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Bacteriological and Physiochemical Characterization of Brewery Effluents

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ABSTRACT

The brewing industry is one of the largest users of water. It has been documented that approximately 3 to 10 litres of wastewater is generated per litre of beer. Bacteriological and physicochemical quality assessments were carried out on samples collected from 3 different sampling sources: effluent (E), contaminated soil (S) and uncontaminated soil (CL). Bacteria colony counts from sample E (brewery effluent) ranged from 4.0 x 108 cfu/ml to 19.0 x 108cfu/ml and its receiving farm land (S) from 16.0 x 108cfu/mi to 25.0 x 108cfu/ml, while the uncontaminated farm land (CL) from 9.0 x 108cfu/ml to 15.0 x 108cfu/ml respectively. Microorganisms isolated from sample E include *Bacillus* sp, *Staphylococcus* sp, *Escherichia coli, Enterobacter* sp and *Proteus* sp. While those isolated from samples S and CL include *Salmonella* sp, *Bacillus* sp, *Staphylococcus* sp, *Escherichia coli, Klebsiella* sp, *Enterobacter* sp, and *Proteus* sp and *Micrococus* sp. Results obtained indicated that the effluent samples had unpleasant odour and was dark in colour while pH were within the Federal Ministry of Environment permissible limit of 6 to 9 but the pH were below the permissible level of pH values for effluent discharge into the body of water by the World Health Organization (WHO) which is pH of 6.5 to 8.5 and the water must be colourless and odourless. The concentrations of all heavy metals in the effluents were within acceptable limit stipulated by the World Health Organization (WHO) and the Federal Ministry of Environment (FME) except iron and nitrate which were above th3e acceptable limit stipulated by the WHO and FME while concentration of heavy metals on contaminated soil was slightly higher than the uncontaminated soil. In this study, even though the level of some heavy metals were within permissible limit, the discharge of such effluent with unpleasant odour near residential house may make people residing there uncomfortable and may also serve as a breeding site for mosquitoes.

Keywords: Bacteriological, Physiochemical, Characterization, Brewery, Effluentts

INTRODUCTION

The United States Environmental Protection Agency (USEPA) defined effluent as the "wastewater-treated or untreated – that flows out of a treatment plant, sewer, or industrial outfall. It is mainly refers to as liquid wastes discharged into surface waters" (Environmental Protection Agency, 1997). Effluents is a form of point pollution and can be said to be liquid waste discharged into the environment [1]. It can also be referred to as wastewater discharge on farm land.

The brewery industry is one of such establishment that produces reasonably huge quantities of wastes such as spent hops, spent grain, and yeast. Though, the majority of these wastes are environmental friendly because they are agricultural products and they can be recycled and reused without problems. Consequently, comparing to other industrial wastes the brewing industry waste tends to be more pleasant in the environment [2]. There is report that about 3 to 10 litres of wastewater are being generated per litre of beer produced [3].

In developing countries, the fraction of treated effluents being discharged into water bodies such as rivers has increased resulting in high densities of pathogenic bacteria such as *Salmonella typhimurium, Vibrio cholera, Legionella, Escherichia coli*

O157:H7 and *Campylobacter jejuni* in these water bodies [4]. The existence of disease causing microorganism in water constitutes a major health concern.

Wastes are generated by various human activities, and inappropriate management of the large quantities of these wastes poses serious problems to underdeveloped countries. More challenging is the insecure discharge of these vast wastes into the immediate environments. Water bodies, particularly freshwater reservoirs are mainly affected. This has frequently makes these water bodies inappropriate for some purpose and activities carried out in water [5].

This effluent generated from industrial activities requires efficient treatment before disposal into the immediate environment due to its extremely polluting properties [6]. If these wastes are discharged into the body of water without proper treatment, they would cause severe environmental pollution.

Effluent from brewing industry evolves from liquors pressed from grains and yeast recovery and have distinctive odour of fermented malt and a little bit acidic [7]. Brewery effluent samples contain elevated amount of carbohydrates; nitrogen and the washing and cleaning detergents have been confirmed pollutants of water. Disposal of such effluent with elevated organic material and major nutrients into the body of water can change the microflora of the water bodies [8]. It is obvious that water is a natural resource that has various uses, for instance transportation, recreation, power generation, domestic, commercial and industrial uses and therefore should be managed well. Numerous forms of life are supported by water and therefore can directly of indirectly affect health and lifestyle of individual as well as economic well being of any nation.

The fact that the effect of constant industrial effluent discharged on our agricultural soil has not been given the appropriate attention it deserves, may be due to lack of knowledge of its consequence [9]. It is possible that these industrial that use vast quantities of water for some activities have the ability to contaminate water bodies through the release of this waste into rivers and streams or by run-off and percolation of water disposed on the farm land into the ground water sources.

Industrial effluent sample that are responsible for pollution of aquatic environment has become main problem of underdeveloped as well as highly populated country like Nigeria. Fresh water bodies, which are the main drinking water sources in Nigeria, are frequently polluted by the actions of the adjoining populations and industrial activities. Water bodies are the main way for discharge of wastes, especially the brewery effluent samples, from the brewery establishments that are close to them. This brewery wastewater sample from brewing industry have enormous influence on the pollution of the water body and can change the chemical, biological and physical characteristics of the water body that receives it. As a result water bodies have been extremely contaminated. The consequential problem of this on public health and the environment are typically immense in magnitude [10].

Elevated quantities of effluent in water bodies can cause an increase in chemical oxygen demand (COD), biological oxygen demand (BOD), total suspended solids (TSS), total dissolved solids (TDS), toxic metals such as Cadmium, Chromium, Nickel and Lead and faecal coliforms and therefore render the water unfit for various purposes they are used for [11]. Wastewater disposal processes in Nigeria are very poor and society is in danger, particularly the developed parts of our country. [12] pointed out that the ministry of environment established to curb these problems associated wit the litigation of our environment has had little or no impact on the pollution control in our cities because of corruption.

Even though, more than three quarters of the earth's surface is made up of water, only 2.8% of water on earth is accessible for human consumption [13]. Fresh water is a limited resource, vital for industry, human existence and agriculture, without fresh water of sufficient amount and

value, sustainable development will not be obtainable. Rivers function essentially in conveying of urban and industrial wastes and run-off from agricultural land, the former cause the steady polluting source whereas the later is a seasonal phenomenon [14].

LITERATURE REVIEW

Industrialization is one the essential factors that improves the growth of a country's economy, via the instigation of firm and factories. Brewery discharge is the combination of different substances such as non-biodegradable organics matter, biodegradable organics matter, disease-causing organisms, metals, and suspended inorganic solids. The type and nature of effluent introduced into the environment by brewery industry is in line to its different activities [15]. Such discharges can affect the natural balance of the receiving environment if not properly treated before discharge and could bring about discolouration, odour and death of aquatic organism, despite rendering the water unsafe for various other activities. However, effluent from brewery industries could be disposed on the farm land and as well as bodies of water and thus really cause severe problems to human health and proper functioning of the environment [16].

Brewery industries have been documented to cause serious environmental problems through disposal of their waste in the immediate environment [17]. Water body is mainly affected and this is a serious problem worldwide, so, it should be seen as a matter of priority on how to control this problem as health of individual is related to the accessibility of potable water [18]; [19].

Constituents of Brewery Effluent

The constituents of brewing effluents vary significantly as it depends on different processes that take place within the brewery, but the amount of wastewater depends on the water consumption during the process. The main constituents of brewing liquid waste are chemical oxygen demand (COD), biochemical oxygen demand (BOD), total suspended solid, heavy metals, pathogens and others [20]. Inappropriate treated liquid wastes normally have chemical oxygen demand (COD) from 1800-3000 mg/l, nitrogen from 30-100mg/l, biochemical oxygen demand (BOD) from 1,000-1,500mg/l and suspended solids from 10-60 mg/l [21]. According to the work of [22], brewery effluent contains biochemical oxygen demand (BOD) in the range 20-260mg/l, total suspended solids in the range (TSS) 40-260 MG/L. [23] reported that brewery effluent contains 2.30mg/l biochemical oxygen demand (BOD) and 12.0mg/l total suspended solids (TSS). [24] reported that excess BOD level in a stream may cause reduction of dissolved oxygen level to the extent of causing stress to some stream lives and it may lead to high competition for oxygen with the ecosystem.

Metals

Cadmium (Cd)

Cadmium is one of the heavy metals that can affect life of terrestrial and aquatic organisms. Cadmium can cause a series of problems including cancer, malformation of embryo/fetus, toxic to fetus and also can cause increased blood sugar level among others [25]. Moreover, Cadmium has been reported to cause damages to both kidney and liver and malformation of skeletal structure [26]. According to the work of [27], the level of cadmium in brewery effluent range from 0.000-0.216mg/l. According to [28] who documented that liquid waste disposed from oxidation ditch into the immediate environment such as river had bring about high level of pollutants such as Cadmium on such river and therefore affecting the river seriously and cause severe problems to people that uses such rivers.

MATERALS AND METHODS

Materials

Equipment

The equipment used include, autoclave (Health Medical YX2800a, UK), weighing balance (Ohaus Triple beam No.2.7.29439, USA), Incubator (Newlife Medical NL-9052-1, UK), microscope (Olympus optical B0459781, Germany), hot air oven (Equitron Medical, 7025.JB.20, India), refrigerator (Haier Thermocool BD 198,

Germany), wire loop, gloves, conical flasks, Durham's tube, glass slides, Bijou bottles, glass slides, cover slip, Pasteur pipette, sterile test tubes, spatula, petri dishes, measuring cylinder, staining rack, test tube rack, marker pen, tripod stand, cotton wool, syringes, beakers, Bunsen burner, wire gauze, masking tape.

Media

The media used include; Nutrient agar MacConkey agar, Simmon's citrate agar (BIOTECH Laboratories Ltd, UK), Motility medium, Urea medium, and Indole medium.

Reagents

The reagents used include; ethanol, sodium hydroxide (NaOH), ethylenediamine tetraacetic acid (EDTA), normal saline, oxidase reagent, crystak violet stain, Lugol's iodine, acetone – alcohol decolourizer, kovac's reagent (Fischer Scientific Company, USA), peptone water.

METHODS

Study Area Description

Enugu is the capital of Enugu State in Nigeria. It is one of the states in Southeastern Nigeria with latitude 6^{0}28'N and longitude of 7033'E [29]. The city is well populated with inhabitants of 722.664 according to the 2006 Nigerian census. Enugu is located in a tropical rain forest zone with a derived Savannah [3]. They city has a tropical savanna climate. Enugu's climate is humid and this humidity is at its highest between March and November. For the whole of Enugu State, the mean daily temperature is 26.7 ^{0}C (80.1 °F) [12]. As in the rest of West Africa, the rainy season and dry season are the only weather periods that recurs in Enugu. The average annual rainfall in Enugu is around 2,000 millimetres (79 in), which starts sporadically and becomes very heavy during the rainy season [5]. Other weather conditions experienced in Enugu include harmattan, a dusty trade wind lasting from ending of November to January. Like the rest of Nigeria, Enugu is hot all year round.

Ama brewery is one of Nigeria breweries located at Amaeka Ngwo. 9th mile, Enugu East, Enugu, Nigeria. It began brewing on 22nd March, 2003 and their products are distributed all over Nigeria. Some of their brands are Star lager, Gulder lager. Legend extra stout, and Heineken lager.

Collection of Samples

Samples were collected from three (3) different sources: sample E (effluent), sample S (contaminated soil) and sample CL (control or uncontaminated soil). For the effluent sample, the samples were collected into 1 litre keg pre-disinfected with 75% ethanol and rinsed with sterile distilled water. Effluent from each sample source, sample E1-E6 was used to rinse the properly labeled keg twice before sample collection. For the soil samples, the samples were -~.td in a randomized manner into a sterile polythene bag with the aid of sterilized trowel. Samples El and E3 were collected directly from tanker which conveys the effluent from brewery before discharge on the farm land and time of collection was at 1 lam and 3pm respectively.

Samples E2 and E5 were collected immediately after discharge on the farm land and time of collection was at 10am and 1pm respectively.

Sample E4 was collected 2 hours after discharged on the farm land and time of collection was 2pm.

Sample E6 was discharged on the day time and allowed to stay overnight before the sample was collected on the following day at 11am.

Sample S1-S6 and sample CL1-CL6 were collected in a randomized manner and time of collection ranged from 3pm, 10am, ll:30am,2pm, 1pm and 11am respectively. All samples were transported in cooler boxes containing ice packs to Applied Microbiology Laboratory. Ebonyi State University, Abakaliki for analysis. All samples were analyzed within 24hrs of sample .Election [7].

Sample Processing

Ten fold serial dilutions were done and samples were collected from 10^{-7}, 10^{-8} and 10^{-9} dilution. Approximately 1 ml aliquot of each dilution was plated out on Nutrient and MacConkey agar and incubated at 37°C for 24hr. Growth was further sub-culture to obtain a pure culture [12].

Purification of Bacterial Isolates

Pure cultures were obtained by streaking different and distinct colonies onto sterile

nutrient agar plates. The pure cultures obtained were then transferred onto Nutrient agar slants in McCartney bottles and incubated at 37°C for 24 hours. The bottles were then stored in the refrigerator.

Characterization and Identification of Bacterial Isolates

The purified bacterial cultures were characterized on the basis of their colony morphology (colour, shape, elevation and optical characteristics), motility test and Gram's staining [1].

The identification of the test organisms were also done using biochemical techniques such as catalase test, coagulase test, indole test, oxidase test, Voges Proskauer test, and sugar fermentation test like glucose, lactose, maltose and sucrose.

Gram Staining

Gram staining was carried out according to the description of [14]. A drop of sterile distilled water was placed on clean grease - free slide with sterile wire loop sterilized by flaming. Small portion of each of the isolates was transferred into the sterile distilled water at the center and smears made. The smears were allowed to air dry and heat fixed after which they were flooded with crystal violet for 30 seconds. The smear was washed off with clean running tap and flooded with Lugol's iodine for 60 seconds. The smears were again washed off with clean running tap and acetone reagent was applied drop wise until no dye is coming off from the smears. The smears were covered with safranine for 30 seconds and then washed off under running tap. The slides were placed on a draining nick for the smears to air dry and examined microscopically with oil immersion at x100 objective.

Motility Test

The hanging drop method was used in which a small drop of the bacterial broth culture was placed on the cover slip using a sterile wire loop [6]. A thin film of vaseline was applied around the edge of the depression of the cavity slide. The cavity slide was

Gently inverted over the cover slip and pressed down, in order to make it airtight. The cavity slide was then observed under x 40 objective lens of the microscope. The directional movement of bacterial cells indicated motility while no movement of the bacterial cells indicated non-motility [14].

Biochemical Tests

Urease Test

Urea a common organic nitrogen source for many microorganisms, can be hydrolyzed to ammonia and carbon (IV) oxide. Urease is an enzyme that catalyzes the breakdown of urea to ammonia and carbon (IV) oxide, represented by the following equation:

$$H_2NCONH_2 \xrightarrow{\text{urease}} 2NH_3 + CO_2.$$

Bijou bottles containing 3 ml of sterile modified Christensen's urea broth was prepared by slanting. The slants were seeded with isolates and incubated at 37°C for 24 hours. The appearance of pink colour indicated a positive result [7].

Cataiase Test

This test was used to distinguish organisms that produce enzyme catalase from non-catalase producing bacteria. A 24-hour old culture was used to perform the test. A sterile wire loop was used to make a uniform suspension on a slide. A drop of hydrogen peroxide (H_2O_2) was applied to the suspension and the appearance of effervescence showed a positive reaction while its absence signifies a negative reaction [9].

Citrate Test

The test is used to prove the capacity of some bacteria to make use of citrate as their main .e of carbon. Slants of Simmon's citrate agar were prepared in Bijou bottles according to the manufacturer's description. Using a sterile straight wire loop, a saline suspension of the test Organism was first streaked on the slant and then stabbed (to create anaerobic condition). The bottles were incubated at 37°C for 72 hours. Alkaline pH shown by intense blue colour indicated citrate utilization [13].

Coagulase Test

This test was carried out as described by [18]. A slide was marked sections and a loopful of normal saline was placed on each of the marked sections. Smear of the colony of the test organisms was made on the slide and mixed until homogenous suspensions were obtained. A drop of

human plasma was added to one of the suspensions and observed for clumping after few seconds [23].

Indole Test

Peptone broth was used to carry out the test. The broth was prepared according to the manufacturer's description by adding 15g of peptone powder to 1 litre of distilled water. Five ml of the broth was dispensed into McCartney bottles and autoclaved at 121°C for 15 minutes. The medium was inoculated with the isolates and incubated at 35°C for 48 hours. After incubation, AS significant growth of the isolates. One ml of chloroform was added to the broth culture and shaken gently. Also 2 ml of Kovac's reagent was added and shaked gently. The bottles were left to stand on the bench for 20 minutes to enable the reagent to rise to the top. A red colouration at the top layer indicated indole production while yellow colouration indicated a negative result [2].

Oxidase Test

Freshly prepared oxidase reagent was used to carry out the test. After placing filter paper in a clean Petri dish, 2 drops of the oxidase reagent were placed on the filter paper. Using a sterile glass rod, a colony of each of the isolates was picked and smeared on the filter paper in the Petridish. The development of blue-black colour within few seconds on the filter paper indicated a positive result [4].

Voges Proskauer Test

This test relies on the capacity of the microorganism to produce acetoin from glucose. A kinfepoint of creatine was added to a 24 hour culture to be investigated followed by 5ml of 40% NaOH and snaked strongly for several minutes. The production of pink colour within 2 minutes indicates a positive result (NCCLS, 1999).

Sugar Fermentation Test

This test was used to know the capability of the bacterial isolates to ferment lactose, sucrose, maltose and glucose. Some quantity (0.5%) of each sugar was mixed with 5 ml of peptone water in different test tubes and 4 drops of 0.01% phenol red indicator was added and inverted Durham tubes were dropped into the test tubes to identify gas production. The test tube contents were autoclaved at 121°C for 15 minutes, after which they were inoculated with the pure isolates and incubated at 37°C for 5 days. Presence of gas at the bottom of Durham tube and colour change from red to yellow from the bottom to the top indicated positive result [6]; [7].

Physicochemical Analyses

In situ measurement of pH and temperature of the effluent was taken at the sampling points with a digital pH meter and mercury-in-glass thermometer respectively. Also colour was determined on site and other physicochemical parameters such as nitrate, phosphate, sulphate, zinc, lead, cadmium, copper, iron, manganese, chromium and nickel in the effluent samples were determined using Atomic Absorption Spectrophotometer (AAS) (Buck Scientific Model 210 VGP) at the PRODA, Enugu.

RESULTS

Table 1: Colony morphology of isolated cultures

Purified culture name	Shape (Bacteria)	Colour	Shape (Colony)	Elevation	Optical Characteristics
E1.C	Rod	Cream	Spherical	Convex	Translucent
E1.WY	Cocci	Whitish-yellow	Spherical	Convex	Translucent
E2.W	Rod	White	Spherical	Convex	Opaque
E2.WY	Cocci	Whitish-yellow	Spherical	Convex	Translucent
E3.DW	Rod	Dirty-white	Spherical	Flat	Translucent
E3.W	Rod	White	Spherical	Convex	Opaque
E4.C	Rod	Cream	Spherical	Convex	Translucent
E5.C	Rod	Cream	Spherical	Convex	Translucent
E6.WY	Cocci	Whitish-yellow	Spherical	Convex	Translucent
E6.C	Rod	Cream	Spherical	Convex	Translucent
S1.W	Rod	White	Spherical	Convex	Opaque
S2.Y	Cocci	Yellow	Spherical	Convex	Translucent
S2.M	Rod	Milky	Spherical	Convex	Opaque
S3.M	Rod	Milky	Spherical	Convex	Opaque
S4.GW	Rod	Grayish-white	Spherical	Convex	Opaque
S4.C	Rod	Cream	Spherical	Convex	Translucent
S4.DW	Rod	Dirty-white	Spherical	Convex	Translucent
S5.C	Rod	White	Spherical	Convex	Translucent
S5.DW	Rod	Yellow	Spherical	Flat	Translucent
S6.W	Rod	Milky	Spherical	Convex	Opaque
S6.Y	Cocci	Yellow	Spherical	Convex	Opaque
S6.M	Rod	Milky	Spherical	Convex	Opaque
CLI.C	Rod	Cream	Spherical	Convex	Translucent
CLI.DW	Rod	Dirty-white	Spherical	Flat	Translucent
CL2.C	Rod	Cream	Spherical	Convex	Translucent
CL2.WY	Rod	Whitish-yellow	Spherical	Convex	Translucent
CL3.W	Rod	White	Spherical	Convex	Opaque
CL4.M	Rod	Milky	Spherical	Convex	Opaque
CL5.Y	Cocci	Yellow	Spherical	Convex	Opaque
CL6.GW	Rod	Grayish-white	Spherical	Convex	Opaque

Key: E= Effluent, S = Soil contaminated with effluent, CL = Control or uncontaminated soil, GW= Grayish-white, DW – Dirty-white, M = Milky, C = Cream, W = White, Y = Yellow, WY = Whitish-yellow, - = Negative, + = Positive

Table 2: Identify of isolates from effluent base on Gram's stain, Motility and Biochemical tests

Sample source	Gram reaction	Cell morphology	Oxidase test	Citrate test	Catalase test	Indole test	Urease test	Coagulase test	Vp test	Motility test	Fermentation	Glucose	Sucrose	Lactose	Maltose	Tentative organism
E	+	R	-	-	+	-	-	-	+	+	F	A	-	A		*Bacillus* sp
E	+	C	-	+	+	-	-	+	-	-	F	A	A	A	A	*Staphylococcus* sp
E	-	R	-	-	-	+	-	-	-	+	F	A	-	A/G	A	*Escherichia coli*
E	-	R	-	+	+	-	+	-	+	+	F	A	A	-	A	*Proteus* sp
E	-	R	-	+	+	-	-	-	+	+	F	A/G	-	A/G	A/G	*Enterobacter* sp

Key: E= Effluent, - = Negative reaction, + = positive reaction, F = Fermentation, A = Acid, A/G = Acid and Gas, R = Rod, C = Cocci

Table 3: Identity of isolates from effluent contaminated soil base on Gram's stain, Motility and Biochemical test

Sample source	Gram reaction	Cell morphology	Oxidase test	Citrate test	Catalase test	Indole test	Urease test	Coagulase test	Vp test	Motility test	Fermentation	Glucose	Sucrose	Lactose	Maltose	Tentative organism
S	+	R	-	-	+	-	-	-	+	+	F	A	-	A	A	*Bacillus* sp
S	-	R	+	+	+	-	+	-	-	+	O	A	-	-	-	*Pseudomonas* sp
S	+	C	-	+	+	-	-	+	-	-	F	A	A	A/G	A	*Staphylococcus* sp
S	-	R	-	-	-	+	-	-	-	+	F	A	-	A/G	A	*Escherichia coli*
S	+	R	-	-	+	-	-	-	+	-	F	A	A	A	A	*Micrococcus* sp
S	-	R	-	+	+	+	-	-	-	+	F	A/G	-	-	A/G	*Salmonella* sp
S	-	R	-	+	-	-	+	-	+	-	F	A/G	A/G	A/G	A/G	*Klebsiella* sp
S	-	R	-	+	+	-	+	-	+	+	F	A	A	-	A	*Proteus* sp
S	-	R	-	+	+	-	-	-	+	+	F	A/G	-	A/G	A/G	*Enterobacter* sp

Table 4: Identity of isolates from soil uncontaminated with effluent base on Gram's stain, Motity and Biochemical tests

Sample source	Gram reaction	Cell morphology	Oxidase test	Citrate test	Catalase test	Indole test	Urease test	Coagulase test	Vp test	Motility test	Fermentation	Glucose	Sucrose	Lactose	Maltose	Tentative organism
CL	+	R	-	-	+	-	-	-	+	+	F	A	-	A	A	*Bacillus* sp
CL	-	R	+	+	+	-	+	-	-	+	O	A	-	-	-	*Pseudomonas* sp
CL	+	C	-	+	+	-	-	+	-	-	F	A	A	A/G	A	*Staphylococcus* sp
CL	-	R	-	-	-	+	-	-	-	+	F	A	-	A/G	A	*Escherichia coli*
CL	+	R	-	-	+	-	-	-	+	-	F	A	A	A	A	*Micrococcus* sp
CL	-	R	-	+	+	+	-	-	-	+	F	A/G	-	-	A/G	*Salmonella* sp
CL	-	R	-	+	-	-	+	-	+	-	F	A/G	A/G	A/G	A/G	*Klebsiella* sp
CL	-	R	-	+	+	-	+	-	+	+	F	A	A	A	A	*Proteus* sp
CL	-	R	-	+	+	-	-	-	+	+	F	A/G	-	A/G	A/G	*Enterobacter* sp

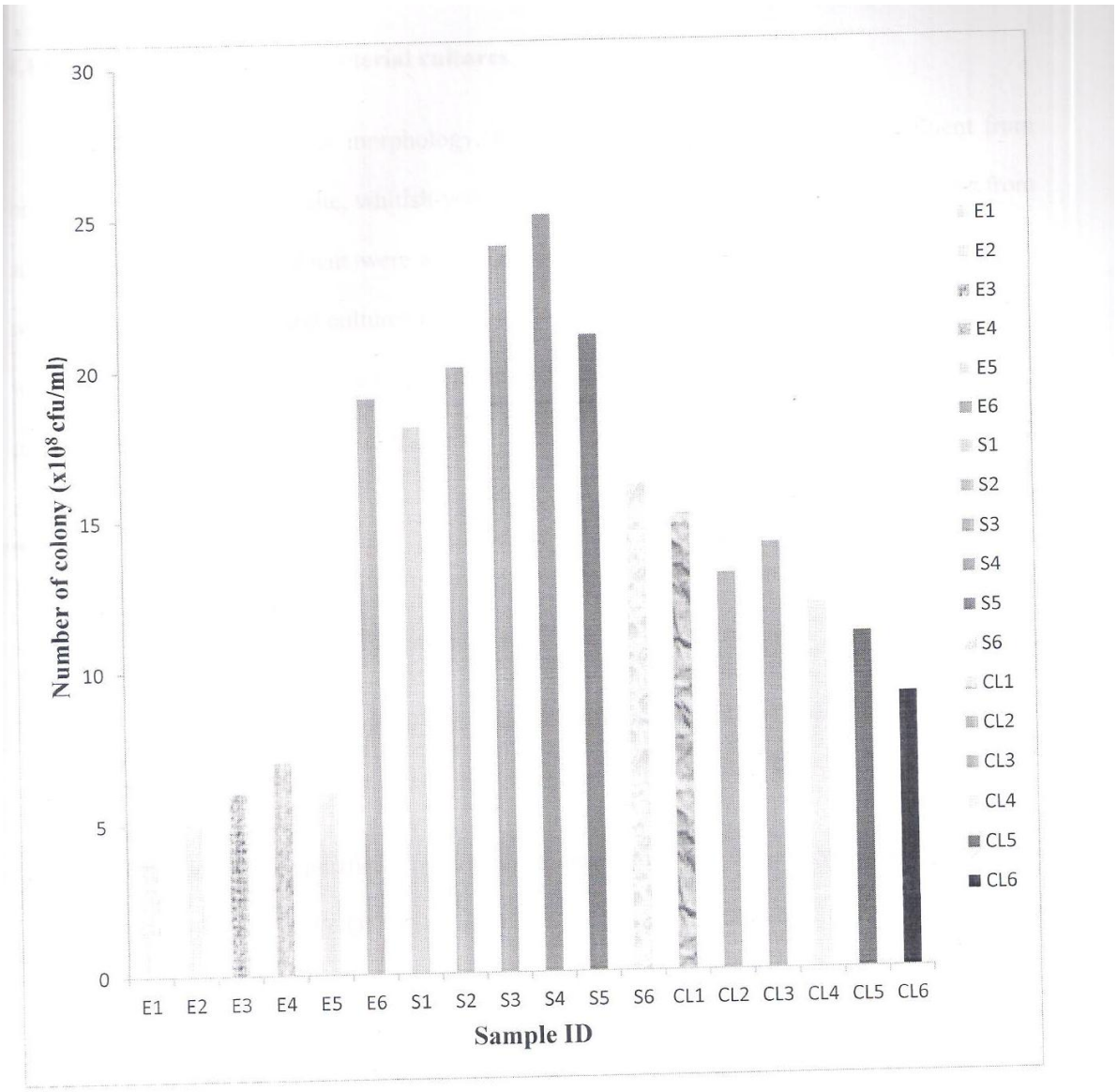

Fig. 1: Number of colony from effluent, contaminated soil and uncontaminated soil samples

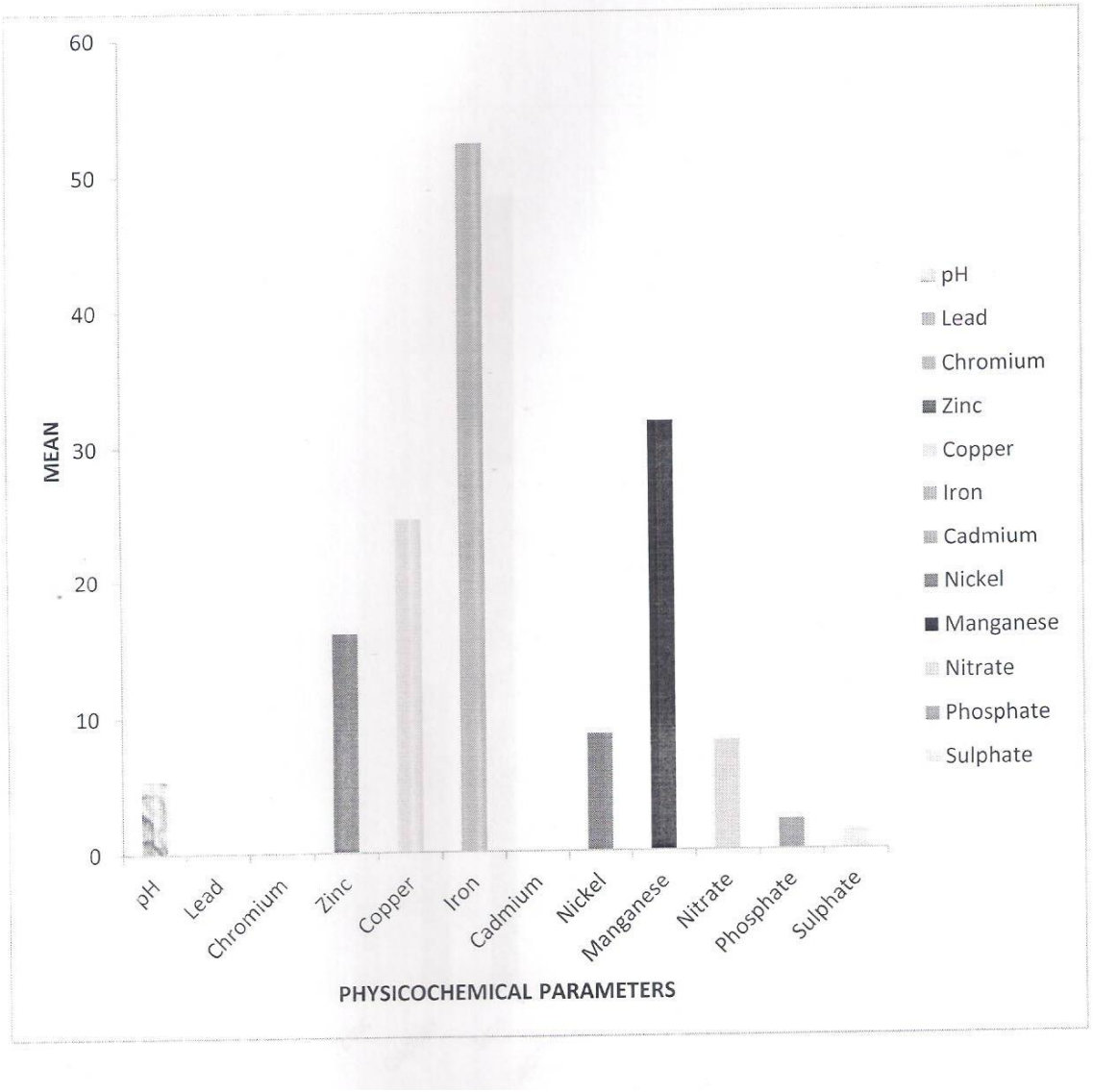

Fig. 2: Physicochemical parameters of soil contaminated with brewery effluent

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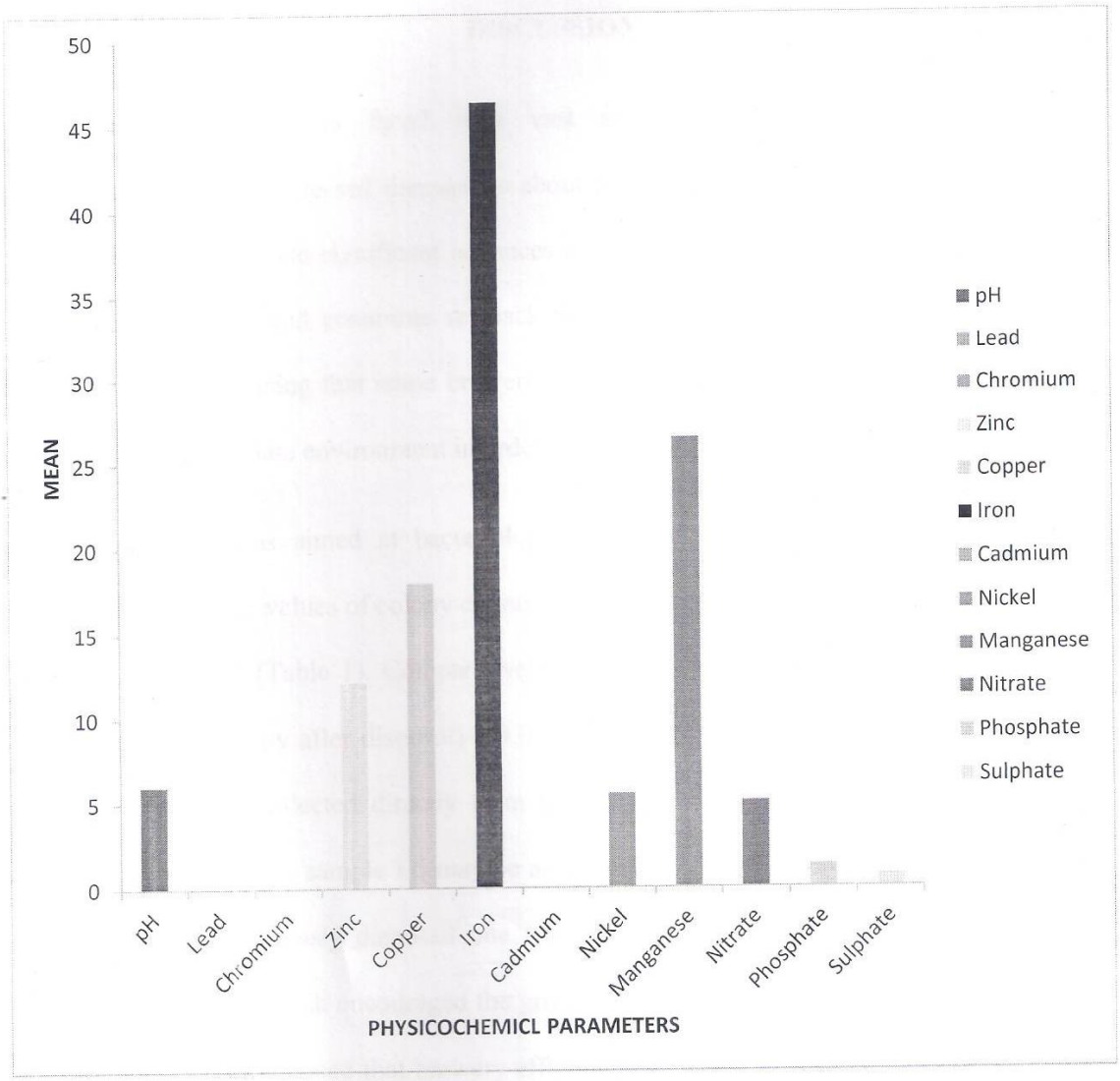

Fig. 3: physiochemical parameters of soil uncontaminated with brewery effluent

DISCUSSION

The environment is faced with vast and diverse problems of deterioration environmentalists have stressed themselves about issues such as air pollution, water pollution and soil pollution. Despite significant advances on technology over years, wastewater disposal remain environmental and economic set back that directly or indirectly affect soil and water bodies. It is not surprising that some breweries may discharge untreated or improperly treated effluent on the immediate environment in order to reduce production cost.

This study was aimed at bacteriological and physicochemical examination of Ama Brewery effluent. The values of colony counts for brewery effluent ranged from 4.0 x 10^8 cfu/ml a: 19.0 x 10^8 cfu/ml (Table 1). Comparatively, high colony count was observed in sample E6 (effluent collected a day after disposal) (19.0 x 10^8cfu/ml). Low colony count was observed in sample El (effluent collected directly from tanker) (4.0 x 10^8 cfu/ml). The reasonably high colony count obtained in sample E6 may be as the result of the mixture of the freshly disposed effluent with the previously disposed one and degradation of organic matter content of the brewery liquid waste which encouraged the growth of microorganisms. [8] [9] stated that brewery effluent contains organic materials like spent grains, waste yeast, spent hops and grits.

The relatively low colony count obtained in sample El could be as a result of effectiveness of effluent treatment plant. The bacterial strains isolated from the effluent samples belong to the genera *Bacillus. Staphylococcus, Escherichia, Proteus* and *Enterohacter.* This was in agreement with the work of [12] on the effects of Dashen Brewery waste water treatment effluent on the bacteriological and physicochemical quality of Shinta River in Gondar,

North West Ethiopia. The isolation *of Bacillus* species and *Enterobacter* species was in line with the work of [25]. *Bacillus* sp identified in this research was also in agreement with the research carried out by [22] on bacteriological and physico - chemical qualities of wastewater from a bottling company in Owerri, Nigeria. Some of these organisms were implicated in infections of burns, wounds, ulcer and the urinary tract infection.

The absence of faecal coliform such as *Escherichia* sp on Eland E3 could be an indicative of non-faecal contamination of effluent collected directly from tanker before disposal. This was in agreement with the work of [20] who reported the absence of faecal coliform from brewery effluent. The identification of organisms especially *Staphylococcus* sp and *Enterobacier* sp in the examined brewery effluent samples is alarming as the presence of these bacteria is indicative of the potential presence of pathogens.

The brewery effluent was dark in colour and had unpleasant odour. This was not in agreement with the conclusion of [11] who reported that brewery effluent was colourless and odourless. The odour of the effluent samples may be as a result of dissolved organic and inorganic contents of the effluent. This was in line with the work of [2] who reported that most obnoxious odours related with contaminated water were due to the presence of inorganic and organic compound of nitrogen, sulphur and phosphorus which arose from putrefaction of proteins and other organic materials present in wastewater.

The result of the physicochcmical parameters analyzed in this study showed that the effluents from Ama brewery met the permissible limit set by FEPA for safe discharge to surface water with the exception of iron and nitrate (Federal Ministry of Environment, 2001).

The temperature values recorded for the brewery effluents were within the permissible limits stipulated by the World Health Organization (2010) and Federal Ministry of Environment 2001). The pH range was also within the FEPA permissible limit of 6-9 and below permissible limits stipulated by the World Health Organization (2010).

All the mean heavy metal values recorded for the respective effluent samples with the exception of iron and nitrate were within the limits stipulated by Federal

Ministry of Environment (2001). The high value of iron may be from canals and sewers that convey both untreated and treated effluent. [4] documented that liquid waste disposed from oxidation ponds into the river cause elevated level of toxic elements in the river therefore affecting the river negatively and causing harm to people that uses such rivers. The high level of nitrate may be due to the presence of organic matter in the discharged effluent.

The values of colony counts of soil contaminated with effluent and uncontaminated soil ranged from 9.0 x 10^8 cfu/ml to 25.0 x 10^8 cfu/ml (Table 1). The relatively high colony count obtained in sample M (contaminated soil) might be as a result of organic matter content of the wastewater disposed on the soil which enriched the soil and encouraged the proliferation of soil microorganisms. According to [10] who documented that carbohydrates and nitrogen are highly present in brewery effluents and the disposal of this effluent, high in major elements can bring about changes in the microflora of the receiving environment.

However, the identification of microorganisms such as *Pseudomonas* sp, *Staphylococcus* sp, *Escherichia coli, Micrococcus, Salmonella* sp. *Klebsiella* sp, *Proteus* sp, and *Enterobcicter* sp could be as a result of the recent faecal contamination of the soil and soil as a natural habitat for some microorganisms.

The low colony count observed in sample CL6 (uncontaminated soil) could be due to non-disposal of the liquid waste high in organic matter content on the soil. The result also showed heavy metals of contaminated and uncontaminated soil during the study. Contaminated soil had high value of heavy metals when compared to uncontaminated soil. The slightly increase in heavy metals concentration on contaminated soil may be due to disposal of effluent on the soil. Even though several toxic metals including copper, zinc, manganese as well as iron are important in plant nutrition, lots of them do not play any beneficial role in plant physiology. If plants are allow to absorb these toxic metals mainly leafy vegetables can serve as an avenue of their entry into human food chain with detrimental effects on human health [19]. Excess level of nickel in the soil has been reported to be toxic and can affect soil fauna such as earthworm which are adjuncts to the microflora inorganic matter decomposition. The lungs, nasal cavity and tissues are the target organs affected by respiratory carcinogen when human are to nickel poisoning [28]. Also, if effluent high in heavy metals are disposed on the soil, it can be an avenue through which ground waters contamination can occur.

CONCLUSION

Good health of people living in an area depends on the cleanliness of the surrounding. Therefore, discharge of effluent with unpleasant odour close to residential house may serve as source of disease transmission and also make people living within that area uncomfortable. Since the discharged effluent is stagnant, it can serve as a breeding site for mosquito which carries causative agent of malaria, *Plasmodium.* For this reasons, the brewery industry should stop discharge their effluents close to residential houses. Even though some physicochemical parameters examined were within the World Health Organization (WHO) Permissible Limit, high level of iron in the effluent can affect colour of water when it comes in contact with water.

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