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Microbiological and Physicochemical Characteristics of Effluents from Cocoa Processing Industry, Ile-Oluji, Nigeria

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Abstract

This study investigated the microbiological and physicochemical properties; and heavy metal content; of effluent from the Cocoa Processing Industry, Ile-Oluji, Nigeria. Effluent samples from the reservoir (sample A) and discharge point (sample B) were aseptically taken and analysed for microbial properties, pH, conductivities (EC), total dissolved solids (TDS), total suspended solids (TSS) and heavy metal contents using standard methods. The data generated were subjected to Analysis of Variance and significance accepted at $p<0.05$. The probable organisms isolated from the two effluent samples and their percentages of occurrence were *Escherichia coli* (25%), *Salmonella* spp (25%), *Shigella* spp (12.5%), *Staphylococcus aureus* (12.5%) and *Proteus* spp (25%). The pH, EC, TDS and TSS were 6.77 and 7.90; 3420.33 and 52019.00 mS/cm; 480.33 and 400.00 mg/L; 40.00 and 18.50 mg/L for samples A and B, respectively. The freshly discharged effluent (Sample B) was higher in all the heavy metals, except nickel, than effluents from reservoir (sample A). The results from this study suggested that the effluents from the industry should be adequately treated before being discharged into the environment to forestall outbreak of diseases and heavy metal poisoning.

Keywords – Cocoa; effluent; microbiological and physicochemical characteristics

I. INTRODUCTION

Undoubtedly, industrialization in the developing world is accompanied by economic prosperity through wealth and job creation but concurrently exerts stress on life supporting systems such as atmospheric air and water bodies [1]. Industrialization aggravates environmental degradation [2]. While, effluents from industries discharged into a water body represent significant source of pollution in many Nigerian rivers. They have considerable deleterious effects on aquatic macro- and microflora alike [3]. Reference [4] noted that heavy metals from industries remain a major source of contamination of groundwater system in developing countries. Also, pathogenic microorganisms constitute the major contributors to numerous water-borne disease outbreaks with costly long-term effects. To mitigate this, indicator organisms are frequently used as a tool for determining the magnitude of risk the presence of a particular pathogen in wastewater can pose [5].

A number of studies have been conducted on effluents from cocoa processing industries. For instance, Akinnusotu and Arawande [6] conducted a study on the physicochemical properties, heavy metals and microbial load of effluents from different three cocoa processing factories in Nigeria, while Ogunleye and Izuagie [7] assessed the heavy metal contents in some industrial effluents including a cocoa processing industry. Hitherto, there is scanty work on the microbiological and physicochemical properties of effluents from different points within a cocoa processing industries. Therefore, this work was geared towards characterizing the effluents from reservoir and discharge point with a view of assisting the cocoa processing industries to take informed decisions as regards disposal of effluents for environmental safety.

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II. MATERIALS AND METHODS

A. Study area

The effluent samples were collected from cocoa processing industry, Ile oluji, Nigeria. The town lies between longitudes 6º40 N, and 7º14 N, and latitudes 4º38 E and 4º53 E.

B. Preparation of media

The media used (nutrient agar and citrate agar) were weighed and prepared according to manufacturer's specification. The prepared media was carefully packed into the autoclave and sterilized at 121 ºC for 15 minutes. Prior to use, the media were cooled to about 45 ºC.

C. Isolation and identification of isolates

Plate growths were noticed after 24 hours of incubation, the isolates were then sub-cultured on fresh media plates until pure isolates were observed. The pure culture of isolates were stocked into MacCartney bottles. The isolates were identified based on their morphological appearance [8].

D. Gram staining techniques

A thin smear was made by emulsifying a little portion of organisms picked from stocked colony of 18–24 hours old pure culture into a drop of sterile distilled water on a grease free slide. The smear was air dried and heat fixed by passing it slightly over flame. The slide was carefully placed on the staining rack and was flooded with primary stain (crystal violet) for 30–60 seconds. Gram's iodine was added (mordant) for 30 seconds. The smear was gently rinsed with tap water. 70% ethanol was applied as decolouriser for 10–30 seconds; it was the stained with the secondary stain (safranin) for 30 seconds before rinsing with tap water and was allowed to dry. The smear was examined under the microscope using oil immersion objective (x100). Gram positive organisms appeared purple while Gram negative appeared red.

E. Biochemical characterization of the isolates

These tests were carried out to further identify and classify the isolates. They include; catalase test [8], citrate utilization test [9], indole Test [10], methyl red test, urea hydrolysis (urease test) [9], Voges Proskaurer test, sugar fermentation test (glucose, sucrose, lactose, galactose, maltose and fructose) [11], respectively.

F. Sugar fermentation test

The carbohydrate fermentation test is used to determine whether or not bacteria can ferment a specific carbohydrate. Carbohydrate fermentation patterns are useful in differentiating among bacterial groups or species. It tests for the presence of acid and/or gas produced from carbohydrate fermentation. Basal medium containing a single carbohydrate source such as glucose, lactose, sucrose or any other carbohydrate is used for this purpose. A pH indicator bromothymol blue (BTB), is also present in the medium; which will detect the lowering of the pH of the medium due to acid production. Small inverted tubes called Durham tube is also immersed in the medium to test for the production of the gas (hydrogen or carbon dioxide). It is a positive test for all members of *Enterobacteriaceae*.

G. Indole test

This test is used to identify microbes that can break down tryptophan to indole. It is used to identify bacteria of the family *Enterobacteriaceae*. Innocuate sterilized tubes containing tryptophan broth (4 ml) and incubate tubes for 24–28 hrs. After which 0.5 ml of Kovac's reagent is added. Presence/absence of ring indicates positive/negative test.

H. Citrate utilization test

This test is often used to differentiate organisms that are capable of utilizing citrate as a carbon source. Simmon's citrate agar medium was prepared in bijou bottle and allowed to set in a slanting position. A sterile wire loop was used to inoculate the test organism on to the slant medium and incubated at 37 ˚C for 48 hours after which it was examined for color change. A bright blue color in the medium gave a positive citrate test.

I. Catalase test

This test is used to identify organisms that produce the enzyme catalase. This enzyme detoxifies hydrogen peroxide (H_2O_2) by breaking it down into water and oxygen gas. This test demonstrates the presence of catalase, an enzyme characterized with the release of oxygen from hydrogen peroxide. A drop of 3% hydrogen peroxide solution was added to the sterile slide containing a loopful of the organism. Foaming or bubble indicates a positive result.

J. Urease test

This is used to identify those organisms that are capable of hydrolysing urea (bacteria that produce urease) to produce ammonia and carbon dioxide. It is primarily used to distinguish urease-positive protease from other Enterobacteriaceae. Organisms that hydrolyze urea rapidly (Proteus spp., *Morganel lamorganii*, and some *Providencia stuartii* strains) will produce strong positive reactions within 1 or 6 hours of incubation; delayed positive organisms (e.g. *Klebsiella* spp.and *Enterobacter* spp.) will produce weak positive reactions in the slant in 6 hours of

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incubation which will be intense during further incubation. The culture medium will remain a yellowish color if the organism is urease negative e.g. *Escherichia coli*. If organism produces urease enzyme, the color of the slant changes from light orange to magenta. If organism do not produce urease the agar slant and butt remain light orange (medium retains original color).

K. Methyl red test

Methyl red broth was prepared and autoclaved in a test tube at 121 ºC for 15 min. The broth was allowed to cool and a colony of organism was inoculated into it and incubated at 37 ºC for 24 hours. About 2-3 drops of methyl red reagent were added to it. A positive result shows the presence of red ring in the test tubes while a negative result does not.

L. Voges-Proskaurer test

Voges-Proskaurer test was carried out by preparing MRVP broth or Clark and Lub's media in test tubes. The broth was inoculated aseptically with 2 loopful of respective bacterial culture. The test tubes were labelled with the name of organism inoculated. This was followed by incubation at 37 ºC for 48 h. Barrit's reagent A (α-napthol) and Barrit's reagent B (40% KOH) in the ratio 3:1 were added. The cotton plug was removed and the tubes were shaken for aeration. The result was observed after 15 min. Pinkish red colour at the surface was taken to be positive result.

M. Determination of physicochemical properties

1) Determination of pH

The pH of the collected samples were determined in the laboratory to cross check the field results using pH meter (Jenway 3505 model) after calibration with standard buffer solutions of pH 4 and pH 7 [12].

2) Determination of electrical conductivity

Electrical conductivity (EC) was measured using a portable Hanna Combo (combined) meter.

3) Determination of total suspended solid (TSS)

Filter paper was weighed and labelled A and B, dried in oven at 105 ºC for 30 mins and weighed again. 50 ml of the samples were measured and allowed to pass through the filter paper into a beaker and the filter paper was dried in the oven and was weighed.

4) Determination of total dissolved solid (TDS)

Evaporating dish was weighed and 50 ml sample was dispensed into the evaporating dish and the evaporating dishes were oven dried and weight of respective dishes were measured.

5) Determination of total solid (TS)

A 50 mL sample was weighed in petri-dish. The petri-dish and the sample was then transferred into the oven and dried at 105 ºC to a constant weight for 24 hours. The petri-dish and its content was later transferred into dessicator, cooled, weighed and calculated as described by Hewitt [12].

N. Determination of selected minerals

The selected minerals were determined as described by AOAC [13] by using atomic absorption spectrophotometer (Buck scientific, USA).

O. Statistical analysis

All determinations were carried out in triplicates and error reported as standard deviation from the mean. All data was subjected to analysis of variance and significance accepted at $p<0.05$. The means were separated using Fisher's least Significant difference test with Minitab statistical software package (version 21.1.0).

III. RESULTS

The cultural and biochemical characteristics of the isolates from cocoa processing effluents are presented (Table 1). The probable organisms and their percentage occurrence were *Escherichia coli* (25%), *Salmonella* spp. (25%), *Shigella* spp (12.5%), *Staphylococcus aureus* (12.5%) and *Proteus* spp. (25%).

The physico-chemical properties of the effluent samples are presented (Table 2). The pH of the two effluents ranged from 6.77 to 7.90 with effluents from reservoir (Sample A) having the lower value and the freshly discharged effluent (Sample B) having the higher value.

For electrical conductivities (EC) were 3420.33 mS/cm and 52019.00 mS/cm for sample A and B, respectively.

While, the total dissolved solids (TDS) for samples A and B were 480.33 mg/L and 400.00 mg/L, respectively. The total suspended solids (TSS) were 40.00 and 18.50 mg/L for samples A and B, respectively.

The selected heavy metals in the effluents are presented in Table 3. The freshly discharged effluent (Sample B) was higher in all the metals, except nickel, than effluents from reservoir (sample A).

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Table 1. Cultural and biochemical characteristics of the isolates from effluents in Cocoa Industry, Ile-Oluji

Test	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6	Isolate 7	Isolate 8
Gram staining	- rod	+ cocci	- rod	- rod	- rod	- rod	- rod	- rod
Catalase test	+	+	+	+	+	+	+	+
Methyl red test	+	+	+	+	+	+	+	+
Voges proskaurer	-	+	-	-	-	-	-	-
Urease	-	+	+	-	+	-	+	+
Citrate	-	+	+	-	+	-	+	+
Gas production	+	+	+	+	+	+	+	+
Indole	+	-	+	+	-	-	-	+
Galactose	+	+	+	+	v	v	v	+
Fructose	-	+	+	-	v	v	v	+
Sucrose	+	+	-	+	-	-	-	-
Mannitol	+	+	-	+	+	+	-	-
Organism	*Escherichia coli*	*Staphylococcus aureus*	*Proteus* spp.	*Escherichia coli*	*Salmonella* spp.	*Shigella* spp.	*Salmonella* spp.	*Proteus* spp.

Key: - = negative; + = positive; v = variable

Table 2. Physicochemical properties of effluents in Cocoa Industry, Ile-Oluji

Parameter	Sample A	Sample B
pH	6.77 ± 0.05^{b}	7.90 ± 0.05^{a}
Total suspended solid (mg/L)	40.00 ± 0.00^{a}	18.50 ± 0.10^{b}
Conductivity (mS/cm)	3420.33 ± 0.57^{b}	52019.00 ± 1.00^{a}
Total dissolved solids (mg/L)	480.33 ± 0.57^{a}	400.00 ± 0.00^{b}
Total solids (mg/L)	520.33 ± 0.57^{a}	418.50 ± 0.10^{b}

Values are Mean± SEM
Values with different alphabets within the row are significantly different $P<0.05$

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Table 3. Selected heavy metals (mg/Kg) of effluents in Cocoa Industry, Ile-Oluji

Heavy metal	Sample A	Sample B	FME limit	NESREA limit
Cu	0.833±0.06[a]	0.407±0.01[b]	<1.00	0.5
Cd	0.002±0.00[b]	0.098±0.00[a]	-	0.2
Ni	0.010±0.00[b]	0.120±0.00[a]	-	0.01
Cr	0.002±0.00[b]	0.170±0.00[a]	-	0.05
Pb	0.000±0.00[b]	0.053±0.00[a]	<1.00	0.05

Values are Mean± SEM
Values with different alphabets within the row are significantly different $P < 0.05$
Cu = copper, Cd = cadmium, Ni = nickel, Cr =chromium, Pb = lead
FME = Federal Ministry of Environment, NESREA = National Environmental Standard and Regulation Enforcement

IV. DISCUSSION

The organisms have been reported by several authors to be causative organisms for several human and animal diseases. *Escherichia coli*, *Salmonella typhi* and *Shigella* species, are the most common food-borne disease causing organisms in developing countries [14]. The diseases such as diarrhea, dysentery and arthritis are caused by *Staphylococcus*, *Salmonella*, *Shigella* [15]. While hemorrhagic uremic syndrome could be caused by *Escherichia coli* and *Shigella* spp. [15]. *Proteus* spp., apart from being implicated in urinary tract infection, is one of the most important bacterial species associated with histamine poisoning in fish [15]. In most cases, foodborne illnesses are fatal as well as cause suffering, discomfort, and debilitation among the survivors with associated huge economic losses [15]. Therefore, effluents from cocoa processing industries should be treated before being discharged into the environment to forestall outbreak of diseases as indiscriminate discharge of untreated or poorly treated domestic and industrial wastewater effluents are major contributors to surface water pollution with its attendant health implications [16, 17].

The pH range is similar to the range (7.03 - 8.98) reported for wastewater from sewage works [18] but higher than the range (6.00 - 6.60) for effluents from some cocoa processing industries [6]. However, the pH values are within the recommended effluent discharge limit (6.00 – 9.00) stipulated by environmental regulatory agencies in Nigeria [19].

The high EC, especially for sample B, obtained in this study could be as result of high amount of dissolved ions in the effluents. The EC of the surface water is a valuable indicator of salinity with total salt content [18]. The EC values are higher than 60 - 1,095 mS/m reported for wastewater effluents in some communities in Africa [18] and 480 - 660 µs/cm for effluents from some cocoa processing plants [6].

The TSS for the samples were within the range (60-701 mg/L) reported for wastewater effluents in some communities in Africa [18], higher than the range (198 – 320 mg/ L) reported for effluents from some cocoa processing factories in Nigeria [6], and lower than range (848 – 1840 mg/L) for textile industries' effluents in Kaduna (Yusuf and Sonibare, 2004). However, the values for both samples are lower than the recommended limit (500 mg/L) stipulated by the National Environmental Standards and Regulation Enforcement Agency [20]. A high TDS could be lethal to aquatic organisms, leading to osmotic shock thereby, affecting the osmoregulatory strength of the organism [21]. The concentrations of TDS in irrigation water hinder plant growth, crop yield, and quality of product [22].

The difference in the TSS values could be as a result of precipitation of some solutes during stop-over of the effluent in the reservoir. The values for both samples are comparable to the range (24.80 – 30.20 mg/L) reported by Akinnusotu and Arawande [6] for effluents from different cocoa processing factories in Nigeria. While the TSS value for sample A is higher than the recommended limit (30 mg/L) that of sample B was lower than the limit stipulated by the Federal Ministry of Environment [6]. Meanwhile, the total solids (TS) for samples A and B were 520.33 mg/L and 418.50 mg/L, respectively. These values were lower than 116.60 – 350.20 mg/L reported by Akinnusotu and Arawande [6] for effluents from different cocoa processing factories in Nigeria. The high TSS and TS is an indication of contamination as

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the solid materials may play host to different microorganisms.

For heavy metal contents, leaving the effluents in reservoir for a while could lead to reduction of the minerals in the effluents. The heavy metal contents of both samples are lower than the values (2.32, 0.14, 0.41, 1.00 and 0.9 mg/Kg for copper, cadmium, nickel, chromium and lead, respectively) reported by Okunola et al. [20] for wastewater from cocoa processing plant. The values for both samples are within the limits stipulated by the Federal Ministry of Environment. For sample A, the copper (Cu) content was above the limit stipulated by National Environmental Standard and Regulation Enforcement (NESRA) for effluent discharge. In the case of sample B, copper and cadmium contents were lower, while nickel (Ni), chromium (Cr) and lead (Pb) contents were higher, than the limits stipulated by NESRA for effluent discharge. Several authors have reported on the harmful effects of heavy metals including the ones involved in this study. For instance, Pb is highly toxic which has adverse effects on the neurological, biological, and cognitive functions in the bodies [23]. Cr can cause dermal, renal, neurological, GI diseases; and several cancers including lungs, larynx, bladder, kidneys, testicles, bone, and thyroid [24]. While Cd contamination of food and water supplies causes painful degenerative bone disease, kidney failure, GI and lungs diseases [25]. There is therefore, a need for treating the effluents, especially sample A, to reduce the levels of the heavy metals to meet the stipulated standards by regulatory agencies as they have harmful effects on human health [26].

V. CONCLUSION

This study showed that effluents from cocoa processing industry, Ile-Oluji, were laden with pathogenic microorganisms such as *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus* and *Proteus* spp. Although, the pH of the effluents were within the stipulated limits set by regulatory agencies, the TSS and TS values of both sample were high. The heavy metal content of sample B was higher than the stipulated limits set for nickel, chromium and lead by NESRA for effluent discharge. The effluents should be adequately treated before being discharged into the environment to forestall outbreak of diseases and heavy metal poisoning.

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