

Assessment of Bioaccumulating Ability of *Mariscus longibrateatus* and Effects of Effluents from Kaduna Refinery and Petrochemical Company on the Plant

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ABSTRACT

The operations of Kaduna Refinery and Petrochemical Company (KRPC) lead to the generation of effluents. Plants growing in the drain are in constant exposure to these effluents. *Mariscus longibrateatus*, which is the most abundant plant species growing in the drain, was studied to determine the effects of the effluents on the plant. Plant samples and soil on which the plant grows, were analyzed for heavy metals. Manganese (1.30mg/g) and copper (1.30mg/g) had the highest concentration of heavy metals in the roots. The plant samples had thinner leaves than the control plant, which may be attributed to dehydration and some hidden injuries. The leaf whole vascular bundle of the studied plants ranged from 19085–20790 μm^2 whereas phloem and xylem area ranged from 3995 – 4290 μm^2 and 6584–7004 μm^2 respectively. Transfer and bioaccumulation factors revealed that heavy metals were not effectively transferred from the root to the stem, but the plant was able to survive in the drain containing KRPC effluents. It is concluded that the effluents caused some changes in the plant. It can be inferred that *Mariscus longibrateatus* are tolerant to the toxicants in the effluents, and therefore, this plant species is recommended for bioremediation study because of its tolerant ability to heavy metals.

Keywords: Bioaccumulation, effluents, heavy metals, *mariscus longibrateatus*.

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I. INTRODUCTION

Water pollution is of great concern due to the presence of toxic and recalcitrant compounds which persist in the environment for a long time, hence are taken up by organisms such as plants. The major causes of water pollution include sewage, industrial waste, marine dumping, radioactive waste, oil pollution, underground storage leakages, atmospheric deposition and global warming. Industrialization has increased effluent generations with consequent effects on water quality and habitat quality. The impact of industrial effluents on aquatic and terrestrial ecosystems has drawn a lot of attention worldwide because of its overwhelming environmental significance (Achudume, 2009). Adeniyi and Afolabi (2002) documented that industrial effluents are not only concentrated but also plentiful, so the pollution potential of industrial effluents is by far greater than that of domestic effluents. Most industrial effluents are usually extremely complex mixtures containing inorganic as well as organic compounds (Edema *et al.*, 2008). The complexity makes it almost impossible to

carry out a hazard assessment based on chemical analysis (Edema, 2009). Due to the ineffectiveness of purification systems, effluents have become seriously dangerous, leading to the accumulation of toxic products in receiving water bodies with potentially serious consequences on the ecosystem (Beg *et al.*, 2003). Both developing and industrialized countries are struggling to solve water pollution problems resulting from its toxicity by heavy metals (Edema *et al.*, 2008).

Effluents released by crude oil processing and petrochemical industries are characterized by the presence of large quantities of crude oil products, polycyclic and aromatic hydrocarbons, phenols, metal derivatives, surface active substances, sulfides, naphthylenic acids and other chemicals (Rana, 2005). Various studies have shown positive correlation between pollutants from refinery effluents and the health of aquatic organisms. A study demonstrated the accumulation of heavy metals with accompanying histopathology in *Oreochromis niloticus* exposed to supposedly treated petroleum refinery effluents

from the Nigerian National Petroleum Corporation, Kaduna (Onwumere & Oladimeji, 1990).

Crude oil contamination of soil leads to the build-up of essential (organic carbon, phosphorus, calcium, magnesium) and non-essential (manganese, lead, zinc, iron, cobalt, copper) elements in soil and the eventual translocation in plant tissues (Vwioko *et al.*, 2006). The contamination of soil by oil has been reported to cause growth retardation in plants (Anoliefo & Edegbai, 2000; Anoliefo *et al.*, 2003). This reduction in plant growth has been attributed to the presence of heavy metals at toxic concentrations in soil (Anoliefo & Edegbai, 2000). The accumulation of heavy metals in plants causes physiological and biochemical changes (Fisher *et al.*, 1981; Singh *et al.*, 1988). Some plants usually adapt to high pollutant concentrations and unfavorable environmental conditions which is likely to result in different morphology and anatomy (Vivan *et al.*, 2012). Heavy metals and obnoxious compounds in crude oil effluents also cause genetic toxicity when taken up by plants (Vwioko *et al.*, 2006).

Kaduna Refinery and Petrochemical Company (KRPC) discharges effluents from the refinery through a 4km drain into River Romi. Some plants species such as *Mariscus longibrateatus*, are found growing inside this drain, thereby maintaining constant contact with these effluents. Plants species could absorb, translocate and bioaccumulate the toxicants in the effluents into their different organs (Beg *et al.*, 2003; Gielwanowska *et al.*, 2005). However, the capacity to absorb, bioaccumulate differs from plants to plants. Unfortunately, the accumulation of these heavy metals in plants causes morphological, anatomical and genetic changes in some plants (Fisher *et al.*, 1981; Singh *et al.*, 1988). Most researches have been centred on edible crop like *Allium cepa*. There is dearth of research on wild plants growing inside effluents from Refinery and Petrochemical Companies in Nigeria. This work therefore showed whether the effluents caused structural changes in *Mariscus longibrateatus*. The objective of this study was to investigate the bioaccumulating ability of *Mariscus longibrateatus* and effect of effluents from KRPC on the plant.

II. MATERIALS AND METHODS

A. Site Description

This study was conducted in Kaduna Refining and Petrochemical Company (KRPC). The coordinates of the study area are defined by latitude 10° 41' 15.90" N and longitude 07° 49' 65" E. KRPC is located on a land area of 2.89 km² approximately 15km Kaduna South, Kaduna State, Nigeria. All industrial wastes, untreated or minimally treated are discharged in the drain, which runs immediately downstream and eventually ends up in Romi River.

B. Sample Collection

A long tape (1000 m) was laid along the KRPC discharged drain; and at every 5m interval, one squared metre length space was marked out. *Mariscus longibrateatus*, which was the most abundant plant species was singled out, authenticated and identified at the botanical herbarium, Nigerian Defence Academy, Kaduna. The plant

and the corresponding soil sample growing around it were collected randomly from three sampling points and then taken to National Geosciences Research Laboratory (NGRL), Kaduna, Nigeria, for heavy metal analysis in their roots, stems and leaves. KRPC effluents was also analysed for heavy metal content. The plant used as control was identified as *Mariscus longibrateatus* growing in upland. Triplicate of the plant was collected for morphological, anatomical and molecular studies.

C. Physicochemical Analysis of the Effluents

Physicochemical analysis of the effluents was carried out to ascertain the quality of the water. Electrical conductivity (EC) of the water samples was determined using conductivity meter. Temperature and pH were analysed using HANNA instrument. Total dissolved solute (TDS) and Dissolved Oxygen (DO) were analysed using TDS meter and DO meter respectively. Chemical Oxygen Demand (COD) was determined by reflux digestion and titration method according to APHA (APHA, 1998). Oil and grease were measured using Gravimetric method. Heavy metals analysis which included Cadmium (Cd), Lead (Pb), Chromium (Cr), Copper (Cu), Manganese (Mn), Iron (Fe) and Zinc (Zn), were determined using Atomic Absorption Spectrophotometer (AAS), according to APHA (1998). All analyses were carried out using distilled water as control.

D. Chemical Analysis of Plant and Soil Samples for Heavy Metals

The plant samples were first washed in running tap water to remove adhering soil and other foreign materials followed by dipping in dilute hydrochloric acid (0.1 M HCl). After washing, plant samples were separated into root, stem and leaf. They were air-dried on filter paper and then oven dried at 105°C for 24 hours before crushing. The crushed leaf material was passed through 2mm mesh sieve and stored in air-tight polythene bags for subsequent chemical analysis. Chemical analysis was carried out for the estimation of heavy metals such as Cd, Pb, Cr, Cu, Mn, Fe and Zn. Adopting the method of APHA (1998), heavy metals in the various parts of the plants (root, stem and leaf) were analyzed using atomic absorption spectrophotometer (AAS).

Also, the soil samples which were collected from the drain were air-dried, crushed with wooden mortar and pestle and sieved through 2mm sieve. The sieved samples were labelled and stored in polythene bags for subsequent chemical analysis. Wet oxidation of the soil samples was employed to digest the samples. Determination of Heavy Metals (Fe, Mn, Cu, Zn, Cd, Pb, and Cr) was carried out by Atomic Absorption Spectrophotometer (APHA, 1998).

E. Morphological Assessment of the Plant

A morphological assessment was carried out by measuring the height of the plant from the soil level to the collar of the uppermost leaf. The leaf area was determined by measuring the length and width of each leaf. The product multiplied by a correction factor of 0.75 to cater for leaf shape (Szabo *et al.*, 2006).

F. Anatomical Examination of the Plant

Anatomical examination of the plant samples was carried out at Department of Biological Sciences, Ahmadu Bello

University, Zaria. Plant samples collected from the sites were kept fresh by putting them into a solution of Formalin-Acetic-Alcohol (FAA). The transverse sections of the roots, stems and leaves of these plant samples were examined anatomically. Paraffin wax mixed with chloroform in the sample bottle and then fixed in embedding oven set at 60°C. The sections were stained with methylene blue for 5 minutes, then washed to remove excess stain and mounted with a drop of 100% glycerin on a clean slide. Cover slips were placed on the preparations and examined under the microscope. The same plant spp. growing on upland out of contact with the KRPC waste water was used as control.

G. Molecular Identification of the Plant

Analysis of genomic DNA of the plant samples was carried out to determine the effect of the effluents on the plant. The DNA of the plant samples were extracted and analyzed according to the method of Zhang *et al.* (2007). The plant samples were pulverized to expose the cells of the plant. An equal volume of chloroform/phenol, 50 µl of RNase and 35 µl of pronase were added into the tube and incubated at 37°C for 15 minutes until lysis completely took place. The aqueous DNA layer was pipetted; The DNA was dried at room temperature and dissolved in 50 µl Tris-EDTA (TE) buffer. The purity of the DNA preparation was determined by calculating the ratio of the absorbance at 280nm to 260nm, a ratio of 1.8 or above indicated a pure sample of DNA. Polymerase chain reaction (PCR) amplification was performed using DNA taq polymerase (Thompson *et al.*, 1994). The PCR conditions was 95 °C for 2 min to denature the template, followed by temperatures of about 55-62°C for 30 sec for primer annealing and 72 °C for 10 minutes for extension.

DNA fragments were separated by agarose gel electrophoresis using 1 % w/v agarose in Tris-acetate-EDTA (TAE) buffer. DNA was visualized by adding 10 µg/ml ethidium bromide to the gel and viewing under ultraviolet. DNA was sequenced using an automated sequencer. Sequences were compared with other sequences in the GenBank databases using the Basic Local Alignment Search Tool (BLAST). For phylogenetic tree construction, multiple sequences were obtained from GenBank.

H. Statistical Analysis

Significant difference in the heavy metal content of the soil, root, stem and root was determined using Analysis of Variance (ANOVA) in the Control site, Site 1, 2 and 3. Correlation analysis of the heavy metals was carried out in the three sampling sites (Site 1, 2 and 3). ANOVA was employed to determine significant difference in the heavy metal of the different sites, in their soil, root, stem and leaf. The analyses were done at 0.05 significant level.

III. RESULTS

A. Physicochemical Parameters and Heavy Metal Concentration of the Effluents

The physicochemical parameters and heavy metal concentration of the effluents from the three different sampling points are shown in Table I. The Electrical conductivity ranged from 1050 to 2170 µS/cm. There was

not much difference in their temperature as it ranged from 28.7 to 29.7 °C. The pH of the effluents from the three sampling points was alkaline (7.9 – 8.7). Total dissolved solute ranged from 950 – 1205 mg/L. Dissolved oxygen and Chemical oxygen demand ranged from 6.5 to 7.2 mg/L and 62.0 to 84.5 mg/L respectively. The oil and grease present in one litre of the sample was between the ranges of 0.98 to 1.55 mg/L.

Heavy metal analysis showed a concentration of 0.18 to 0.57 mg/L for Manganese, 0.07 to 0.92 mg/L for Lead, 0.56 to 1.43 mg/L for Cadmium and 0.87 to 1.28 mg/L for Zinc. Silver concentration ranged from 0.08 to 0.92 mg/L. A relatively high concentration of Chromium (2.56 – 3.52 mg/L), Copper (2.11 – 2.85 mg/L) and Iron (1.90 – 3.45 mg/L) was observed in the effluents.

TABLE I: PHYSICOCHEMICAL PARAMETERS AND HEAVY METAL CONCENTRATION OF THE EFFLUENTS

Parameter	Site 1	Site 2	Site 3
EC (µS/cm)	2170	1420	1050
Temp. (°C)	29.5	28.7	29
pH	8.7	8.2	7.9
TDS (mg/L)	1110	950	1205
DO (mg/L)	6.5	7.2	6.9
COD (mg/L)	84.5	62	71.4
Oil and grease (mg/L)	1.26	0.98	1.55
Mn (mg/L)	0.22	0.18	0.57
Fe (mg/L)	2.31	1.9	3.45
Cd (mg/L)	0.56	0.98	1.43
Pb (mg/L)	0.11	0.07	0.92
Cr (mg/L)	3.52	2.56	3.01
Cu (mg/L)	2.85	2.39	2.11
Zn (mg/L)	0.87	1.28	0.95
Ag (mg/L)	0.08	0.92	0.23

Key: EC - Electrical conductivity; TDS- Total Dissolved Solutes DO- Dissolved Oxygen; COD- Chemical Oxygen Demand; Mn – Manganese; Fe – Iron; Cd- Cadmium; Pb- Lead; Cr- Chromium; Cu- Copper; Zn-Zinc.

B. Heavy Metal Analysis of the Soil

Result showed that iron (4.79 mg/g) had the highest concentration in the soil of the control site while copper was least (0.04 mg/g) in soil and root of control site. Lead also had a concentration of 0.04 mg/g in site 1 (Table II). Chromium (3.34 mg/g) was highest in the soil of site 1 while iron (4.65 mg/g) was highest in the soil of site 2. Lead accumulated most in the soil of site 2, with a concentration of 2.79 mg/g whereas iron had the highest concentration (4.79 mg/g) in the control site. Analysis of variance revealed that there was no significant difference in the soil heavy metals of the control site, site 1, 2 and 3, having a P-Value of 0.9465 (Table III). Comparison of heavy metal content in the sampling sites showed that chromium differed significantly in site 1 and 2 (Table III). Manganese varied between site 1 and other sites. There was significant difference between silver concentration in site 2 and 3 (Table III). As shown in Table IV, manganese correlated positively with cadmium ($r = 0.994$) and chromium ($r = 0.989$). There was a strong negative correlation between copper and zinc (-1.000) at 0.05 level of significance.

C. Heavy Metal Concentration in the Root

Comparison of heavy metals in the different sites showed that site 2 had the highest accumulation of most heavy metals in the roots of plants (Table II). The concentration of cadmium, lead, iron, zinc and silver were 2.34, 2.45, 4.00 and 1.50 mg/g. Chromium (2.21 mg/g) was the most

accumulated heavy metals in the roots of the plants in site 1. Manganese (1.30 mg/g) and copper (1.30 mg/g) had the highest concentration heavy metals in the roots of the plants in site 1 and site 3 respectively.

D. Heavy Metal Concentration in the Stem

A comparison of heavy metals between sites, Iron (2.65 mg/g) had the highest concentration in the stems of plants in site 2 and lowest in the stem of plant in the control site (0.87mg/g). Chromium (2.30 mg/g) was highest in site 1 and in the control site (1.25mg/g). Concentration of copper (1.00 mg/g) and cadmium (1.87 mg/g) in site 3 were highest. Control site had zinc (1.23 mg/g) as the most concentrated heavy metal (Table II).

E. Heavy Metal Concentration in the Leaf

Iron has the highest value in the leaves from site 2 (4.60 mg/g), zinc (1.66 mg/g) and silver (1.42 mg/g) followed respectively. Chromium (1.95 mg/g) had the highest concentration in site 1 while the lowest value recorded was lead (0.04mg/g). The concentration of lead (2.00 mg/g) and copper (0.87 mg/g) were highest in the leaves of the plant in control site (Table II).

F. Transfer Factor from Root to Stem

Transfer factor is the ratio of the concentration of heavy metal in the stem to that of the root of the plant. The transfer factor of the heavy metals from the root to the stem is presented in Table V. Transfer factor greater than one, showed that metals were effectively translocated to the stem from the root (Fayiga & Ma, 2006). The maximum rate of transfer factor was recorded for copper (4.55) in site 2. The transfer factor for chromium (1.02) and lead (1.13) were greater than one, in the control site. Zinc and silver also showed transfer factor greater than one in site 1 and 3 respectively.

G. Bioaccumulation Factor

Bioaccumulation factor is the ratio of the concentration of heavy metal in the stem to the heavy metals in the soil. Bioaccumulation factor for copper at control site was equal to one whereas bioaccumulation factor for silver and copper at site 1 and 2 respectively, were greater than one, indicating an effectively bioaccumulation transfer (Table VI).

TABLE II: HEAVY METAL CONTENT OF SOIL, ROOT, STEM AND LEAF

Heavy metals (mg/g)	Control				Site 1				Site 2				Site 3			
	Soil	Root	Stem	Leaf	Soil	Root	Stem	Leaf	Soil	Root	Stem	Leaf	Soil	Root	Stem	Leaf
Cadmium	1.43	1.00	0.85	0.66	2.45	1.56	1.23	0.90	2.00	2.34	1.20	0.60	1.98	1.90	1.87	0.25
Chromium	2.34	1.23	1.25	0.99	3.34	2.21	2.30	1.95	1.33	1.20	1.10	0.78	1.78	1.75	0.96	0.54
Manganese	0.80	0.74	0.50	0.88	2.21	1.30	1.51	1.42	0.90	0.95	0.65	0.10	1.00	1.00	0.54	0.30
Lead	2.71	0.40	0.45	2.00	1.90	0.25	0.12	0.04	3.31	2.45	2.05	0.65	2.79	2.00	1.65	0.70
Iron	4.79	0.99	0.87	0.98	1.85	2.20	1.25	1.57	4.65	4.00	2.65	4.60	2.34	2.30	2.00	1.65
Zinc	2.38	1.55	1.23	1.24	1.67	0.65	0.71	0.84	1.65	1.65	1.00	1.66	0.89	0.96	0.77	0.86
Copper	0.04	0.05	0.04	0.87	0.10	0.12	0.09	0.65	0.12	0.11	0.50	0.10	1.45	1.30	1.00	0.24
Silver	1.20	0.67	0.55	0.86	1.00	1.41	1.31	0.42	1.67	1.50	1.25	1.42	0.99	0.76	0.89	0.52

TABLE III: ANALYSIS OF HEAVY METAL CONTENT IN THE SOIL

Site	Cd	Cr	Mn	Pb	Fe	Zn	Cu	Ag
Control	0.99	1.45	0.73 ^a	1.39	1.92	0.54	0.41	0.28
1	0.67	2.45 ^a	0.41 ^{abc}	0.58	0.40	0.48	0.27	0.45
2	0.79	0.23 ^a	0.39 ^b	2.12	0.93	0.33	0.20	0.17 ^a
3	0.83	0.61	0.35 ^c	1.79	0.32	0.08	0.54	0.20 ^a
P-Value	0.6132	0.0172	0.0051	0.2163	0.0448	0.0533	0.0325	0.0270

Values on the same column that have the same letter in the superscript are significantly different at 0.05 level using Tukey's Significant Difference Test

TABLE V: TRANSFER FACTOR FROM ROOT TO STEM

Heavy Metals (mg/g)	Control	Site 1	Site 2	Site 3
Cadmium	0.85	0.79	0.51	0.98
Chromium	1.02	0.04	0.92	0.55
Manganese	0.68	0.16	0.68	0.54
Lead	1.13	0.48	0.84	0.83
Iron	0.88	0.57	0.66	0.87
Zinc	0.79	1.09	0.61	0.80
Copper	0.80	0.75	4.55	0.77
Silver	0.82	0.93	0.83	1.17

TABLE IV: CORRELATION ANALYSIS OF SOIL HEAVY METAL IN THE SAMPLING SITES

	Cd	Cr	Mn	Pb	Fe	Zn	Cu
Cr	0.968						
Mn	0.994*	0.989*					
Pb	-0.917	-0.987	0.954				
Fe	-0.606	-0.785	0.687	0.873			
Zn	0.551	0.325	0.460	0.172	0.330		
Cu	-0.543	-0.316	0.451	0.163	0.339	1.000**	
Ag	-0.456	-0.664	0.548	0.773	0.984	0.492	0.500

**P < 0.05, *P < 0.10

TABLE VI: BIOACCUMULATION FACTOR FROM ROOT TO STEM

Heavy Metals (mg/g)	Control	Site 1	Site 2	Site 3
Cadmium	0.59	0.50	0.60	0.94
Chromium	0.53	0.69	0.83	0.54
Manganese	0.63	0.68	0.72	0.54
Lead	0.17	0.06	0.62	0.59
Iron	0.18	0.68	0.57	0.85
Zinc	0.52	0.43	0.61	0.80
Copper	1.00	0.90	4.17	0.77
Silver	0.46	1.31	0.75	0.90

H. Morphological Characteristics of the Plant Samples

A morphological study of *Mariscus longibrateatus* from the three sampling sites in the discharged drain showed that the plants had similar height, shape, size and colour of stem, roots and leaves, while control sample from upland were all green including their numerous inflorescences. Table VII shows the plant heights, length, width and area of the leaves. The height (38.6 cm) of the control plant was more than that of the studied plants, and was greener and fresher. The studied plants from the sampling sites had thinner leaves than the control plant. The length of leaf of the studied plant samples ranged from 13.2 – 15.0 cm while the control was 15.8 cm.

TABLE VII: MORPHOLOGICAL CHARACTERISTICS OF THE PLANT SAMPLES

Morphological characteristics	Control	Site 1	Site 2	Site 3
Leaf length (cm)	15.8	13.2	15.0	14.8
Leaf width (cm)	4.0	3.5	3.1	3.5
Plant height (cm)	38.6	33.1	34.5	34.0
Leaf area (cm ²)	47.40	34.65	34.88	38.85

I. Anatomical Characteristics of the Plant Samples

The anatomical characteristics of the vascular bundles of the leaves, stems and roots of the plant samples are shown in Table VIII. The whole vascular bundle area of leaf, stem and root of the control plant were greater than that of studied plants. The leaf whole vascular bundle of the studied plants ranged from 19085 – 20790 μm^2 whereas phloem and xylem area ranged from 3995 – 4290 μm^2 and 6584 – 7004 μm^2 respectively. The range of stem whole vascular bundle area was 19950 – 21549 μm^2 while phloem and xylem were 3829 – 4156 μm^2 and 6748 – 8100 μm^2 respectively. Root whole vascular bundle area ranged from 20650 – 21811 μm^2 . The ranges of area of phloem and xylem were 3263 – 3886 μm^2 and 6495 – 6754 μm^2 respectively.

TABLE VIII: ANATOMICAL CHARACTERISTICS OF THE VASCULAR BUNDLES OF THE LEAVES, STEMS AND ROOTS OF THE PLANT SAMPLES

Leaf	Control	Site 1	Site 2	Site 3
Phloem area (μm^2)	4421	4101	4290	3995
Xylem area (μm^2)	7405	6980	7004	6584
Whole vascular bundle area (μm^2)	21856	19350	20790	19085
Mesophyll (μm)	156	112	109	110
Parenchyma (μm)	74	58	51	65
Epidermis (μm)	25	12	17	14
Stem				
Phloem area (μm^2)	4560	4000	4156	3829
Xylem area (μm^2)	8500	7870	6748	8100
Whole vascular bundle area (μm^2)	22548	19950	20056	21549
Root				
Phloem area (μm^2)	3965	3488	3886	3263
Xylem area (μm^2)	7852	6495	6732	6754
Whole vascular bundle area (μm^2)	22870	21625	21811	20650

J. Molecular Identification of the Plant Samples

Results revealed the difference between the plant spp. growing in the drain and the plant growing upland with respect to their molecular identity (Table IX). Phylogenetic analysis showed that *M. longibrateatus* growing in the drain is 97% similar to *Cyperus filiculmis*, indicating effect of KRPC effluents on the plant.

TABLE IX: BLAST ANALYSIS OF THE PLANT SAMPLES

Organism	Plant spp. Growing in the Drain	Plant spp. Growing Upland
GenBank Relative Accession Number	<i>Cyperus filiculmis</i> KY267395.1	<i>Mariscus longibrateatus</i> AB889399.1
Coverage	98%	100%
E-Value	2e-111	2e-116
Identity	97%	100%
Accession Length	412	429

IV. DISCUSSION

The effluent temperature range (28.7 °C – 29.5 °C) was within the acceptable Federal Ministry of Environment limit (< 40 °C). Usman *et al.* (2012) documented a similar value of temperature (28 – 32 °C) on KRPC discharge effluent in River Romi. Temperature controls the rate of all chemical reactions, and affects the activities of aquatic ecosystem. The pH values of the effluent were within the permissible Federal Ministry of Environment limit of 6-9. This finding is in agreement with the report of Uzoekwe and Oghosanine (2011) who recorded a pH of 6.5 – 8.5 from Warri refinery effluent, Nigeria.

Electrical Conductivity is a function of the concentration of soluble ionic salt present in the wastewater. The conductivity values in the three sampling points were above World Health Organization and Federal Ministry of Environment permissible limits of 500 $\mu\text{S}/\text{cm}$ for surface waters. Similarly, Usman *et al.* (2012) obtained high conductivity values on their assessment of refinery effluents from Kaduna refinery petrochemical company (KRPC). The high conductivity level at the sampling points could also be linked to leaching of inorganic contaminants as observed by Harison (1992). High conductivity could lead to changes that reduce biodiversity and alter community composition of aquatic flora and fauna. Rosenberg (2008) reported in their study that crude oil pollution has also been associated with increase in nutritive salts (CO_3^{2-} , SO_4^{2-} , NH_4^+ and NO_3^-) and salinity level of aquatic ecosystem. Total dissolved solids (TDS) in site 1 and 3 were high when compared with World Health Organization standard limit for good water quality (1000 mg/L). The increased level of dissolved solute may be as a result of high deposit of organic and inorganic matters from the effluent discharge. The relatively low value of TDS in site 2 might have occurred as a result of dilution factor as the river flows (Chapman, 1996).

Dissolved oxygen (DO) value with water body gives direct and indirect information such as bacterial activity, photosynthesis, availability of nutrients, stratification (Premlata, 2009). Dissolved oxygen (DO) concentration was above the WHO acceptable limit of 5mg/L. The result conforms to the findings of Momba and Kamika (2003).

Rosenberg (2008) reported that conductivity, pH and DO are dependent upon water temperature. At high temperature, the rate of dissolution of atmospheric oxygen in the water is usually low and this affects the sustainability of the aquatic habitats due to the reduction in the level of dissolved oxygen. Also, the excessive production of organic matter leads to the building up of sludge and mineralization process which consumes all dissolved oxygen from the water column and this causes death to aquatic organisms (Osibanjo & Adie, 2011). Similarly, McNeely *et al.* (1979) reported that large amounts of degradable organic materials results in low level of dissolved oxygen.

The chemical oxygen demand (COD) values exceeded the Federal Ministry of Environment acceptable limit (40 mg/L). This high level of COD might be due to the discharge of chemical oxidants from the refinery into the receiving water. McNeely *et al.* (1979) reported that high COD could deplete available oxygen; create septic conditions, generate foul-smelling hydrogen sulfide, which in turn can precipitate iron and any dissolved salts, turning the water black and highly toxic for aquatic life in the receiving river system. High COD could likely cause nutritive fixation in the soil resulting in reduced rate of nutrient availability to plants (Ubwa *et al.*, 2013).

Vwiokoet *et al.* (2006) reported that plants growing in crude oil polluted site could accumulate and translocate heavy metals in all parts of the plants. Heavy metals such as Lead, manganese, iron, chromium, zinc, cadmium, copper and silver, were accumulated by the plants. This is consistent with the report of Beauford *et al.* (1977) who reported that *Mariscus longibrateatus* growing in industrial effluent concentrated toxic metals. In this study, higher concentrations of heavy metals were reported in the roots of plants. Logendra *et al.* (1999) observed that heavy metals are accumulated more in the roots of plants, and they associated the observation to the fact that the roots are in direct contact with the metals in the soil environment. The effect of crude oil effluent on plant is of great concern as it causes damage to different parts of the plant that are vital for its wellbeing and survival and hence obstructs development and growth.

Anoliefo and Edegbai (2000) showed that the leaves of plants affected by crude oil effluent changed colour and show a general sign of chlorosis, indicating water deficiency. Plants exposed to pollutants can present changes in their morphology, anatomy, physiology and biochemistry (Gabara *et al.*, 2003; Reig-Armiñana *et al.*, 2004). The morphological characteristics of *M. longibreatus* from the three sampling sites have shown that the plants have slightly different, but similar height, shape, size and colour of leaves, stem and roots. However, morphological study of the plant samples revealed that the leaves of *M. longibreatus* growing in the drain are significantly thinner than that from the leaves from upland (Control). Gielwanowska *et al.* (2005) reported that reduction in leaf area growing in the vicinity of effluents observed in many plants may be due to dehydration. Also, some hidden injury or physiological disturbance might have occurred which caused reduction in morphological and anatomical characters of other plant species (Makbul *et al.*, 2006). There was a difference in colouration of leaves of *M. longibrateatus* growing in the

drain from the one growing outside the drain. Observable colour change in *G. americana* leaves exposed to pollutants were also related by Silva *et al.* (2005) who classified this species (based on morphological changes), in comparison with other four plant species, as moderately injured when exposed to pollutants. Uaboi-Egbenni *et al.* (2009) observed that the water-soluble fraction of oil had a high wetting capacity and penetrating power and when in contact with root, the oil would enter and affect the anatomical features of the root and bring about root stress. Gielwanowska *et al.* (2005) found that the water-soluble fraction of the oil was translocated to other parts of the plant, leading to changes in the anatomical structures and considerable reduction in plant height, plant girth and even leaf area.

Anatomical studies of *M. longibreatus* are mainly focused on leaf, stem and root features. The anatomical analysis of injuries caused by pollutants on plant species has been used in various studies to assess the real damage caused by pollutants (Hara, 2000). Several authors have related the deleterious effects of pollutants on the anatomy and ultra-structural leaf characteristics (Gabara *et al.*, 2003; Reig-Armiñana *et al.*, 2004). Leaf anatomy of the studied plant showed a reduction in mesophyll, parenchyma and epidermis as compared to leaves (control) collected from upland. Results from other authors revealed that significant reduction or change in shape and anatomical structures of leaves were due to continuous exposure to heavy metal pollutants (Szabo *et al.*, 2006). Similarly, Jahan and Zafar (1992) have reported significant reduction in mesophyll and flattening of leaf parenchyma cells in heavy metal polluted environment.

The vascular system (including xylem, phloem, and the bundle sheath) is the most important architectural component in plant tissues and is responsible for the transport of water and nutrients (Steudle & Frensch, 1996; Cholewa & Griffith, 2004). The vascular bundle size and the density of bundle sheath cells are strongly positively correlated with translocation and height of plants (He & Zhang, 2003). In this study, *M. longibrateatus* growing in the drain showed differences in the size and area of their whole vascular bundles when compared with *M. longibrateatus* growing outside the drain. A study carried out by Ogle (2003) revealed that change in the area of whole vascular bundle of plants were due to their prolonged exposure to effluents. This suggests that a modification in the vascular system greatly determines the ability of plants to tolerate heavy metals, thus hyperaccumulators of heavy metals. Logendra *et al.* (1999) proposed that hyperaccumulator species can be identified by collecting plants from the areas where soil contains greater than usual amount of heavy metals as in the case of polluted areas such as drain. *M. longibrateatus* growing in the drain accumulated heavy metals in their various organs. Prasad (1995) had reported that some of the prevalent mechanisms of metal-tolerance were accumulation, sequestration, synthesis of metal-binding complexes and their stabilization by sulphide ions. Prevalence of some plant species in one location but not in the other may be attributed to environmental factors. Some of these factors affect plant growth including the topography of the land, soil type, drainage characteristics, climate and the level of

contaminants present in the environment (Imevbore & Adeyemi, 1981).

A relatively small group of hyperaccumulator plants are capable of sequestering heavy metals in their shoot tissues at high concentrations (Yang *et al.*, 2005). Heavy metal tolerance, as observed in *M. longibrateatus* growing in the drain can be suggested to have hyperaccumulator ability. This phenomenon has implications in phytoremediation (Prasad & Freitas, 2003).

V. CONCLUSION

Physicochemical parameters of the effluents from the three sampling points varied. Bioaccumulation factor showed that there was no effective accumulation of the heavy metals by *Mariscus longibrateatus* but it demonstrates the ability to survive in KRPC drain. *Mariscus longibrateatus* from the three sampling points showed similar morphology but different from that of the control. The anatomy of the studied plant species was greatly affected in comparison to that of the control. The Kaduna Refinery and Petrochemical Corporation (KRPC) should employ more effective and efficient techniques in treating effluents before discharge into the water bodies. Regulatory agencies should reassess their standard of effluent limitations and take into consideration living organisms especially plants. There is need for other plants growing in the drain to be studied in order to measure the level of impact of effluents on aquatic biota. *M. longibrateatus* growing in KRPC drain should be studied for phytoremediation because of their tolerant ability to heavy metals.

CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest.

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