	A	*Vibrio* sp.	*Vibrio* sp.
Azu	+	R	-	-	-	-	-	-	+	+	-	F	A	A	A	A	*Lactobacillus* sp	*Lactobacillus* sp
Azu	-	R	-	-	+	+	-	-	-	+	-	F	A	-	-	A	*Escherichia* sp.	*Escherichia* sp.
Azu	-	R	+	+	+	+	-	+	+	+	-	O	A	A	A	A	*Aeromonas* sp.	*Aeromonas* sp.
Azu	-	R	-	-	+	-	+	+	+	-	-	F	A/G	A/G	A/G	A/G	-	*Klebsiella* sp.
Azu	+	C	-	+	+	-	-	+	+	-	-	F	A	A	A	A	-	*Micrococcus* sp

Key: Azu= Azuiyiokwu Creek, - = negative reaction, C= Cocci, R= Rod, CR= Curved Rod, + = Positive, F= Fermented, O= Oxidized, A/G= Acid and Gas, A= Acid, O = Oxidized, Ox.= Oxidase, Cat.= Catalase, Cit.= Citrate, Ind.= Indole, Ure.= Urease, Met.= Methyl, V.P= Voges-Poskauer

Table 3. Bacteria Isolated from Ebonyi Fertilizer and Chemical Plant (EFCP) in Abakaliki during Dry and Rainy seasons

Sample Source	Gram React.	Cell Morph	Ox. Test	Cat. Test	Cit. Test	Ind. Test	Ure. Test	Met. Red	VP. Test	Mot. Test	Spore Form.	Ferm.	Glu.	Suc.	Mal.	Mann.	Tentative Identification	
																	Dry Season	Rainy Season
EFCP	-	R	-	-	+	-	-	+	+	+	-	F	A/G	A/G	A/G	A	*Enterobacter* sp.	*Enterobacter* sp.
EFCP	+	R	-	+	-	-	-	-	+	+	+	F	A	-	-	A	*Bacillus* sp.	*Bacillus* sp.
EFCP	-	R	+	+	+	-	+	+	-	+	-	O	A	-	-	-	*Pseudomonas* sp.	*Pseudomonas* sp.
EFCP	-	R	-	+	+	-	+	+	-	+	-	F	A/G	A/G	A/G	A/G	*Citrobacter* sp.	*Citrobacter* sp.
EFCP	-	R	-	+	+	-	+	+	+	-	-	F	A	A	-	-	*Acinetobacter* sp.	*Acinetobacter* sp.
EFCP	-	R	+	+	-	+	-	-	-	-	-	F	A/G	-	-	-	*Flavobacterium* sp.	*Flavobacterium* sp.
EFCP	+	C	-	+	+	-	-	-	-	-	-	F	A	A	A	-	*Staphylococcus* sp.	*Staphylococcus* sp.
EFCP	-	R	-	+	+	+	-	+	-	+	-	F	A/G	A/G	A/G	A/G	*Salmonella* sp.	*Salmonella* sp.
EFCP	+	R	-	-	-	-	-	-	+	+	-	F	A	A	A	A	*Lactobacillus* sp	*Lactobacillus* sp
EFCP	-	R	-	-	+	+	-	-	-	+	-	F	A	-	-	A	*Escherichia* sp.	*Escherichia* sp.
EFCP	-	R	+	+	+	+	-	+	+	+	-	O	A	A	A	A	*Aeromonas* sp.	*Aeromonas* sp.
EFCP	-	R	-	-	+	-	+	+	+	-	-	F	A/G	A/G	A/G	A/G	*Klebsiella* sp.	*Klebsiella* sp.
EFCP	+	C	-	+	+	-	-	+	+	-	-	F	A	A	A	A	*Micrococcus* sp	*Micrococcus* sp

Key: - = negative reaction, C= Cocci, R= Rod, CR= Curved Rod, + = Positive, F= Fermented, O= Oxidized, A/G= Acid and Gas, A= Acid, O = Oxidized, Ox.= Oxidase, Cat.= Catalase, Cit.= Citrate, Ind.= Indole, Ure.= Urease, Met.= Methyl, V.P= Voges-Poskauer

Table 4. Bacteria isolated from the control Creek in Abakaliki during Dry and Rainy seasons

Sample Source	Gram React.	Cell Morph	Ox. Test	Cat. Test	Cit. Test	Ind. Test	Ure. Test	Met. Red	VP. Test	Mot. Test	Spore Form.	Ferm.	Glu.	Suc.	Mal.	Mann.	Tentative Identification	
																	Dry Season	Rainy Season
CTRL	-	R	-	-	+	-	-	+	+	+	-	F	A/G	A/G	A/G	A	*Enterobacter* sp.	*Enterobacter* sp.
CTRL	+	R	-	+	-	-	-	-	+	+	+	F	A	-	-	A	*Bacillus* sp.	*Bacillus* sp.
CTRL	-	R	+	+	+	-	+	+	-	+	-	O	A	-	-	-	*Pseudomonas* sp.	*Pseudomonas* sp.
CTRL	-	R	-	+	+	+	+	+	+	-	-	F	A	A	A	-		*Proteus* sp.
CTRL	-	R	-	+	+	-	+	+	-	+	-	F	A/G	A/G	A/G	A/G		*Citrobacter* sp.
CTRL	-	CR	+	-	+	+	-	+	+	+	-	F	A	A	A	A		*Vibrio* sp.
CTRL	-	R	-	-	+	-	+	+	+	-	-	F	A/G	A/G	A/G	A/G	*Klebsiella* sp.	
CTRL	+	C	-	+	+	-	-	-	-	-	-	F	A	A	A	-		*Staphylococcus* sp.
CTRL	-	R	-	+	+	+	-	+	-	+	-	F	A/G	A/G	A/G	A/G	*Salmonella* sp.	*Salmonella* sp.
CTRL	+	R	-	-	-	-	-	-	+	+	-	F	A	A	A	A	*Lactobacillus* sp	*Lactobacillus* sp
CTRL	-	R	-	-	+	+	-	-	-	+	-	F	A	-	-	A	*Escherichia* sp.	*Escherichia* sp.
CTRL	-	R	+	+	+	+	-	+	+	+	-	O	A	A	A	A	*Aeromonas* sp.	*Aeromonas* sp.

Key: CTRL= Control Creek- = negative reaction, C= Cocci, R= Rod, CR= Curved Rod, + = Positive, F= Fermented, O= Oxidized, A/G= Acid and Gas, A= Acid, O = Oxidized, Ox.= Oxidase, Cat.= Catalase, Cit.= Citrate, Ind.= Indole, Ure.= Urease, Met.= Methyl, V.P= Voges-Poskauer

Table 5. Colonial Morphology of Isolates

Sample No.	Shape	Size (mm)	Opacity	Colour	Edge	Elevation	Genera
1.	Circular	2	Translucent	Cream	Entire	Convex	*Enterobacter* sp.
2.	Circular	5	Translucent	Dirty white	Rough	Flat	*Bacillus* sp
3.	Circular	4	Opaque	Dirty white	Smooth	Convex	*Klebsiella* sp
4.	Circular	3.5	Opaque	Creamy with grayish yellow pigmentation	Rough	Convex	*Pseudomonas* sp
5.	Circular	2	Opaque	Yellow	Smooth	Convex	*Micrococcus* sp
6.	Circular	Irregular	Opaque	White	Entire	Convex	*Proteus* sp
7.	Circular	3	Translucent	White	Entire	Convex	*Citrobacter* sp.
8.	Circular	2	Translucent	Whitish yellow	Entire	Convex	*Staphylococcus* sp
9.	Circular	3	Opaque	Grayish-white	Smooth	Convex	*Salmonella* sp
10.	Circular	3	Opaque	Yellow-brown	Smooth	Convex	*Vibrio* sp.
11.	Circular	2	Opaque	Cream	Rough	Convex	*Lactobacillus* sp.
12.	Circular	2	Translucent	Cream	Entire	Convex	*Escherichia* sp.
13.	Circular	3	Opaque	Cream	Smooth	Convex	*Aeromonas* sp.

Table 6. Physicochemical Parameters detected from Azuiyiokwu Creek in two different seasons (ppm)

Physicochemical Parameter	Samples in Dry Season				Samples in Rainy Season			
	No. of Trials			Average	No. of Trials			Average
	1	2	3		1	2	3	
pH	7.900	7.900	7.900	7.900	6.900	6.900	7.900	7.233
Ca	0.102	0.105	0.103	0.103	0.500	0.500	0.500	0.500
Mg	0.041	0.041	0.041	0.041	0.073	0.073	0.073	0.073
Ni	0.020	0.021	0.020	0.020	0.050	0.050	0.050	0.050
P	2.100	2.101	2.001	2.067	5.125	5.112	5.012	5.083
Mn	0.063	0.063	0.063	0.063	0.332	0.330	0.330	0.331
Fe	0.057	0.057	0.057	0.057	0.504	1.500	0.500	0.501
Zn	0.023	0.021	0.020	0.021	0.301	0.300	0.300	0.300
Cu	0.014	0.014	0.014	0.014	1.001	1.000	1.000	1.000
Pb	0.003	0.003	0.003	0.003	0.021	0.021	0.020	0.021
As	0.002	0.002	0.002	0.002	0.012	0.012	0.010	0.011
Al	0.001	0.001	0.001	0.001	0.021	0.021	0.021	0.021
Cr	0.060	0.060	0.060	0.060	0.200	0.200	0.200	0.200
Hg	0.040	0.041	0.039	0.040	0.060	0.060	0.060	0.060
Co	0.001	0.001	0.001	0.001	0.005	0.005	0.005	0.005
T (^{o}C)	27.000	27.000	27.000	27.000	29.000	29.000	29.000	29.000

Key: pH= hydrogen ion concentration Ca= Calcium, Mg= Magnesium, Ni= Nickel, P= Phosphorus, Mn= Manganese, Fe= Iron, Zn= Zinc, Cu= Copper, Pb= Lead, As= Arsenic, Al= Aluminum, Cr= Chromium, Hg= Mercury, Co= Cobalt, T= Temperature

Table 7. Physicochemical Parameters detected from Ebonyi Fertilizer and Chemical Plant Effluents (ppm)

Physicochemical Parameter	Samples in Dry Season				Samples in Rainy Season			
	No. of Trials				No. of Trials			
	1	2	3	Average	1	2	3	Average
pH	5.240	5.240	5.240	5.240	6.010	6.010	6.010	6.010
Ca	1.500	1.510	1.504	1.504	0.930	0.930	0.930	0.930
Mg	0.360	0.360	0.360	0.360	0.300	0.301	0.301	0.301
Ni	0.147	0.145	0.145	0.146	0.100	0.100	0.100	0.100
P	78.000	78.001	78.101	78.034	69.800	69.800	69.800	69.800
Mn	3.296	3.295	3.296	3.296	2.010	2.000	2.010	2.007
Fe	4.059	4.059	4.059	4.059	2.110	2.110	2.110	2.110
Zn	1.226	1.226	1.226	1.226	1.210	1.210	1.210	1.210
Cu	6.690	6.690	6.690	6.690	5.000	5.010	5.010	5.007
Pb	0.387	0.389	0.389	0.388	0.290	0.290	0.290	0.290
As	0.375	0.376	0.376	0.376	0.240	0.239	0.240	0.239
Al	0.079	0.079	0.079	0.079	0.030	0.040	0.031	0.034
Cr	1.299	1.297	1.300	1.299	1.010	1.010	1.010	1.010
Hg	1.504	1.550	1.560	1.538	1.031	1.031	1.031	1.031
Co	2.356	2.356	2.356	2.356	0.911	0.921	0.900	0.911
T (°C)	28.300	28.000	28.000	28.100	27.100	27.100	27.100	27.100

Key: pH= hydrogen ion concentration Ca= Calcium, Mg= Magnesium, Ni= Nickel, P= Phosphorus, Mn= Manganese, Fe= Iron, Zn= Zinc, Cu= Copper, Pb= Lead, As= Arsenic, Al= Aluminum, Cr= Chromium, Hg= Mercury, Co= Cobalt, T= Temperature

Table 8. Physicochemical Parameters detected from Onuebonyi Control Creek (mg/l)

Physicochemical Parameter	Samples in Dry Season				Samples in Rainy Season			
	No. of Trials				No. of Trials			
	1	2	3	Average	1	2	3	Average
pH	7.200	7.100	7.200	7.160	7.900	8.000	7.800	7.900
Ca	0.104	0.005	1.183	0.097	0.501	0.520	0.530	0.517
Mg	0.021	0.022	0.021	0.021	0.037	0.034	0.034	0.035
Ni	0.017	0.018	0.010	0.015	0.020	0.020	0.020	0.020
P	1.100	1.101	1.101	1.101	2.000	2.000	2.000	2.000
Mn	0.033	0.033	0.033	0.033	0.042	0.043	0.030	0.038
Fe	0.056	0.054	0.058	0.056	0.506	0.501	0.500	0.502
Zn	0.030	0.011	0.022	0.021	0.305	0.290	0.279	0.291
Cu	0.008	0.008	0.008	0.008	0.061	0.060	0.060	0.060
Pb	0.001	0.001	0.001	0.001	0.020	0.021	0.021	0.021
As	0.001	0.001	0.001	0.001	0.007	0.007	0.006	0.007
Al	0.001	0.001	0.001	0.001	0.020	0.021	0.021	0.021
Cr	0.061	0.060	0.061	0.061	0.200	0.200	0.200	0.200
Hg	0.013	0.011	0.009	0.011	0.020	0.018	0.018	0.019
Co	0.002	0.001	0.001	0.001	0.004	0.006	0.005	0.005
T (°C)	29.000	29.100	29.100	29.100	29.000	31.000	29.500	29.800

Key: pH= hydrogen ion concentration Ca= Calcium, Mg= Magnesium, Ni= Nickel, P= Phosphorus, Mn= Manganese, Fe= Iron, Zn= Zinc, Cu= Copper, Pb= Lead, As= Arsenic, Al= Aluminum, Cr= Chromium, Hg= Mercury, Co= Cobalt, T= Temperature

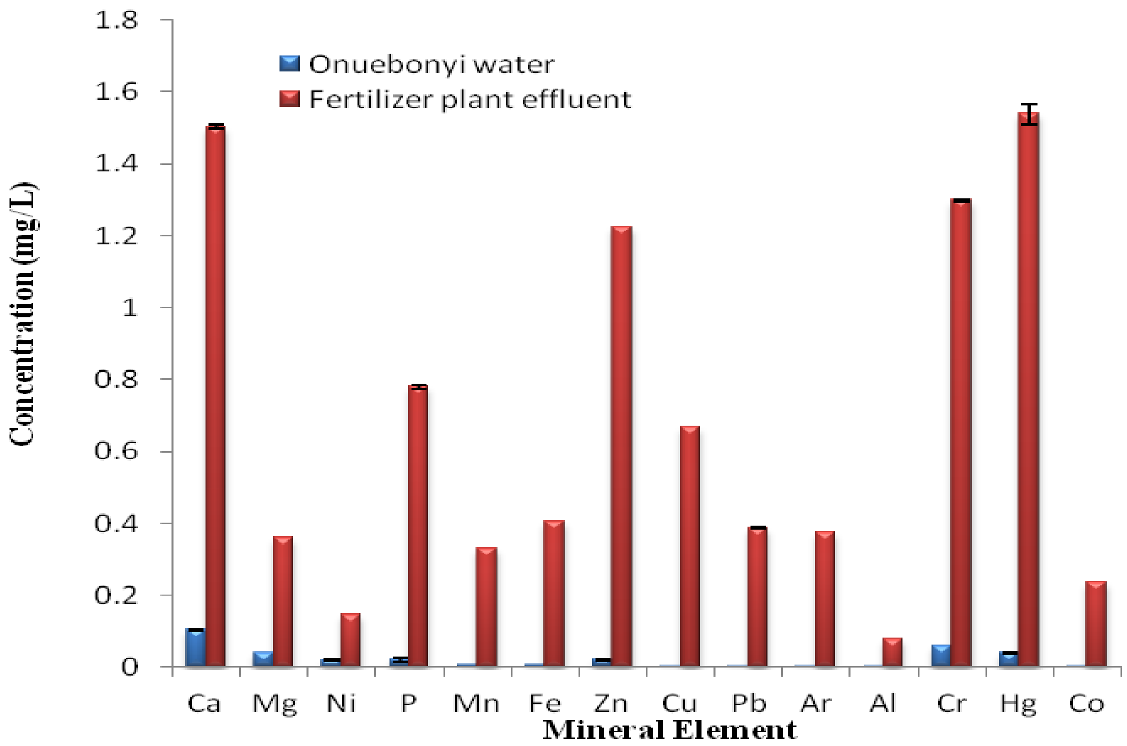

Figure 1. Comparison of the mineral elements status of Onuebonyi River water and Ebonyi Fertilizer and Chemical Plant effluent in dry season between 2010 and 2011 (values of Fe, Cu, Mn and Co are in x 101, while P is in x 102)

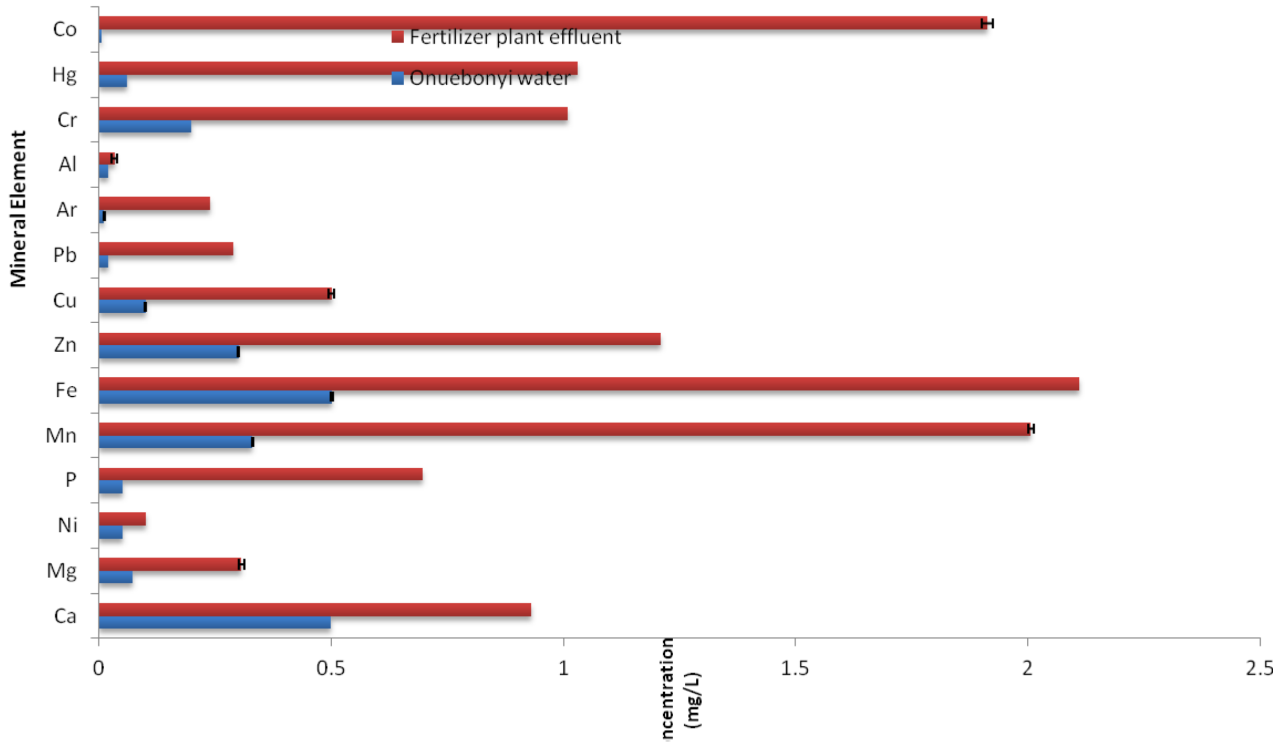

Figure 2. Comparison of the mineral elements status of Onuebonyi River water and Ebonyi Fertilizer and Chemical Plant effluent in rainy season between 2010 and 2011 (values of P are in x 102, while Cu are in x 101)

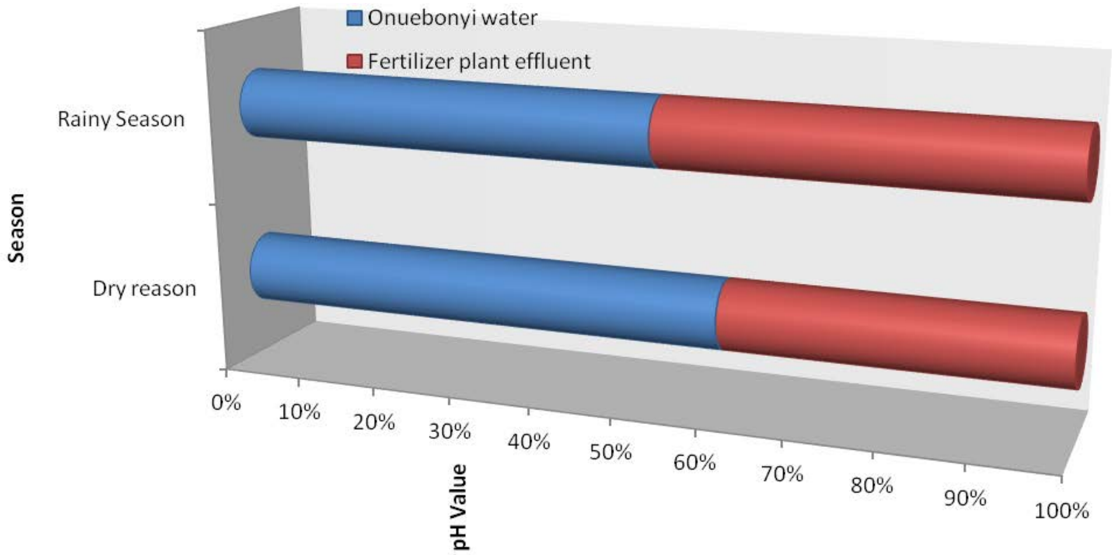

Figure 3. Comparison of the pH values of Onuebonyi River water and Ebonyi Fertilizer and Chemical Plant effluent in rainy season between 2010 and 2011

4. Discussion

The study analyzed bacteriologically and physicochemically, the Ebonyi Fertilizer and Chemical Plant effluent and the nearest water body, Azuiyiokwu Creek with a view to determining the microbial load of the effluents and their physicochemical contents.

The results from the control creek revealed that there was a gradual increase in bacterial load and some metal elements in Azuiyiokwu along the location of EFCP which could be as a result of infiltrations of effluents from EFCP. The control result also showed that some of the elements like Ca, Co, Fe, Zn, Cr, Ar, and Al were relatively at the same range of value in both Azuiyiokwu and the Control Creeks, but considerably high in EFCP effluent, while pH was relatively higher in Azuiyiokwu and control Creek through the seasons; this shows that the effluent was highly acidic. This could be as result of metabolic processes going on in the effluents between microorganisms and nutrients.

By Pearson simple correlation analysis of the metals detected from the fertilizer plant and Azuiyiokwu Creek showed that strong positive correlations were observed between Mg and Ca (r=0.99959), which also varies with other metals, Ni/Ca (r=0.001). All the elements had negative correlations with pH showing that they do not affect the pH. Statistically, there was a significant difference ($P \leq 0.05$) in comparison between the Azuiyiokwu creek and EFCP both bacteriologically and physicochemically. The bacterial load of Azuiyiokwu creek fluctuated seasonally whereas that of EFCP effluent was high in the rainy season and decreased in the dry season. Low bacterial load observed from the Azuiyiokwu Creek in dry season could be as a result of self purification process taking place in the water and reduction of water in-flow from EFCP effluents. This could be as a result of low fertilizer production in dry season.

Low bacterial count of 4.9 x10^6 cfu/ml was observed from the effluent sample in dry season. This could be attributable to some factors like adverse weather condition including high temperature, pH, and pressure, due to lack of space both for growth and metabolic activities. The condition can lead to formation of spores by spore-formers and many other bacteria organisms going into dormant stage. On the other hand, unfavorable condition cannot only affect the growth of microorganism but can lead to death of some organisms.

The microbial count was reasonably greater in the rainy season. Microbial count of 9.7 x10^6 cfu/ml was observed from the same sample in the rainy sample. This increase could be attributable to increase in production, favorable weather condition and availability of nutrients. When the temperature and other physical parameters were at their optimum requirement, the multiplication and metabolic processes could increase microbial load. These favorable conditions can bring about germination of the spores, making available favorable environment for microbial growth. This result agreed with the report of Obire *et al*. [2] who reported reduction in the population of organisms in the NAFCON effluents in the dry season, as a result of some environmental changes such as increased temperature and excessive water evaporation leading to reduction of the effluent volume, which later increased as a result of rain falls.

The high microbial count of 8.2 x10^6 cfu/ml of Azuiyiokwu in rainy season against 1.9 x10^6 cfu/ml observed in the dry season shows that rain could be a major agent of transport between the fertilizer plant effluent and town drainage systems into the Azuiyiokwu Creek. The result of the Control Creek revealed that there was a sudden increase of physicochemical parameters (Mg, Mn, Fe, Cu, and Hg) of Azuiyiokwu creek in the rainy season. Comparing the Azuiyiokwu results with the control and EFCP which was constantly high in physicochemical parameters and bacterial load, it could be said that the location of EFCP could be responsible for these changes in bacterial and physicochemical qualities of Azuiyiokwu Creek. On the other hand, it could be said that self purification contributed in the reduction in the number of microorganisms, thereby encouraging the residence organisms in the degradation processes in dry season. The isolation of such organisms as *Bacillus* sp, *Pseudomonas, Klebsiella, Proteus, Samonella Staphylococcus*, *Escherichia*, *Aeromonas* and *Vibro* from both the effluents and the Creek accounted for the autochthony of these organisms. Gram negative rods were more prevalent in both the effluents and the creek. This could be attributed to the abundance of enterobacteriaceae family which were major contaminating organisms.

The prevalence of Coliform bacteria like *E. coli* and *Salmonella* in the Creek in rainy season could be attributed to a high level of faecal contamination of the Creek. There was a significant difference ($P\leq 0.05$) in the total bacterial count of isolates as shown by the results of this study. The health implication follows that when they are ingested through drinking, consumption of raw vegetables washed with these contaminated water from the Creek, or by swimming, they can cause illnesses to the consumers. For example, *Salmonella* sp causes typhoid fever, E. *coli* is part of the gastrointestinal flora of normal humans but is also a common cause of urinary tract infections, and traveler’s diarrhea to mention but a few while *Vibro* sp causes a diarrheal disease called cholera.

This research revealed the physicochemical parameters of EFCP, Azuiyiokwu and the control Creek. Statistically, there was a significant difference ($P\leq 0.05$) between physicochemical parameters of EFCP effluents, Azuiyiokwu Creek and the control. The physicochemical analysis revealed that phosphorus and some heavy metal in their oxidized form entered the Creek from different sources, which could be natural or from human activities. The fertilizer and chemical plant sited near the Creek is considered to be one of the sources of infiltrations of these metals, since the result of EFCP effluents showed heavy content of these elements. This was in agreement with the research of carried out at Okirika Creek in Rivers State, Nigeria [2].

There was complete content of detected heavy metals in the dry season at Azuiyiokwu Creek while the concentrations of the metals at the EFCP effluents were very high and the least from the control Creek. This result was contrary to the report of Obire *et al.* on Okirika which revealed that the concentrations were high in dry seasons were in dry season [2].

In the rainy season, the concentration of the metals were high in the Azuiyiokwu water samples compared with the very low quantities obtained during the dry season, while the volume of the effluents at Ebonyi Fertilizer and chemical plant was increased and the quantities of the metals obtained from the samples were less in comparison with the high quantities of the metals when the volume of the effluents was low and highly concentrated (dry season).

The sudden increase of the metals in Azuiyiokwu Creek samples could be attributed to the washing off of the effluent materials from EFCP, town drainage systems and form the surrounding farmlands into the Creek by rain water. It could also be said that reduction in production in the dry season, reduced the rate at which effluents are being discharged and evaporations could be some of the contributing factors [2]. Rain water serves as a major vehicle that conveys discharged pollutants to the environment.

Water salinity, which has a significant influence on the species composition of the microbiota, was considerably normal, capable of accommodating varieties of organism. Most metals like nickel, mercury, zinc, copper, lead, magnesium, and iron analyzed, were present in an infinitesimal amounts (less than 1.0 mg/l) in all the water samples from Azuiyiokwu Creek against the maximum standards stated by WHO [21] which ranged as follow (Fe=0.1-1.0, Zn=5.0-1.5, Hg=0.1, and Pb=0.05). The case was different in the Ebonyi Fertilizer and chemical plant effluents: iron, Zinc, copper, mercury, phosphorus, chromium and cobalt were very high, (4.059 mg/l, Zn=1.226 mg/l, Cu=6.69 mg/l, Co=2.356 mg/l, and Hg=1.504 mg/l). This result reveals that EFCP produces large quantity of these metals which endangers the surrounding environment.

5. Conclusion

The survival of man in a given geographical area depends on the availability and quality of water in the area. Despite the volume of water bodies in Nigeria, there is still problem of water as a result of man and natural activities which make the available water unavailable for use. Naturally, water is second only to air among the most important resources for human existence, but is the most threatened aspect of life. Results from this study shows that the effluents received by Azuiyiokwu creek have low bacteriological and physicochemical qualities and the range of microorganisms isolated in this study raise more serious concern about the public health implications. This reflects the possible pathetic conditions of most water bodies in Nigeria. Therefore, adequate measures such as proper effluents management, intermittent microbiological analysis and constant monitoring of water bodies receiving industrial effluents, as well as educating the populace on appropriate waste management techniques are hereby recommended as they would help to ameliorate the deplorable state of our water bodies.

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