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Efficacy Assessment of Brewery Wastewater Treatment Practice using Physico-chemical Characterization and Toxicity Markers in African Mud Catfish (*Clarias gariepinus* Burchell, 1822) and African Brackish Water Shrimp (*Palaemonetes africanus* Balss, 1916)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Industrial activities and urbanization in developing countries have gradually led to the deterioration of the environment in recent years. Large quantities of wastewater effluent are inadequately treated or untreated before discharge into receiving waterbodies, resulting in environmental health concerns. Thus, this study investigated the efficacy of a brewery wastewater treatment system in Lagos by assessing the physicochemical characteristics, acute toxicity, and oxidative stress induction capabilities of the influent (untreated) and effluent (treated) on African mud catfish, *Clarias, gariepinus* and brackish water Shrimp, *Palaemonetes africanus*. The test organisms were acutely and sub-lethally exposed to predetermined concentrations of treated and untreated brewery wastewater. Results from the physicochemical assessment of the respective wastewater indicated that the treatment process was relatively effective in improving acidity levels (80.0%

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reduction), suspended solids (88.0% reduction), total nitrogen concentration (77.0% reduction), chemical oxygen demand (24.05% reduction), and biological oxygen demand (30.0% reduction), while nickel and cobalt concentrations reduced by 50%. Also, there was improvement of dissolved oxygen by about 250%. The 96 h LC_{50} value of the Catfishes and shrimps exposed to treated wastewater were 12.926 ml/L and 1.095 ml/L respectively compared to and 10.371 mg/L and 0.061 mg/L for the untreated wastewater, indicating an improvement factor of 1.23 and 17.95 for both species, correspondingly. Inhibition of the levels of activities of anti-oxidative enzymes (Reduced glutathione GSH, Superoxide dismutase SOD, and Catalase CAT) and increased lipid peroxidation damage (Malondialdehyde MDA) were recorded in all treatments for both test species, relative to the unexposed control groups. The enzymes however did not show significant trend with respect to the wastewater treatment status. Overall, it can be concluded that the wastewater treatment improved the effluent quality and such a process should be encouraged and enforced to ensure sustainable use of our water bodies.

Keywords: Antioxidative stress enzymes; brewery effluent; environmental health; influent; toxicity markers; wastewater.

1. INTRODUCTION

For centuries, Environmental pollution has been a global problem. Industries like petroleum refinery, soap and detergent [1], food and beverage, brewery, textiles and apparels, building materials, timber products, wood and leather works, metal works, chemicals and plastic industries are currently operational in Nigeria [1,2]. Various effluents are being discharged into the environment by these industries. Most large cities in Nigeria are feeling the nip of pollution from industrial effluents [3]. It is needless to talk of tons of effluents disposed indiscriminately into lagoons, rivers, streams and open land. It is evident that the discharge of untreated or partially treated wastes contain algal nutrients, non-biodegradable organics, heavy metals and other toxicants, and this hurtle the deterioration of receiving waterbodies [2,3]. Increasing awareness has led to acceptance and effective treatment of various effluents before they are being discharged into waterbodies [4]. Sadly, in most developing countries like Nigeria, effluent quality standards imposed by legislation (where they exist) are sometimes easily flouted due to unavailability of regulators to investigate standards [5].

Beer production have been a culprit of large volume discharge of extremely polluting effluents, especially because large volumes of water are used during its production [6]. It is estimated that for every 1 L of beer produced, about 3-10 L of effluent is generated [6]. Beer is the fifth most consumed beverage in the world and its industry constitutes an important economic segment of any country. Beer production involves brewing and packaging of the finished product via series of steps (malt production, wort production, beer production and general cleaning process). Spent grains, surplus yeast, grits and spent hops generated from these production processes are key contributors to brewery effluent pollution [7]. Also, general cleaning activities such as cleaning of bottles, tanks, machines and production areas results in discharge of high volumes of polluted water [6]. Effluents from individual brewing processes vary. Bottle washing for example results in discharge of large volumes of wastewater, however, it contains only slight parts of the total organics from brewing processes. Effluents from fermentation and filtration processes on the other hand, are high in organics and BOD, accounting for about 97% of BOD which are contained in 3% of the total wastewater volume [6]. The discharge of wastewater from breweries has over the years included direct introduction into waterways (oceans, rivers, lakes, streams or lagoons), municipal sewer systems without prior treatments or discharge into waterways and municipal sewer systems after total or partial treatment. The disposal of untreated or partially treated effluents into waterbodies constitutes severe pollution to them due to presence oxygen degrading organic compounds [7].

Existing studies have shown that brewery effluents can alter the physicochemical properties of receiving waterbodies. For instance, in a study conducted by Alao et al. [8], the discharge of brewery effluents critically altered the physicochemical properties of surface water in Majawe. In the same study, Lead was also detected in the surface water sample. In a separate study, the mean physicochemical properties of surface water from three different

brewery industry sites in Nigeria were observed to be well above designated regulatory limits [9]. It was also observed that alterations in the BOD and COD parameters of waterbodies can affect aquatic life [2, 9], plant morphology [10] and the microbiological quality of waterbodies [11, 12]. In as much as these studies suggest that brewery effluents poise a potential to negatively impact the environment, there is a dearth in information regarding toxicological assessments of these effluents to marine organisms in Nigeria, 'a major requirement in most developed countries' [13]. Thus, the present study sought to incorporate classic biological methods to routine toxicity assessments in assessing the efficacy of a brewery plant by evaluating the acute toxicity, morphological alterations and biochemical indices of African mud catfish (*Clarias gariepinus)* and African brackish water shrimp (*Palaemonetes africanus)* exposed to treated and untreated brewery effluents. This is done in order to ensure comprehensive results which safeguards the environment are made available.

2. MATERIALS AND METHODOLOGY

2.1 Study Design

The study involved an initial assessment of the physicochemical properties and acute toxicity of Brewery wastewater; treated (effluent) and untreated (influent) for 96h followed by a 32-day sub-lethal toxicity assay, using *C. gariepius* and *P. africanus*. After acute toxicity assays, test concentrations were calculated based on literature for definitive sub-lethal toxicity study. After 32 days of sub-lethal exposure, the fishes were examined for biomarkers of oxidative stress in liver and Gills of the catfishes and Shrimps.

2.2 Test Organisms: Collection and Acclimatization

Fingerlings and juveniles of *C. gariepinus* and that of African brackish water shrimp (*P. africanus)*, used in the present study were purchased from the Marine Science Department of the University of Lagos, Lagos State, Nigeria. They were transported to the Ecotoxicology and Conservation Laboratory unit, University of Lagos, in plastic containers. The respective organism groups were transferred into 40 L capacity holding tanks (l x w x h = 60 cm x 35 cm x 30 cm) half filled with culture water from their respective habitats. The holding medium were aerated with a 220-v Cosmo aquarium air pump (double type 12000) to ensure maintained levels of dissolved oxygen. Holding tanks were cleaned and culture water renewed at 48-hour interval to prevent the build-up of metabolic wastes [14]. The average mean weight and total length of test organisms were: 3.40 ± 0.43 g and 4.2 ± 0.7 cm (*C. gariepinus* fingerlings), 6.89 ± 0.22 and 8.77 ± 0.12 cm (Juveniles), 0.06 ± 0.04 g and 15.01 ± 18 mm respectively (*P. africanus* fry). Organisms were acclimatized to laboratory conditions for a period of 7 days prior to exposure (based on OECD Test 203 Guideline), during which they were fed with Coppens® fish feed containing 45% crude protein, provided daily at 1% of body weight [15]. The mean physicochemical parameters of culture water in the holding tank were measured using a Horiba U50 water quality monitor. The parameters measured were; dissolved oxygen, pH, salinity, total dissolved solids (TDS) and specific conductance. Organisms were exposed to their natural photoperiod (12 h daylight and 12 h darkness). Feeding in holding tanks stopped 24 h prior to exposure.

2.3 Test Chemical

Untreated (Influent) and treated (Effluent) brewery wastewater samples were obtained from a reputable brewery in Lagos, Nigeria. The wastewater samples were collected in June, 2019 from the entry and discharge points of the wastewater treatment plant into 5 L pre-cleaned plastic containers and refrigerated in the laboratory at 4˚C until use for bioassays. Portions of the wastewater samples were transported the Chemistry laboratory at the University of Lagos for physicochemical characterization.

2.4 Physicochemical Analysis

The physicochemical and heavy metals analyses of the effluent were conducted according to APHA [16]. The physicochemical parameters analysed were colour, pH, temperature, acidity, alkalinity, chloride, Sulphate, Sulphide, Sodium, Ammonia Nitrogen, Total Nitrogen, Free Chlorine, Total Phosphorus Nitrate, Turbidity, Dissolved Oxygen (DO), oil and grease, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Solids, Total Suspended Solids (TSS), and Total Dissolved Solids (TDS). The heavy metals were analysed using Flame Atomic Absorption Spectrophotometer (Perkin Elmer A Analyst 200) and metals analysed were, lead; zinc; iron; copper; manganese; cobalt; cadmium, chromium

and nickel. Phenol, oil and grease and presence of detergents were also measured.

2.5 Laboratory Bioassay Techniques

2.5.1 Relative acute toxicity of wastewater samples against *Clarias gariepinus* and *Palemonetes africanus*

A static bioassay procedure (no renewal of test media) was adopted for toxicity tests. Depending on the test concentrations, 1000 ml of dechlorinated water was measured into bioassay containers for each concentration, and the exact value of water in equal proportion to the test concentration amount was removed (displacement method). Preliminary tests were carried out at first to determine suitable range concentration for the bioassay. Concentrations derived and used were; 1 ml, 3 ml, and 7 ml (untreated) and 2 ml, 4 ml, 6 ml, 8 ml and 10 ml (treated) for both *C. gariepinus* and *P. africanus*. Each concentration was carefully measured and dispensed into bioassay aquariums (l x w x h = 6 cm × 6 cm × 7 cm) for each wastewater sample. Five (5) active individuals of *C. gariepinus* were randomly selected with introduced into bioassay aquariums containing experimental media. Ten (10) individuals of *P. africanus* were also picked at random from the holding tank and introduced into bioassay media. Each treatment was in duplicate including the control media, totalling 10 fingerlings, and 20 shrimps per concentration. Exposed organisms were monitored for mortality at 24, 48, 72, and 96 hours. Organisms weren't fed during the experiment [17] and were confirmed dead by their failure to respond to stimulus even when prodded gently with a glass rod. All experiments were conducted under the same laboratory conditions as acclimatization. Behavioural changes on exposure were also observed and reported.

2.5.2 Chronic toxicity study

In this series of experiment, three (3) juveniles of *C. gariepiuus* and ten (10) fry of shrimps were exposed in replicates to sub-lethal concentrations ($1/100^{th}$ and $1/10^{th}$ of the respective 96 h LC50 values) of the test effluents and an untreated control for a period of 32 days in a static-renewal bioassay. The test media was renewed every 48 h to prevent the accumulation of metabolic wastes [18]. At the end of the exposure period (32 days post treatment), fishes and shrimps were harvested from the test media and immobilized through spinal puncture and dissected to extract samples. Liver and gill samples from the fishes and whole shrimp samples were collected and immediately transferred into universal sample bottles and preserved ice cold. Samples were transported to the laboratory for analysis. Analysis was carried out in Lagos University Teaching Hospital, Idi-Araba. Biochemical analyses were conducted on homogenized gills, liver and shrimp samples respectively.

2.5.3 Antioxidant enzymes assay

The activities of superoxide dismutase (SOD) were measured based on its ability to inhibit the auto-oxidation of epinephrine and quantified by recording absorbance at 460 nm for 5 min [19].

Catalase (CAT) activities were evaluated by the method of Aksenses and Najaa [20]. This was achieved by comparing the absorbance at 240 nm of both samples and phosphate buffer blank, each introduced into 30 mM of H_2O_2 and subsequently calculating the enzyme activity using a molar extinction coefficient of 40 M^{-1} cm^{-1}.

The reduced glutathione (GSH) levels in the homogenates as nonprotein sulfhydryls were assessed by treating the supernatant produced following centrifugation in 10 % TCA with Ellman's reagent (19.8 mg 5,5- dithiobisnitro benzoic acid in 100-mL 0.1 % sodium nitrate) as well as 3.0-mL 0.2 M phosphate buffer (pH 8.0) [21].

Lipid peroxidation levels were determined vis Thiobarbituric acid reaction (TBARS assay) by measuring the levels of malondaildehyde in homogenates using a spectrophotometer at absorbance levels of 535 nm according to the technique of Yagi [22].

2.6 Data Analysis

Observed mortality in each treatment and control groups during acute assays were computed and analyzed using probit regression analysis as described by Finney [23]. The indices of toxicity measurement derived from these analyses were LC_{50} (Median concentration that causes 50% mortality of exposed organisms), Toxicity Factor (T.F) which is a measure of relative potency of toxicants.

> Toxicity Factor (TF) = (LC_{50} value of other chemicals / LC_{50} value of the most toxic chemical)

Analysis of variance (ANOVA) followed by post hoc (Duncan's test) was carried out at 5% ($p<0.05$) level of significance for MDA, SOD, CAT, GST and GSH activities. All statistical analysis was performed using the SPSS Version 23 software (IBM).

3. RESULTS

3.1 Physicochemical Characterization

The result from the physicochemical characterization of the treated brewery wastewater showed that pH value was 7.2 indicating a neutral medium. Sodium had the highest concentration of 70.97 mg/L, followed by Zn with a concentration of 0.41 mg/L. Mn and Ni were detected each at concentrations of 0.01mg/L while the DO levels was at 7.0 mg/L. The COD and BOD levels were 60.0 mg/l and 28.0 mg/L respectively and TDS was 995.0 mg/L (Table 1).

For the untreated brewery wastewater, the pH value was 5.4 indicating slight acidity. Na had the highest concentration of 23.66 mg/L, followed by Fe with a concentration of 0.612 mg/L and Mn having the lowest concentrations of 0.01 mg/L. The DO content of the treated effluent was 2.0mg/l. The COD and BOD levels were 79.0 mg/L and 40.0 mg/L respectively while TDS levels was at 774.0 mg/L (Table 1).

With respect of the wastewater treatment efficiency, it was observed that there were significant improvements in most of the assessed parameters. For instance, the acidity levels improved by 80.0% while suspended solids reduced by 88.0%. Total nitrogen concentration reduced by 77.0% while nickel and cobalt concentrations reduced by 50 % (Table 1).

3.2 Acute Toxicity Responses

For treated brewery wastewater, the Catfish exposure resulted in an LC_{50} value of 12.92 mg/L compared to a value of 1.09 mg/L for the exposed shrimps (Table 2). More so, for the untreated wastewater, the estimated 96h LC_{50} value was 10.37 mg/L for the exposed Catfishes compared to an LC_{50} value of 0.06 determined from the shrimps (Table 3). The findings showed that the wastewater was more toxic to shrimps compared to Catfishes with a toxicity factor of 11.80 and 170.01 for the treated and untreated wastewater respectively.

Regarding treatment efficacy, there was an overall reduction in the toxicity of wastewater to both test species, with an improvement factor of 1.2 and 18.0 for the Catfish and the Shrimp respectively (Table 4).

3.3 Antioxidative Stress Enzyme Activities

Findings from antioxidative stress enzyme assay revealed that gill and liver samples of *C. gariepinus* showed comparable trend of antioxidative stress enzyme activities in organisms exposed to both treated and untreated wastewater. The measured levels of GSH, SOD and CAT activities showed significant ($p<0.05$) inhibition between untreated and treated wastewater. The mean (±SD) levels of enzyme activities at $1/10^{th}$ 96h LC_{50} and $1/100^{th}$ 96h LC_{50} of the gill and liver samples of untreated and treated wastewater are as follows; GSH: 3.44±0.58 (untreated), 4.65±0.06 (treated), 3.62±0.12 (untreated), 5.26±0.21 (treated) and 2.67±0.13 (untreated), 3.66±0.05 (treated), 2.70±0.10 (untreated), 4.98±0.15 (treated) U/mg protein respectively (Figure 1); SOD: 2.64±0.10 (untreated), 3.83±0.04 (treated), 2.74±0.06 (untreated), 3.09±0.09 (treated) and 3.71±0.27 (untreated), 4.64±0.05 (treated), 3.41±0.04 (untreated), 3.90±0.15 U/mg protein (treated) respectively (Figure 2); CAT: 21.48±3.40 (untreated), 52.18±1.62 (treated), 39.60±1.22 (untreated), 33.17±1.28 (treated) and 25.18±2.76 (untreated), 52.17±1.45 (treated), 30.88±0.91 (untreated), 40.42±1.26 U/mg protein (treated) respectively (Figure 3). For shrimps, measured levels followed similar trend as fishes. The observed levels of GSH, SOD and CAT at $1/10^{th}$ 96hr LC_{50} and $1/100^{th}$ 96hr LC_{50} for *P. africanus* were; GSH: 10.28±0.62 (control), 5.93±0.29 (untreated), 6.28±0.17 (treated), 3.11±0.07 (untreated) and 4.26±0.07 U/mg protein (treated); SOD: 10.28±0.62 (control), 3.91±0.22 (untreated), 5.28±0.11 (treated), 2.24±0.09 (untreated) and 3.12±0.07 U/mg protein (treated); CAT: 52.84±1.60 (control), 48.90±3.13 (untreated), 59.66±1.28 (treated), 23.18±0.50 (untreated) and 31.49±0.90 U/mg protein (treated) correspondingly.

The aldehyde byproduct of lipid peroxidation, MDA, was significantly ($P < 0.05$) lower in the control relative to the $1/10^{th}$ 96hr LC_{50} and $1/100^{th}$ 96hr LC_{50} of the gill and liver samples for both untreated and treated wastewater groups (Figure 4). Specifically, the MDA levels for gills and liver (*C. gariepinus*) samples were between 2.40-2.81 (control), 4.30-5.10 (untreated), 3.46-7.78

(treated), 3.67-3.71 (untreated) and 4.86-5.27 U/mg protein (treated) respectively. For *P. africanus*, the respective mean (±SD) MDA levels at 1/10th 96hr LC_{50} and 1/100th 96hr LC_{50} for both wastewaters were 3.04±0.05 (control), 4.52±0.46 (untreated), 3.48±0.12 (treated), 7.80±0.17 (untreated) and 3.89±0.08 U/mg protein (treated).

Table 1. Physicochemical Parameters of Treated and Untreated Brewery Wastewater

Physicochemical parameters	Untreated	Treated	Percentage efficiency	FMENV Limits
Colour	Light Brown	Colourless	NA	Colourless
Appearance	Not Clear	Clear	NA	
Temperature (ºC)	21.0	20.7	1.43	40
pH	5.4	7.2	-33.33	6.5 – 6.9
Acidity (mg/l)	20.0	4.0	80.00	
Alkalinity (mg/l)	ND	8.0	0.00	150
Chloride (mg/l)	36.0	108.0	-200.00	250
Total Solids (mg/l)	779.0	995.6	-27.80	
Total Suspended Solids, TSS (mg/l)	5.0	0.6	88.00	25
Total Dissolved Solids, TDS (mg/l)	774.0	995.0	-28.55	500
Sulphate (mg/l)	85.0	9.0	89.41	250
Turbidity (NTU)	10.0	4.0	60.00	5
Nitrate (mg/l)	3.5	1.0	71.43	10
COD (mg/l)	79.0	60.0	24.05	60: 90
DO (mg/l)	2.0	7.0	-250.00	
BOD (mg/l)	40.0	28.0	30.00	30: 50
Sulphide (mg/l)	0.1	ND	0.00	0.2
Ammonia Nitrogen (mg/l)	2.0	0.01	99.50	1.0
Total Nitrogen (mg/l)	1.0	0.23	77.00	10
Free Chlorine (mg/l)	ND	ND	0.00	0.5
Total Phosphorus (mg/l)	0.25	0.20	20.00	2.0
Copper, Cu (mg/l)	0.10	0.02	80.00	
Chromium, Cr (mg/l)	ND	ND	0.00	0.005
Lead, Pb (mg/l)	ND	ND	0.00	0.005
Nickel, Ni (mg/l)	0.02	0.01	50.00	0.05
Sodium, Na (mg/l)	23.66	70.97	-199.96	200
Iron, Fe (mg/l)	0.612	0.212	65.36	
Manganese, Mn (mg/l)	0.01	0.01	0.00	0.2
Cadmium. Cd (mg/l)	ND	ND	0.00	1.0
Cobalt, Co (mg/l)	0.02	0.01	50.00	0.05
Zinc, Zn (mg/l)	0.54	0.413	23.52	2.0
Phenol (mg/l)	ND	ND	0.00	0.5
Oil & Grease (mg/l)	ND	ND	0.00	10
Detergent (mg/l)	0.50	0.25	50.00	15
THC	ND	ND	0.00	

FMENV: Federal Ministry of Environment; ND: Not Detected; NA: Not Available

Table 2. Relative acute toxicity of treated brewery wastewater against *Clarias gariepinus* and *Palaemonetes africanus*

Time 96h	LC_5	LC_{50}	LC_{95}	SE	DF	Probit line equation	TF
C. gariepinus	1.575	12.926	106.084	0.905	3	Y = 1.799X – 2.000	11.80
P. africanus	0.000	1.095	4.377E+10	0.356	4	Y = 0.155X – 0.006	1.00

KEY: CL: Confidence Limit, DF: Degree of Freedom, SE: Standard Error, T.F.: Toxicity Factor

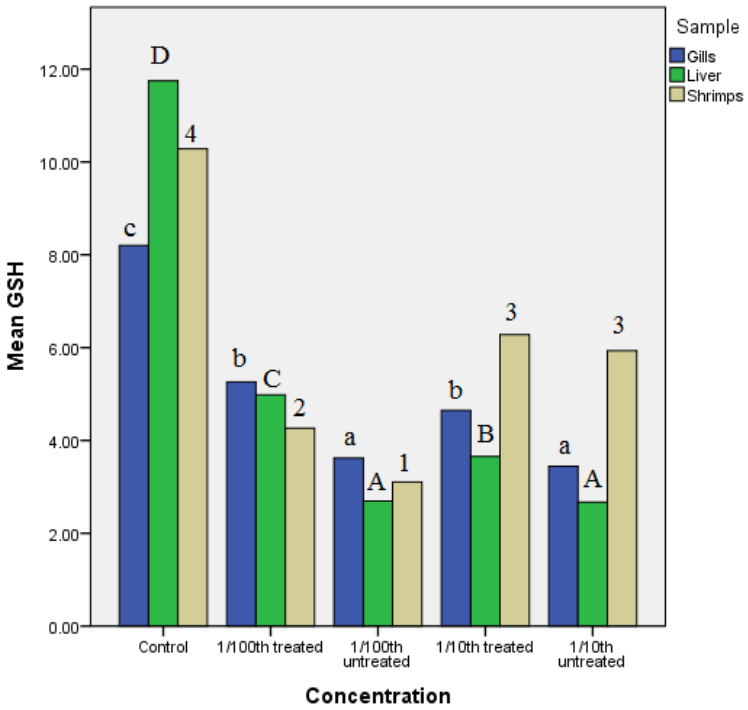

Fig. 1. Levels of Reduced Glutathione GSH in the liver and gills of Catfishes, *Clarias gariepinus* as well as the Shrimp, *Palaemonetes africanus* on exposure to treated and untreated wastewater. Statistically significant differences (p<0.05; Duncan test) are indicated with different letters and numbers

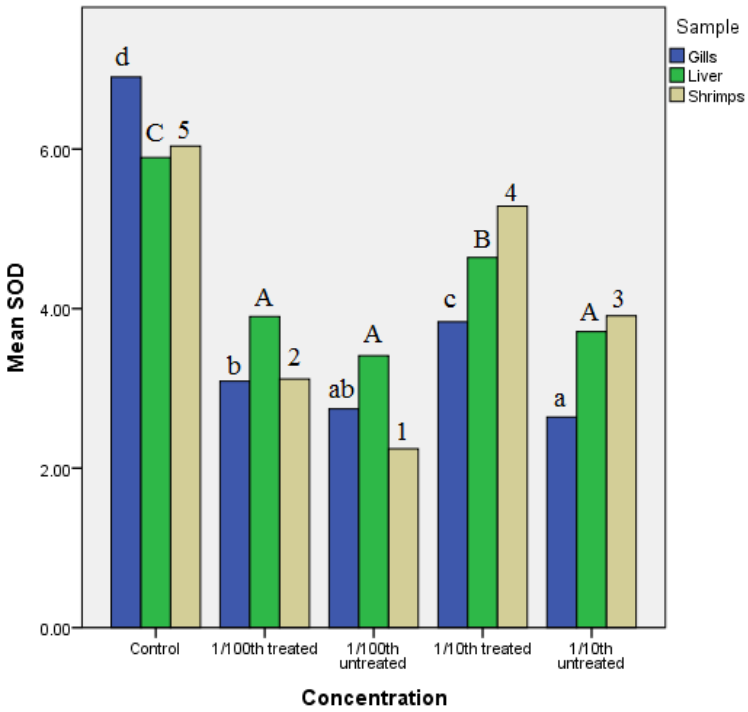

Fig. 2. Activities of Superoxide Dismutase (SOD) in the liver and gills of Catfishes, *Clarias gariepinus* as well as the Shrimp, *Palaemonetes africanus* on exposure to treated and untreated wastewater. Statistically significant differences (p<0.05; Duncan test) are indicated with different letters and numbers

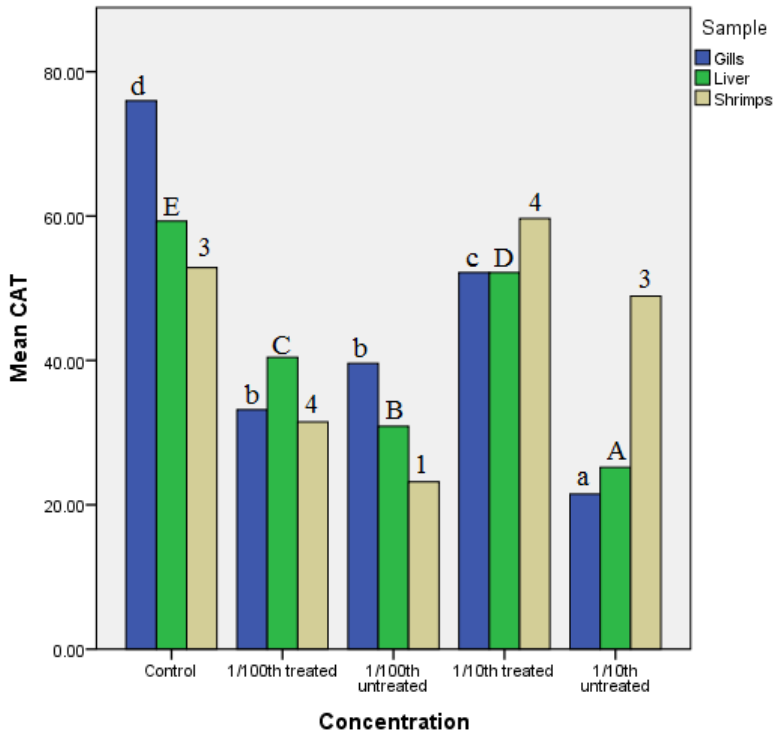

Fig. 3. Catalase (CAT) activities in the liver and gills of Catfishes, *Clarias gariepinus* as well as the whole tissue homogenate of the Shrimp, *Palaemonetes africanus* on exposure to treated and untreated wastewater. Statistically significant differences (p<0.05; Duncan test) are indicated with different letters and numbers

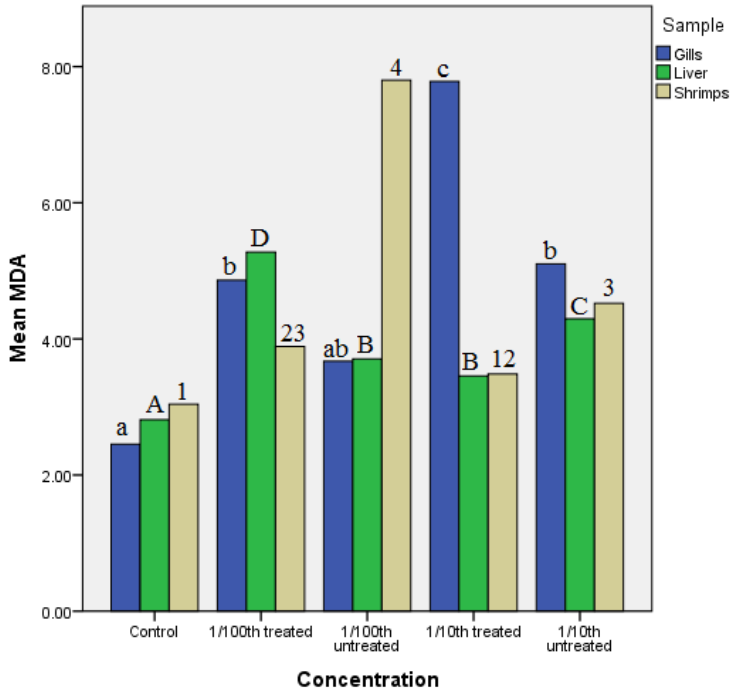

Fig. 4. Levels of Malondialdehyde (MDA) in the liver and gills of Catfishes, *Clarias gariepinus* as well as the whole tissue homogenate of the Shrimp, *Palaemonetes africanus* on exposure to treated and untreated wastewater. Statistically significant differences (p<0.05; Duncan test) are indicated with different letters and numbers

Table 3. Relative Acute Toxicity of Untreated Brewery Wastewater against *Clarias gariepinus* and *Palaemonetes africanus*

Time 96h	LC_5	LC_{50}	LC_{95}	SE	DF	Probit line equation	TF
C. gariepinus	0.350	10.371	307.254	0.873	3	Y = 1.118X – 1.135	170.01
P. africanus	0.000	0.061	12.974	0.390	4	Y = 0.706X + 0.859	1.00

KEY: CL: Confidence Limit, DF: Degree of Freedom, SE: Standard Error, T.F.: Toxicity Factor

Table 4. Relative improvement factor of the Brewery Wastewater Toxicity on *Clarias gariepinus* and *Plaemonetes africanus*

Test organism	Wastewater Status	LC_{50}	Improvement Factors
C. gariepinus	Treated	12.926	1.2
	Untreated	10.371	
P. africanus	Treated	1.095	18.0
	Untreated	0.061	

4. DISCUSSION

Findings from the present study has pointed to the need for every such facility to own a wastewater treatment unit, given the remarkable improvements observed in the physicochemical properties including acidity levels, total suspended solids, nitrates, biological oxygen demand, chemical oxygen demand, heavy metals, amongst others. Unusual physicochemical properties of industrial wastewater are inevitable [24,25], however, effective treatment regimens can make significant improvements. For instance, it was observed that the dissolve oxygen levels improved by 250% and this can be associated with aeration systems in the waste treatment plants which reduced BOD and COD levels by 30% and 24% respectively.

From this study, it was evident that the remediation of treatment efficiency is effective, with a pH improvement of 33.33% which indicates the effluents is within FMENV standards. Hydrogen-ion concentration indicates a parameter of both natural and wastewaters, which is also used to describe the properties of acid or base in wastewater. A pH which is less than 7 in wastewater influent reveals certain conditions, which might be the septic holding tank or sludge tank while the values less than 5 and greater than 10 reveals various components of industrial wastes which are not compatible with biological operations. The pH concentration range for the existence of biological life is quite narrow (typically 6-9). An indication of extreme pH is known to damage biological processes in biological treatment units [25,26].

There are several parameters used to determine significant effect on the characteristics of natural water and effluent and dissolved oxygen (DO) is put into consideration. Dissolved oxygen levels from the present study were found to improve with a percentage efficiency of 250% between influent (2.0) and effluent (7.0), and this indicates that there is appreciable amount of dissolved oxygen required for the respiration of aerobic microorganisms as well as all other aerobic life forms. The actual quantity of oxygen that can be present in solution is governed by the solubility, temperature, partial pressure of the atmosphere and the concentration of impurities such as salinity and suspended solids in the water [26, 28]. There are various components of Oxygen demanded in aquatic ecosystem (benthic and microorganism) for feeding and survival upon the organic solids in wastewater. These demands are in form BOD or COD; BOD percentage efficiency is 30.0 and both influent (40.0) and effluent (28.0) and COD percentage efficiency is 24.05and both influent (79.0) and effluent (60.0) are within FMENV standard [27,29].

The observed turbidity value of the influent was 10.0 while the effluent had a turbidity value of 4.0 which is within the FMENV limits. This is in agreement with the findings of Prasad et al. [30]. The improvements due to the reduced toxicity of the wastewater is also apparent, implying the improved safety factor due to the treatment process. The results of this experiment clearly demonstrate that concentrations of treated and untreated brewery effluents would result in mortality of *C. gariepinus* and *P. africanus*. On the basis of the 96h LC_{50} values, it can be deduced that the untreated brewery effluents had a higher mortality effect on both *C. gariepinus*

and *P. africanus*. Generally, it is clear from the present study that brewery effluents can cause mortality in aquatic organisms. This is in accordance with studies carried out by Adeboyejo et al. [31] on the acute toxicity of industrial effluents from Agbara environs of Ologe lagoon on early life stages of African catfish *C. gariepinus*. From the research, it was observed that industrial effluents had LC_{50} 96h as 34.03 and 19.63 for fingerlings and juveniles of catfish respectively.

The environmental impact of effluent, if not treated adequately can be linked to various health issues. This can be characterized as a phenomenon of bioaccumulation and biomagnifications of contaminants. In the case of bioaccumulation, various substances characterized as low concentrations or barely measurable in water is sometimes found in high concentrations of the plant and animal tissues. They are therefore considered stable substances, which live long chemically, and cannot be broken down easily by digestive processes [32,33]. The concentrations of some contaminants might increase considerably through the food-chain in a case of biomagnification.

Several other parameters as well contributes a great deal to survival of aquatic organisms in the ecosystem. Oxidative stress reflects the tissue damage resulting from an imbalance between excessive generation of oxidant compounds and insufficient antioxidant defence mechanisms. Oxidant compounds are extremely reactive species capable of independent existence that contains one or more unpaired electron, named free radicals (FRs). They are either endogenous and/or exogenous. Because of their high reactivity, they can abstract electrons from other compounds to obtain stability. Thus, the attacked molecule loses its electron and becomes a FR itself, beginning a chain-reaction cascade, which finally damages the organism's structure and functions (Longini et al., 2017). Oxidative compounds are also physiologically relevant in inflammation and tissue repair processes. Hence, they represent some defence mechanisms against microorganisms and malignant cells as well as tissue healing and remodelling [34].

The results from this study indicated a similar trend in the activities of antioxidant enzymes. The level of GSH in the treated wastewater was observed to be higher than that of the untreated wastewater. GSH functions as a detoxifier of ROS, and also helps in the transportation of amino acids and it converts some antioxidant to their active forms [35-37]. Decrease in GSH for *C. gariepinus* and *P. africanus* confirms the quantity or amount of ROS species present in the effluent.

Superoxide dismutase (SOD) is a major and powerful internal antioxidant enzyme in the body, which wrestles against oxidative stress both inside and outside cell membrane. The observed reduction in SOD activity for both catfish and shrimps reveal that the enzymes could no longer protect cells against superoxide radicals, which are considered the most dangerous of all the free radicals. This result is similar to that of [37-39] who observed decrease in SOD activity in fishes from polluted rivers. Antioxidant defence impairment in cells or systems can result in DNA damage, as well as protein and lipid oxidation due to oxidative stress.

The level of SOD in the treated wastewater was also observed to be higher than the level of SOD in the untreated wastewater for catfish gills and liver. Meanwhile in shrimp, CAT activity in $1/10^{th}$ LC50 treated wastewater was higher than the control. Reduction in CAT of catfish when compared to control, reveals the fish was under oxidative stress and this is as a result of increase in production of ROS by pollutants in the effluents. The result is similar to that of Farombi et al. [40], who observed that an increase in ROS production correlated with decreased catalase activity as well as other antioxidants in the cells of *C. gariepinus* in Ogun river. However, CAT was higher in shrimps and this might be an adaptive response to the increase in production of ROS in the shrimps.

Levels of lipid peroxidation marker (MDA) in the treated wastewater were also observed to be higher than that of fish gill and liver in the untreated wastewater. In shrimp, the activity of MDA increased with increase in wastewater concentration. Increase in MDA was significantly higher than control for both treated and untreated effluents and this may be due to generation of ROS pollutants in the media, which is as a result of imbalance between ROS and antioxidants and this thereby causes alteration of the antioxidant enzymes activities [41].

5. CONCLUSION

It is apparent that wastewater generation in industries such as breweries is inevitable as the

present study has made evident that the physicochemical parameters of wastewater effluent is a major criterion for discharging into the waterbodies. However, there remains opportunity to improve the situation in terms of waste reduction and effluent quality improvement. Thus, observed parameters from treated wastewater from the present study conforms with the limits slated for industrial discharge by the FMENV except for the increased value in TDS which is an anomaly and occurs in rare cases. Also, after a conclusive biochemical experiment, it was certain that treated and untreated brewery effluent can significantly alter the activity of oxidative stress enzymes in *C. gariepinus* and *P. africanus*. Therefore, there is need for further studies involving holistic evaluations of the fish diversity as well as chronic toxicological assessment of the whole effluent to aquatic organisms at lower levels of biological organization in order to identify the potential hazards of the wastewaters in a more realistic environmental scenario. Most importantly, wastewater assessments should be treated as a very important aspect of wastewater treatment before discharge and such actions should be backed by adequate monitoring by regulators for efficiency and sustainability.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

AVAILABILITY OF DATA AND MATERIAL

Data generated as part of this study is available upon request from the corresponding author.

ETHICAL APPROVAL

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This study followed the principles in the Declaration of Helsinki on the humane treatment of animals used in research and the principles in the AVMA Guidelines for the euthanasia of animals.

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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