EFFECT OF ARTISANAL CRUDE OIL REFINING ON THE MICROBIOLOGICAL PROFILE CHEMICAL CONTAMINATION OF PLANTS

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Abstract: Artisanal crude oil refining spills crude oil and refined products, in addition, releases soot into the environment. This can impact negatively on plants within the vicinity of pollution. This study aimed to assess the impact of artisanal refinery operations on plants in Igia-Ama, Tombia Kingdom, Rivers State, Nigeria. The concentrations of heavy metals, hydrocarbons and polychlorinated biphenyls (PCB) were determined in plant tissues, using standard APHA methods. The microbial population on the plants was determined using standard plate count. The mean total heterotrophic bacterial count (THBC) for *Chromolaena odorata* during the wet and dry seasons respectively was 6.8 log CFU/g and 7.8 log CFU/g; the mean total fungal count (TFC) was 2.3 log CFU/g and 4 log CFU/g; mean hydrocarbon utilising bacterial count (HUBC) 4.9 log CFU/g and 4.4 log CFU/g and mean hydrocarbon utilising fungal count (HUFC) was 0 log CFU/g and 3.5 CFU/g. Mean THBC for *Ipomoea involucrate* during the wet and dry seasons respectively are 6.6 log CFU/g and 8.1 log CFU/g; TFC 1 1og CFU/g and 1.5 log CFU/g; HUBC 4.8 log CFU/g and 5.9 log CFU/g and HUFC 0 log CFU/g and 3.7 log CFU/g. All the heavy metals monitored (Cd, Cr, Fe, Cu, Ni, Pb, Ti, Se, Zn and Hg) in polluted plant samples, were detected at various concentrations during the dry and wet season. Mean BTEX and PCB concentrations of 0.74±0.26 mg/kg and 1.5±0.06 mg/kg were detected in *Chromolaena odorata*, whereas for *Ipomoea involucrate,* the concentrations were 1.19±0.28 mg/kg and 1.44±0.14 mg/kg. PAH and TPH were not detected in plant samplesPlants in the impacted area were severely tainted with heavy metals and hydrocarbons, therefore making them unsafe for consumption.

Keywords: Artisanal refinery, *Chromolaena odorata*, *Ipomoea involucrate*, heavy metals, hydrocarbons

Introduction

The Niger Delta region has the largest oil and gas resources, the mainstay of Nigeria's economy. While these resources are needed for the development of the nation, there is the problem of striking a balance between harnessing their full benefits and the cost in terms of deterioration of the physical ecosystem and quality of life in this region. Environmental awareness has made the danger of polluting air, water, soil and vegetation of great concern today (Satish, 2012). While attention on environmental pollution in the Niger Delta had been mostly on largescale oil spill from oil and gas exploitation, with local and international oil companies being fingered, the contribution of artisanal crude oil refining to the degradation of this fragile ecosystem, is not going unnoticed.

Artisanal crude oil refinery is a makeshift setup for the separation of petroleum fractions, particularly diesel, petrol and kerosene. The refining process is similar to crude distillation applied in local gin making, which does not require any sophisticated technology. The refineries abound in the creeks of the Niger Delta, where criminal cannibalism of oil pipelines supply of crude oil, which is either sold or refined locally (Onwuna *et al*., 2022a). These substandard illegal refineries, have low-quality refining capacity and invariably contribute to the daily release of hazardous gases, soot, and spilt crude oil into the environment, in amounts that threaten vegetation (Akeredolu and Sonibare, 2015; Onwuna *et al*., 2022b).

Local crude oil refining has become a lucrative but disturbing business in Nigeria's Niger Delta expanse. Camps are built deep within the Niger Delta Forest and used for local crude oil refining. The economic benefits to the refiners are obvious, but the host communities are severely impacted by the activities of the local refiners. As a result of river and estuary pollution, farmlands have been destroyed and fishing villages have been evacuated, resulting in the loss of lives and property.

Pollution is a civil wrong and an environmental crime, as it a wrongful contamination of the environment. It is an offence committed against the community, with immediate and long-term implications in some instances, as seen in the pollution in the Niger Delta. Any solid, liquid or gaseous substances present in such concentration as maybe or tend to be injurious to the environment, is considered a pollutant (Satish, 2012). Any entity, therefore, that is guilty of causing pollution or illegally discharging pollutants into the environment, must be held liable for the damages and compensate for ecological restoration (Satish, 2012). The weakness of the law, lack of enforcement and criminal negligence has made pollution in the Niger Delta to continue unabated. The vicinity around artisanal crude oil refineries portrays the ecological danger of unabashed criminal refining of crude oil in Nigeria, as the area is often bare of vegetation.

Artisanal crude oil refinery waste typically includes unrefined crude, partially refined crude and soot (Onakpohor *et al*., 2020). These pollutants have been reported to impact on vegetation. Soot can reduce the amount of available water in soil by clogging air spaces (Swami, 2018); can affect plants by covering leaf and stem surface (Swami, 2018); can occlude stomata which could hinder transpiration, photosynthesis and respiration (Pourkhabbaz *et al.,* 2010), and could alter the chemical composition and metabolic functions of plant leading to growth retardation, plant stress and eventually death (Swami, 2018**)**. Hazardous effects of hydrocarbons on vegetation include interference with plant nutrient absorption (Alkorta and Garbisu, 2001; Andrade *et al*., 2004; Rahbar *et al*., 2012); reduction of chlorophyll contents and stomatal conductance (Osuagwu *et al*., 2013; Han *et al*., 2016); reduction the amount of essential elements in soil while boosting total microbial biomass

(Shukry *et al*. 2013), metabolic impairment (Shukry *et al*., 2013; Achuba 2014), inhibition of photosynthesis and transpiration (Lin *et al*., 2002; Rahbar *et al*., 2012), inhibition of germination (Oyedeji *et al*. (2012), decline in plant growth and yield (Ali and Abd Ali, 2008; Agbogidi, 2010) and ultimately, depletion of plant communities.

Plants’ bioaccumulation of petroleum contaminants has implication for human health problems upon consumption (Park and Park, 2011; Oyedeji *et al*., 2012). Soot composition can be delivered into the abiotic environment (soil, water, and air) by bioaccumulation or as food intake (trophic transfer) (Chojnacka and Mikulewicz, 2014).

This study aimed to assess the impact of artisanal crude oil refining on plants.

Materials and Methods

Study Area

The study location was Igia-Ama, Tombia, Rivers State, Nigeria located at 40 53’ 12.7’’ North, 70 07’ 30.6’’ East. The area is within the mangrove forest belt of the Niger Delta. The riverine area of Tombia Kingdom is situated in Degema Local Government Area of Rivers State and has a population of approximately 15,000 persons.

The oil-rich region has several oil and gas facilities. Traditionally, the people of Tombia Kingdom are fishermen and women. Subsistence agriculture is also practiced in the area. The creeks of Igia-Ama are sites for artisanal crude oil refining.

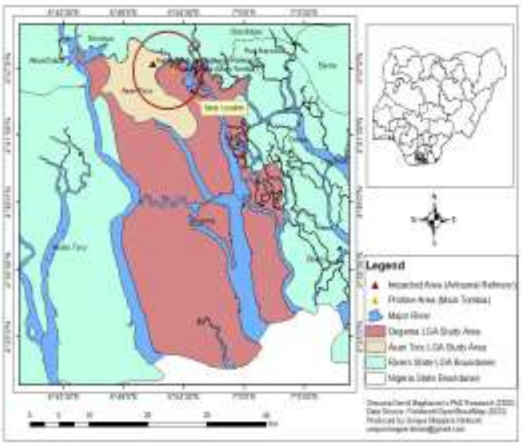

Figure 1: Study area showing sampling points

Samples Collection

Two plant species (*Ipomoea involucrate* and *Chromolaena odorata*) were collected from the control and impacted sites. They were transported to the Plant Horticulture Laboratory, University of Port Harcourt, for plant identification. Furthermore, the plants were processed for microbial identification and enumeration, as well as for physicochemical analysis.

Physicochemical Analysis

Heavy metal contents were determined using APHA (3030E) Atomic Absorption Spectrophotometry (AAS) AA500 PG method APHA (1998). PAH, TPH and BTEX were determined using EPA 8015 U.S. EPA (2003) and EPA 8100 U.S. EPA (1986) methods using Gas Chromatography and Flame Ionization Detector (GC/FID).

Parameters monitored in the plant were biomass and moisture contents.

Microbiological Analysis

One (1) gram of plant samples was macerated and dissolved in 9 ml of distilled water. Aliquots of 0.1ml of the serially diluted sample were plated in duplicates on nutrients agar (for bacteria) and potato dextrose agar (for fungi) using spread plate techniques. Triplicate plates were incubated at room temperature for 24-78 hours. After incubation colonies were counted to obtain colony forming units (CFU) per gram of plant samples as CFU/g. discrete colonies were picked and sub-cultured, and subsequently identified based on their cultural, morphological and biochemical characteristics.

Hydrocarbon utilizing bacteria (HUB) and hydrocarbon utilizing fungi (HUF) were isolated and enumerated using the vapour phase transfer method (Onwuna *et al*., 2022a).

Data Analysis

Information gathered was analysed using descriptive statistics (frequencies and percentages) and inferential statistics. Data for physicochemical and microbiological parameters monitored were subjected to one-way analysis of variance (ANOVA) using SPSS ver 20.

Results

Microbial Load on Plants

Figure 1 shows bacterial and fungal counts in *Chromolaena odorata* and *Ipomoea involucrate*. Mean THBC, TFC, HUBC and HUFC for *Chromolaena odorata* during the wet and dry seasons respectively were 6.8 log CFU/g and 7.8 log CFU/g; 2.3 log CFU/g and 4 log CFU/g; HUBC 4.9 log CFU/g and 4.4 log CFU/g, and HUFC 0 log CFU/g and 3.5 CFU/g. Mean THBC, TFC, HUB and HUF for *Ipomoea involucrate* during the wet and dry seasons respectively were 6.6 log CFU/g and 8.1 log CFU/g; 1 log CFU/g and 1.5 log CFU/g; 4.8 log CFU/g and 5.9 log CFU/g, and 0 log CFU/g and 3.7 log CFU/g.

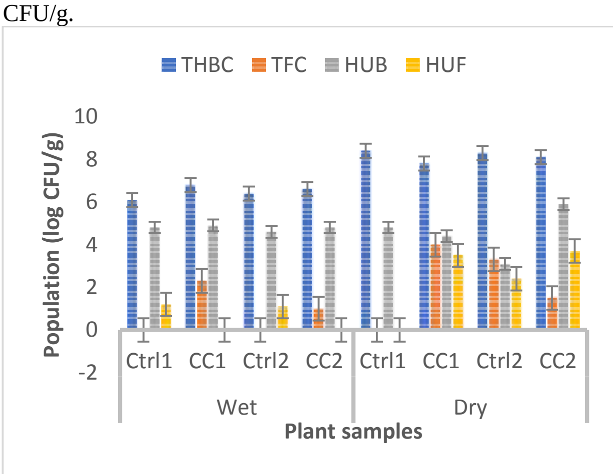

Fig. 1: Bacterial and fungal counts in *Chromolaena odorata* (CC1) and *Ipomoea involucrate* (CC2)

Heavy Metals Concentrations in Plants

Table 1 shows heavy metal concentrations in plants. All the heavy metals monitored in polluted plant samples were detected at various concentrations during the dry and wet season. The analysis of variance (ANOVA) result revealed that in the dry season, the concentration of heavy metal in the sample are significantly different ($p<0.05$) for the following heavy metals Cd, Cr, Fe, Cu, Ni, Pb, Ti, Se and Zn except Hg which was same. Also, in the wet season, samples are significantly different ($p<0.05$) for the following heavy metals Cd, Cr, Fe, Cu, Ni, Pb, Ti, Se and Zn except Hg which was the same.

Table 1: Heavy metals concentrations in plants

	Dry Season				**Wet Season**			
	Control (Plt 1)	Polluted (Plt 1)	Control (Plt 2)	Polluted (Plt 2)	Control (Plt 1)	Polluted (Plt 1)	Control (Plt 2)	Polluted (Plt 2)
As (mg/kg)	0[a]	0[a]	0[a]	0[a]	0[a]	0.47[a]	0[a]	10.67[b]
Cd (mg/kg)	1.84[d]	4.89[f]	2.57[e]	2.11[d]	3.54[b]	0.07[a]	3.3[b]	6.57[f]
Cr (mg/kg)	0[a]	0.34[a]	0[a]	0[a]	0[a]	0.03[a]	0[a]	0.81[b]
Fe (mg/kg)	2396.18[c]	2.36[a]	1814.19[b]	4374.11[d]	244.26[a]	2.43[a]	199.74[a]	396.13[a]
Cu (mg/kg)	0[a]	0.03[a]	0[a]	0.16[b]	0.49b	0.09[a]	1.02[c]	7.86[g]
Ni (mg/kg)	0[a]	0.15[a]	0.43[a]	1.33a	2.59[b]	0.12[a]	4.45[d]	4.34[d]
Pb (mg/kg)	4.28[b]	0.08[a]	0.39[a]	4.24[b]	4.57[c]	0.1[a]	5.1[c]	5.03[c]
Ti (mg/kg)	0[a]	7.71[c]	0[a]	2.29[b]	0[a]	0.07[a]	0[a]	0.66[a]
Se (mg/kg)	0[a]	0.76[a]	0[a]	40.55[b]	0[a]	110.31[b]	0[a]	7.7[a]
Zn (mg/kg)	19.92[c]	0.12[a]	60.79[g]	55.57[f]	7.2[b]	0.32[a]	15.38[d]	18.37[f]

Plt 1 = *Chromolaena odorata* Plt 2 = *Ipomoea involucrate*

PAH, TPH, BTEX and PCB Levels in Plant

Mean BTEX and PCB concentrations of 0.74±0.26 mg/kg and 1.5±0.06 mg/kg were detected in *Chromolaena odorata,* whereas for *Ipomoea involucrate,* the concentrations were 1.19±0.28 mg/kg and 1.44±0.14 mg/kg. PAH and TPH were not detected in plant samples.

Table 2: PAH, TPH, BTEX and PCB contents of Plant

Seasons	**Samples**	**PAH (mg/kg)**	**TPH (mg/kg)**	**BTEX (mg/kg)**	**PCB (mg/kg)**
	Control (Plt 1)	0±0[a]	0±0[a]	0±0[a]	0±0[a]
Wet	Polluted (Plt 1)	0±0[a]	0±0a	0.74±0.26a	0±0[a]
	Control (Plt 2)	0±0[a]	0±0a	0±0a	0±0[a]
	Polluted (Plt 2)	0±0[a]	0±0[a]	1.19±0.28a	1.44±0.14a
	Control (Plt 1)	0±0[a]	0±0[a]	0±0[a]	0±0[a]
Dry	Polluted (Plt 1)	0±0a	57.41±10.85c	0.49±0.36a	0±0[a]
	Control (Plt 2)	0±0[a]	0±0[a]	0±0[a]	0±0[a]
	Polluted (Plt 2)	0±0[a]	55.73±9.52[c]	0.29±0.04[ab]	5.19±0.32b

Plant biomass and moisture content

Table 4 shows mean values for plant biomass and moisture content for both wet and dry seasons. The ANOVA result revealed that samples were significantly (p<0.05) different for plant moisture but were not significant (p>0.05) for biomass.

Table 4: Plant biomass and moisture content (wet and dry seasons)

	Factor	**Biomass**	**Moisture**
Dry	Control	6.68±0[a]	42.37±0.31[a]
	Polluted	23.4±0[ab]	36.42±0.93[a]
Wet	Control	16.06±22.57[ab]	68.2±8[b]
	Polluted	43.84±22.85[b]	73.74±0.15[b]
	ANOVA (p-value) Decision	2.90 (0.102) Not Significant	63.382 (0.000) Significant

Discussion

The microbiological and physicochemical properties of plants within the vicinity of artisanal crude oil refineries in Agia-Ama, Tombia Kingdom, were evaluated in this study. Microbial populations observed on sampled plants (*Chromolaena odorata* and *Ipomoea involucrate*) isolated from polluted sites were variable across dry and wet seasons and differed from the control. Microorganisms by their ubiquity can grow on plant surfaces in free, symbiotic or parasitic associations. Plant-microbes interaction can help in the remediation of hydrocarbon polluted sites (Sharma and Pathak, 2014). In the present study, hydrocarbon utilizers on the plant samples were high (10^3 - 10^4 log CFU/g). This is consistent with the report by Al-

Awadhi *et al* (2012) that plant growing in Kuwait harboured hydrocarbon utilizing bacteria in the same magnitude.

Volatile hydrocarbon components of soot from artisanal refinery sites can be metabolized by hydrocarbon utilizing microorganisms in the phyllosphere, as supported by studies by Pawlik *et al*. (2017) and Al-Awadhi *et al* (2012), particularly by Actinobacteria and Firmicutes. Endophytic bacteria can benefit their host in many ways one, as growth promoters, and with regards to hydrocarbon pollutants, they can aid their eventual metabolism and removal from the plants, thus contributing to plants' adaptation to environmental contaminants (Pawlik *et al*., 2017).

All the heavy metals monitored, Cd, Cr, Fe, Cu, Ni, Pb, Ti, Se and Zn, were detected in the plant samples, except As. The analysis of variance (ANOVA) result revealed that in the dry season, the concentration of heavy metal in the sample are significantly different ($p<0.05$) for the following heavy metals Cd, Cr, Fe, Cu, Ni, Pb, Ti, Se and Zn except Hg which was same. Also, in the wet season, samples are significantly different ($p<0.05$) for the following heavy metals Cd, Cr, Fe, Cu, Ni, Pb, Ti, Se and Zn except Hg which was the same. Heavy metal concentrations in polluted samples were higher than in the control. The study carried out by Onwuna *et al*. (2022b) reported high heavy metal contamination in soil and water samples within the vicinity of artisanal crude oil refinery sites. Plants growing in such environments would invariably accumulate the heavy metals in their tissues. The contribution of soot to heavy metal pollution in the Niger Delta is supported by Yakubu (2018) and Obi and Ugwoha (2022). These metallic elements are considered systemic toxicants that are known to induce multiple organ damage, even at lower levels of exposure (Asati *et al*., 2016; Baghaie and Fereydoni, 2019).

For both wet and dry seasons, BTEX and PCB concentrations in polluted samples were higher than in the control. Hydrocarbons and PCBs are known stressors of plants (Collier *et al*., 2017; Odukoya *et al*., 2019), and could impact on the diversity and survival of plants when their concentrations are high in the environment. Subramanian, *et al*. (2017) reported on the effects of PCBs and hydroxylated PCBs on plants to include inhibition of germination and plant growth. Odukoya *et al*. (2019) inferred in their study that crude oil contamination induces abiotic stresses in plants and eventually affects crop quality and yield. The impact caused by these environmental contaminants is not limited to a reduction in plant yield and economic losses but also to the likely toxicity to living cells upon consumption (Baghaie and Fereydoni, 2019). Indeed, plants can respond to chemical pollution by accumulating large amounts of the pollutants in their tissue (Ogbo *et al*., 2009; Nie *et al*., 2010). Thus, the amount of BTEX and PCB suggests uptake from the environment.

Plant biomass does not appear to be affected by pollutants from the artisanal crude oil operations as ANOVA result revealed that samples are not significant ($p>0.05$) different for biomass. Reports by De Jong (1980) and Adieze *et al*. (2012) suggest that low concentrations of hydrocarbon support plant growth, which translates to an increase in biomass. However, moisture content for both wet and dry seasons was significantly ($p<0.05$) different. Crude oil and soot particles can clog plant stomata and affect transpiration (Pourkhabbaz *et al.*, 2010). Physical obstruction of the stomata depends on the amount and type of pollutant deposited on the plant (Pezeshki *et al*., 2000).

Conclusion

Plants growing in the vicinity of artisanal crude oil refineries harbour microorganisms including hydrocarbon utilizers, which can be beneficial in removing atmospheric hydrocarbon pollutants. The heavy metals, BTEX and PCB concentrations in plant tissues were higher in plant samples from impacted area than in the control sites, and in the wet season than in the dry season.

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