

**HEAVY METALS AND TOTAL HYDROCARBON CONTENTS OF NYPA FRUITICANS GROWN ALONG THE SHORES OF QUA IBOE RIVER, AKWA IBOM STATE OF NIGERIA**

**ABSTRACT**

This study ascertains the amount of heavy metal and hydrocarbon content in an area exposed to crude oil exploration and exploitation activities. The study area is the Qua Iboe River in the Niger Delta region of Nigeria and the study samples were *Nypa palm* (*Nypa fruiticans*) leaves obtained along the shores of the river. The concentration of Pb, Cd, Cu and Ni in the leaves were determined using the Atomic Absorption Spectrophotometer while Total Hydrocarbon Content was determined using Gas Chromatography, coupled with the flame ionization detector (GC-FID). Results obtained showed that in site I, Pb mean concentration was  $1.068 \pm 0.014$  mg/kg, Cd ( $0.017 \pm 0.003$  mg/kg), Cu ( $2.390 \pm 0.204$  mg/kg), Ni ( $0.012 \pm 0.001$  mg/kg) and THC ( $124.361.7 \pm 1120.502$  mg/kg) while for site II: Pb ( $1.076 \pm 0.025$  mg/kg), Cd ( $0.028 \pm 0.003$  mg/kg) Cu ( $0.037 \pm 0.007$  mg/kg) Ni ( $2.049 \pm 0.024$  mg/kg) and THC ( $311,813.4 \pm 2950.291$  mg/kg). Heavy metal concentration in the study sites were largely within the WHO permissible limits with the exception of Cd in site II. Generally, the amount of heavy metals and THC were higher in the study sites than in the control site, which was an area with negligent oil exploitation and industrial activities. Therefore, the elevated concentration of heavy metals and THC in the study sites may be attributed to the various industrial activities sited in the area.

**Keywords:** *Heavy Metal, Total Hydrocarbon, Nypa Fruiticans.*

**1. INTRODUCTION**

The southern region of Nigeria is characterized by a large number of oil and gas fields. The increasing explorative activities of oil companies and transportation of the crude oil to their refining/storage depots have led to a number of incidental discharges or spills into the environment. The oil spills can be attributed to various reasons ranging from failure of production equipment to operational mishaps, or intentional damage to production facilities, otherwise known as sabotage (Iwegbue *et al.*, 2007). One of the methods of determining the incidence of crude oil pollution in an area is by estimating the total hydrocarbon content (THC) and heavy metals in impacted soil. The increased use of metal-based fertilizer in agricultural revolution of government could result in

37 continued rise in concentration of metal pollution in fresh water reservoir due to water  
38 run-off (Ubong *et al.*, 2015). As human population increases, the intensity of anthropogenic threat  
39 exerted on the environment increases as a result of industrialization and agricultural activities (Ubong *et*  
40 *al.*, 2020a) Apart from soil environment and aquatic ecosystem, atmospheric inorganic  
41 contaminants of natural origin or anthropogenic sources that contained heavy metals  
42 and/or trace elements such as Cadmium (Cd), Chromium (Cr), Cobalt (Co), Copper  
43 (Cu), Nickel (Ni), Lead (Pb) and Zinc (Zn) at high concentrations could led to serious  
44 ecological consequences and pose human health risks (Ite *et al.*, 2016). Heavy metals  
45 are potentially hazardous to humans and various ecological receptors because of their  
46 toxicity, persistence, bioaccumulative and nonbiodegradable nature. Therefore,  
47 monitoring and evaluation of heavy metal concentrations in soils, groundwater and  
48 atmospheric environment is imperative in order identify hazards to human health, to  
49 prevent bioaccumulation in the food chain and further degradation of the ecosystem  
50 (Ite *et al.*, 2016) .

51 Organic contaminants are mostly human-induced chemicals entering into natural fresh  
52 water through pesticide use, industrial chemicals, and as by-products of degradation of  
53 other chemicals and persist long enough in the environment to cause harmful effects.  
54 They tend to accumulate in reservoirs such as water, soil, sediments etc. From these  
55 reservoirs, they are remobilized through various processes, switch form or speciation  
56 and become available to the biological food chain. In this way, these contaminants tend  
57 to bio accumulate and bio magnify exhibiting toxicity and other related outcomes –  
58 mutagenicity, carcinogenicity and teratogenicity - resulting into chronic and acute  
59 disorders (Barnes *et al.*, 2008) . Records of THC and heavy metals, taken seasonally  
60 would enhance the ability to confirm the extent of pollution, especially by comparing  
61 the data from virgin areas or available baseline data from regulatory bodies. Usually,  
62 when oil spills on shore or near shore, it inevitably affects the soil ecosystem, a prime  
63 factor in agricultural productivity. This is particularly problematic because most of the  
64 terrestrial ecosystems and shorelines in the oil producing communities encompass  
65 important agricultural land and are under continuous cultivation (Ribes *et al.*, 2003) .  
66 Soil contact with oil may result in the damage of soil properties and plant communities,  
67 as well as microorganisms present in the soil.

68 Qua Iboe river in the Niger Delta of Nigeria is located in an area of intense oil  
69 exploration and exploitation activities (Ikpe *et al.*, 2020). Nypa palm (*Nypa fruticans*),  
70 which is an ubiquitous mangrove along the shores of Qua Iboe River, are constantly  
71 affected by heavy metals and hydrocarbon pollution; therefore, they are used as bio-  
72 indicator to assess the damage caused by oil spill in the ecosystem (Ikpe *et al.*,2020).  
73 Accordingly, the present study examines the concentrations of hydrocarbons and heavy  
74 metals in Nypa palm at Qua Iboe River, Nigeria.

## 75 2. MATERIALS AND METHODS

## 76 Study area and Sampling Site

77 Qua Iboe River Estuary, the relief is characterized by the Atlantic Ocean shoreline and  
78 surf beach. It falls within Latitudes  $4^{\circ} 39'N$  and Longitudes  $7^{\circ} 56'E$ . The region is also  
79 characterized by mangrove swamp and river floodplains mostly found around the Qua  
80 Iboe Estuary and the floodplains in Akwa Ibom State, Nigeria. The river measures about  
81 158.18 kilometers in length and inundates about 7,000 hectares of fresh water  
82 floodplains once a year.

83 The samples were collected from three different sites; two sites were at Nditia  
84 community, Ibeno L.G.A with latitude between  $04^{\circ}34'56.74''N$  and  $04^{\circ}34'02.6''N$  and  
85 longitude between  $07^{\circ}54'50.96E$  and  $07^{\circ}58'25.9E$  respectively, while the third site was  
86 at Ikot Ibok, Etinan L.G.A between latitude  $4^{\circ}47'0.50''N$  and a longitude of  $7^{\circ}52'55.80''E$ ,  
87 which was the control.



96 **Fig. 1** Map showing the Study area

## 97 Sample collection and preservation

98 The *Nypa Fruticans* leaf samples were collected from the three sampling sites, washed  
99 with distilled water to remove dust particles and dried in an oven ( $105^{\circ}C$ ) for 24 hrs.  
100 The dried leaf samples were crushed using mortar and pestle. Finally, the powder was  
101 stored in tightly closed clean sample bottles until analysis.

## 102 Determination of Heavy Metals in *Nypa fruticans*

103 The grounded samples were digested after drying according to Lee, (1995). The  
104 concentration of Pb, Cd, Cu and Ni were determined using bulk scientific model 210VGP  
105 (variable giant pulse) atomic absorption spectrophotometer with different hollow

106 cathode lamp at different wavelength. All reagent used were of analytical grade and  
107 deionized water was used in all preparations. The digestion procedure was as follows;  
108 the samples (1 g) was weighed into digestion flask and 10 ml nitric acid (HNO<sub>3</sub>)/  
109 perchloric acid (HClO<sub>4</sub>) acid mixture (3:1 ratio) was added to the sample; the  
110 suspension was swirled and allowed for some minutes for any reaction to subside. The  
111 digestion flask was mounted on a heating mantle and heated at 70 C until appearance  
112 of whitish dense fumes; digestion ended when a clear solution was obtained. The  
113 digestion flask was removed and allowed to cool. 50 ml of deionized water was added  
114 to the digest filtered and made up to mark of 100 ml standard volumetric flask with  
115 deionized water. Each of the standard flasks with the digest was corked labeled and  
116 refrigerated for AAS analysis (Ogbeifun *et al.*, 2019).

### 117 **Determination of Total Hydrocarbon Contents (THC) in *Nypa fruticans***

118 Total hydrocarbon contents (THC) were determined using Gas chromatography fitted  
119 with flame ionization detector (GC-FID). The samples were cut into pieces and then  
120 crushed using mortar and pestle. The extraction method outlined by Schwab *et al.*,  
121 (1999);(Ekanem *et al.*,2019) was applied to the samples as follows; 10g of each of the  
122 crushed samples were weighed into a 100ml beaker and 60ml of THC extraction  
123 mixture (250ml of acetone and 250 ml of dichloromethane) was then added. The  
124 beaker with its contents was placed on a magnetic stirrer (with heater) and shaken for  
125 about 25 min. at 70<sup>0</sup>C. The extract was later decanted into a flask. 30ml of fresh  
126 extraction solvent was added and the process of shaking on the magnetic stirrer  
127 repeated. 5g of anhydrous sodium sulphate was used to remove water from the extract,  
128 which was concentrated to 3ml with rotary evaporator maintained at 20<sup>0</sup>C.  
129 Subsequently, 1.5 ml of the concentrated extract was loaded on silica gel column. The  
130 silica gel was prepared by loading a 2g glass wool followed by a 30g chromatography  
131 silica gel onto a chromatography column (2cm internal diameter and 10cm long). Each  
132 of the bed was conditioned with 40ml HPLC-hexane to remove any contaminant. The  
133 concentrated was eluted with 30ml HPLC-hexane into a well labeled 100ml beaker to  
134 get the aliphatic hydrocarbon components in the sample. Thereafter, 30ml of  
135 chloromethane was added to elute the aromatic hydrocarbon contents into another  
136 labeled 100ml beaker. 2g of anhydrous sodium sulphate was added to remove any  
137 traces of water left in the extract. The extract were re-concentrated using rotary  
138 evaporator to about 2ml. 1ml of extract was taken and transferred into a well labeled  
139 chromatography vial read for gas chromatography analysis. The samples were stored at  
140 temperature of 4<sup>0</sup>C until GC analysis.

### 141 **Moisture Content Determination**

142 Water content in *Nypa Fruticans* leaves was determined by drying a known weight (1 g)  
 143 in an oven to a constant weight at a suitable temperature of 105°C. The loss in weight  
 144 was due to moisture loss and calculated in terms of percentage weight of the samples  
 145 (1 g of the fresh sample).

146 % Moisture=  $\frac{W_1 - W_2}{W_1} \times 100$

147 Where  $W_2$ = weight of sample after drying

148  $W_1$ = weight of sample before drying

149 **Ash Content Determination**

150 The ash content of the samples was determined by burning the dry samples in an  
 151 enclosed muffle furnace at temperature of 500°C, for 4 hours until the samples turned  
 152 to ash. The organic matter was obtained by subtracting the amount of the ash from the  
 153 dry samples and the value expressed in terms of dry weight of the samples.

154 **3. RESULTS AND DISCUSSION**

155 **Table 1. Proximate analysis**

SAMPLE	pH	TEMPERATURE (°C)	MOISTURE CONTENT	ASH CONTENT
Nypa Palm	6.46±0.01	27.01±0.06	65.023±0.02	4.02±0.05

156

157 **Table 2. Levels of Heavy metals in *Nypa fruticans* leaves**

SITES	METALS	RANGES (mg/kg)	MEAN CONCENTRATIONS (mg/kg)	STANDARD DEVIATIONS
SITE I	Pb	1.051-1.076	1.068	0.014
	Cd	0.014-0.019	0.017	0.003
	Cu	1.001-3.09	2.390	0.204
	Ni	0.011-0.012	0.012	0.001
SITE II	Pb	1.048-1.094	1.076	0.025
	Cd	0.025-0.031	0.028	0.003

	Cu	0.029-0.042	0.037	0.007
	Ni	2.031-2.076	2.049	0.024
<b>SITE III (control)</b>	Pb	0.012-0.029	0.023	0.010
	Cd	BDL	BDL	BDL
	Cu	0.001-0.007	0.002	0.001
	Ni	0.007-0.007	0.007	0.000

158 Detection Limit (DL) of metals studied: Pb (0.25), Cd (0.025), Cu (0.078), Ni (0.125)

159

160

161 **Table 3. Levels of Total Hydrocarbon Contents in *Nypa friuticans* leaves**

<b>SITE</b>	<b>RANGE (mg/kg)</b>	<b>MEAN CONCENTRATION (mg/kg)</b>	<b>STANDARD DEVIATION (mg/kg)</b>
<b>SITE I</b>	123240 - 125481	124361.7	1120.502
<b>SITE II</b>	310009.1 - 315218.11	311,813.4	2950.291
<b>SITE III (control)</b>	1489.00 - 1502.13	1488.08	14.532

162

163 **Table 4. WHO recommended limits in plants (Organization, 1996) (WHO**  
164 **1996)**

<b>METALS</b>	<b>LIMITS (mg/kg)</b>
<b>Pb</b>	2
<b>Cd</b>	0.02
<b>Cu</b>	10
<b>Ni</b>	10

165

166 **Lead:** High Pb concentration in environmental compartments indicates pollution from  
167 oil spill and other industrial activities. According to Osuji & Onojake, (2004), lead is

168 present in crude oil obtained from the Niger Delta, Nigeria. Plants grown in lead-  
169 contaminated soils accumulate low levels of lead in the edible portions of the plant from  
170 adherence of dusts and translocation into the tissues (Finster *et al.*, 2004; Ubong *al.*,  
171 2020). At site I, the mean value of Lead in *Nypa fruticans* ranged from 1.051-1.076  
172 mg/kg with a mean value of  $1.068 \pm 0.014$  mg/kg (Table 2), site II ( $1.076 \pm 0.025$   
173 mg/kg) and III ( $0.023 \pm 0.010$  mg/kg). These values are all below the WHO  
174 recommended limit (Table 4), which indicates a low level of lead pollution. However,  
175 site III recorded the least concentration of lead and it may be attributed to the absence  
176 of oil exploration and other industrial activities at this site. Comparatively, these values  
177 are higher than results recorded by Opaluwa *et al.*, (2012) who investigated the heavy  
178 metals in plants grown around dumpsites in Lafia, Nigeria. However, the Pb  
179 concentrations in the current study are much lower than values obtained by Deribachew  
180 *et al.*, (2015), who investigated the levels of heavy metals in plants produced through  
181 wastewater irrigation in eastern Ethiopia. Lead is a well-known neurotoxin which causes  
182 the impairment of the neurodevelopment in children. Exposure in the uterus and  
183 breastfeeding may all be responsible for the effects. Lead accumulates in the skeleton  
184 and its mobilization from bones during pregnancy and lactation causes exposure to  
185 fetuses and breastfed infants (ATSDR, 2007). The observed study disagrees with the  
186 observation of Ubong *et al.*, (2011) on determination of heavy metals in tissues of  
187 *Callinectes latimanus* from new Calabar river, Nigeria. The results obtained from the  
188 current study show that metal concentration in male *C. latimanus* ranged as follows: Ni  
189 (93.09 – 231.17 mg/kg), Pb (2.73 – 29.76 mg/kg), Cd (0.05 – 4.10 mg/kg) while the  
190 range for female crab species were: Ni (165.63 – 313.53 mg/kg), Pb (4.77 – 37.08  
191 mg/kg), Cd (0.26 – 4.10 mg/kg).

192 **Cadmium:** Cadmium is utilized in several industrial and agricultural activities like  
193 manufacture of herbicides and pesticides; it is also found in trace amounts in crude oil  
194 (Osuji & Onojake, 2004). Table 2 showed that the concentration of Cadmium in *Nypa*  
195 *fruticans* obtained from site I ranged from 0.014 - 0.019 mg/kg while the mean values  
196 of site II  $0.028 \pm 0.003$  mg/kg; the amount of Cd in site III was below the detection limit  
197 (BDL) indicating a negligible degree of Cd pollution. It can be observed that only site II  
198 recorded Cd concentration above the WHO recommended limit of 0.2 mg/kg (Table 4),  
199 which implies that site B has a high degree of Cd pollution. However, all Cd  
200 concentrations recorded in this study are lower than the results by Bal *et al.*, (2013) on  
201 heavy metal accumulation in plants in urban and industrial area of Istanbul, Turkey. Cd  
202 has similar chemical properties like Zn, which may account in part for its toxicity in  
203 biological system. Zn being an essential trace element in plants and animals can be  
204 substituted with Cd and may cause the malfunctioning of metabolic processes

205 (Campbell, 2006). Cd is very toxic to human, and specifically targets the bones and  
206 kidneys.

207 **Copper:** Cu is a micro element which is essential in plant growth and occurs naturally  
208 in soil and sediments. It is an important component of enzymes and is necessary for  
209 normal growth and development. However, anthropogenic activities may raise the level  
210 of Cu in soil above their natural background levels; this may lead to hair and skin  
211 decolorations, dermatitis, respiratory tract disease in humans (Khan *et al.*, 2008) .  
212 From Table 2, the mean values of Copper were  $2.390 \pm 0.204$  (Site I),  
213  $0.037 \pm 0.007$  mg/kg (Site II) and  $0.002 \pm 0.001$  mg/kg (Site III), and were all below the  
214 WHO permissible limit of 10 mg/kg (Table 4). Site III recorded the least Cu  
215 concentration while site I had the highest. Sites II and III gave a lower Cu  
216 concentration than the results of Opaluwa *et al.*, (2012) on the metal concentration in  
217 plants grown around dumpsites (0.36 – 0.71 mg/kg).

218 **Nickel:** Ni is an essential trace element for plants and animals; according to  
219 Hjortenkrans, (2003), it is absorbed easily and rapidly by plants. However, at high  
220 concentration, Ni is toxic to humans and causes severe diseases like weight loss, loss of  
221 vision, heart and liver failures (McGrath, 1990). Nickel in *Nypa fruticans* ranged from  
222 0.011-0.012 mg/kg in site I (Table 2), 2.031-2.076 mg/kg (site II) and 0.007-  
223 0.007 mg/kg (site III) and were all below WHO permissible limit. Higher Ni  
224 concentration in plants was recorded by Bal *et al.*, (2013). According to Ubong *et al.*,  
225 (2020b), Ni in *Tympanotonus fuscatus* and sediments of Iko River ranged from  
226  $0.77 \pm 0.3$  -  $83.6 \pm 0.2$  mg/kg and  $2.42 \pm 0.3$  -  $91.6 \pm 0.2$  mg/kg, respectively, which is  
227 largely higher than the amounts recorded in this study.

228 **THC:** Hydrocarbon is a family of organic compounds or a class of organic chemicals  
229 composed entirely of carbon and hydrogen atoms, which bond together as structure of  
230 the compound. It is considered to be an organic compound of simplest composition and  
231 may be thought to be the parent substance from which other compounds are derived  
232 (McDonald & Ahern, 2002) Hydrocarbons come in four structural classes namely;  
233 aromatic, aliphatic, halogenated and terpenes. This study however assessed the  
234 cumulative amount of all classes of hydrocarbons in the study area. At site I, the mean  
235 value of THC in *Nypa fruticans* ranged from 1.23240-125.481 mg/kg with a mean value  
236 of  $124,361.7 \pm 1120.502$  mg/kg (Table 3); at site II the mean value of THC ranged from  
237 310,009.1-315,218.9 mg/kg with a mean value of  $311,813.4 \pm 2950.291$  mg/kg and at  
238 site III (control) the mean value of THC ranged from 1489-1502 mg/kg with a mean  
239 value of  $1488.08 \pm 14.532$  mg/kg. The THC in *Nypa fruticans* at the different study site  
240 follows the sequence: Site II > Site I > Site III. THC concentration in the study sites  
241 exceed previous study obtained by Numbere, (2019) on the bioaccumulation of THC in  
242 *Nypa fruticans* in the Niger Delta, Nigeria. Furthermore, THC in this research exceed  
243 the concentration recorded in other studies carried out in the Niger Delta as

244 follows;(Osam *et al.*, 2011), (Okop & Ekpo, 2012) , and (Edwin-Wosu & Albert, 2010).  
245 Therefore, it implies that the area under investigation has a high degree of THC  
246 pollution, which will pose a problem to the exposed ecosystem. According to Udoetok *et*  
247 *al.*, (2011), the introduction of petroleum hydrocarbons to the Niger Delta as a result of  
248 the oil spill incident there, may have been responsible for the high level of petroleum  
249 hydrocarbons obtained at the site. This is evident by the high level of total hydrocarbon  
250 content (THC), significant concentration of total petroleum hydrocarbon fractions within  
251 the n-C12 - n-C17 range, especially the n-C13 and n-C17 fractions, the high  
252 concentration of polycyclic aromatic hydrocarbons (PAHs) and the substantial  
253 concentration of the volatile BTEX fractions. Hydrocarbon accumulation in humans leads  
254 to diverse adverse effects as outlined in different literature, which include the  
255 pulmonary and cardiovascular effects (Aitzaz *et al.*, 2019), central nervous system,  
256 gastrointestinal effects, renal effects and dermatological effects (Members *et al.*, 2009).  
257

#### 258 4.0 CONCLUSION

259 The findings showed that the samples under study were contaminated with heavy metal  
260 and total hydrocarbon content. The trend of heavy metal was as follows: Cu > Pb > Ni  
261 > Cd at site I, Ni > Pb > Cu > Cd at site II and Pb > Ni > Cu > Cd at site III which is  
262 the control. The heavy metals in *Nypa* palm were below the permissible limits of world  
263 health organization (WHO), with the exception of Cd in site II. The levels of THC in sites  
264 I and II were much higher than site III, indicating that both sites I and II are highly  
265 contaminated with THC. The heavy metals and hydrocarbon present in the environment  
266 can pose a serious environmental risk and affect crops that are grown in the area. The  
267 results from this research reveals that the flora found on the shores of Qua Iboe River  
268 in Nditia community (Ibena LGA) have a very high level of THC, making the plants unfit  
269 for human consumption. On the other hand, heavy metals and THC in *Nypa friuticans* in  
270 the control site (Ikot Ebok) were very low and within permissible limits; implying very  
271 minimal contamination of the area.

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#### 277 References

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280 Aitzaz, A. M., Kim, J., Kim, T., Park, K. D., & Cho, S. (2019). Electrical characterization of pork  
281 tissue measured by a monopolar injection needle and discrete fourier transform based  
282 impedance measurement. *Applied Sciences*, 9(19), 4049.

283 ATSDR, U. (2007). Toxicological profile for benzene. *US Department of Health and Human  
284 Services, Agency for Toxic Substances and Disease Registry, Atlanta, GA.*

285 Bal, R., Türk, G., Tuzcu, M., Yılmaz, Ö., Kuloğlu, T., Baydaş, G., . . . Tuzcu, Z. (2013). Effects of the  
286 neonicotinoid insecticide, clothianidin, on the reproductive organ system in adult male  
287 rats. *Drug and Chemical Toxicology*, 36(4), 421-429.

288 Barnes, K. K., Kolpin, D. W., Furlong, E. T., Zaugg, S. D., Meyer, M. T., & Barber, L. B. (2008). A  
289 national reconnaissance of pharmaceuticals and other organic wastewater  
290 contaminants in the United States—I) Groundwater. *Science of the Total Environment*,  
291 402(2-3), 192-200.

292 Deribachew, B., Amde, M., Nigussie-Dechassa, R., & Taddese, A. (2015). Selected heavy metals  
293 in some vegetables produced through wastewater irrigation and their toxicological  
294 implications in Eastern Ethiopia. *African Journal of Food, Agriculture, Nutrition and  
295 Development*, 15(3), 10013-10032.

296 Edwin-Wosu, N., & Albert, E. (2010). Total Petroleum Hydrocarbon Content (TPH) As an Index  
297 Assessment of Macrophytic Remediation process of a Crude Oil Contaminated Soil  
298 Environment. *Journal of Applied Sciences and Environmental Management*, 14(1).

299 Ekanen, A., Ikpe, E., & Ekwere, I. (2019) Assessment of Polycyclic Aromatic Hydrocarbons  
300 Levels in Soil around Automobile Repair Workshops Within Eket Metropolis, Akwa  
301 Ibom State, Nigeria. *International Journal of Research and Scientific Innovation (IJRSI)*;  
302 ISSN 2321–2705 6:102

303 Finster, M. E., Gray, K. A., & Binns, H. J. (2004). Lead levels of edibles grown in contaminated  
304 residential soils: a field survey. *Science of the Total Environment*, 320(2-3), 245-257.

305 Hjortenkrans, D. (2003). Diffuse metal emissions to air from road traffic. A case study of Kalmar,  
306 Sweden. *Environ. Sci. Sect. Bullet*, 1-55.

307 Ite, A. E., Ubong, U. U., Etesin, U. M., Nsi, E. W., Ukpong, E. J., Ekanem, A. N., . . . Udo, A. I.  
308 (2016). Heavy metals in epiphytic lichens and Mosses of Oil-Producing Communities of  
309 Eket and Ibeno, Akwa Ibom State-Nigeria. *American Journal of Environmental  
310 Protection*, 4(2), 38-47.

311 Ikpe, E. E., Ekwere, I. O., Ukpong, E. G., Effiong, J. O., Okon, O. E. (2020) Assessment of Heavy Metals  
312 and hydrocarbons in *Rizophora mangle*, *Callinectes sapidus* and Sediment in Qua-Iboe  
313 River Akwa Ibom State Nigeria (2020). *Global Journal of Advanced Engineering  
314 Technologies and Sciences*  
315

316 Iwegbue, C., Nwajei, G., & Arimoro, F. (2007). Characteristic Levels of Total Petroleum  
317 Hydrocarbons in Soil, Sediment and Surface Water of an Oil Impacted Area in the Niger  
318 Delta. *Biological Sciences-PJSIR*, 50(4), 247-250.

319 Khan, S. A., Khan, L., Hussain, I., Marwat, K. B., & Akhtar, N. (2008). Profile of heavy metals in  
320 selected medicinal plants. *Pakistan Journal of Weed Science Research*, 14(1-2), 101-110.

321 Lee, M. (1995). Official methods of analysis of AOAC International (16th edn): edited by Patricia  
322 A. Cunniff, AOAC International, 1995. \$359.00 (North America)/\$399.00  
323 (elsewhere)(xxvi+ 1899 pages) ISBN 0 935 584 54 4. In: Elsevier.

324 McDonald, S., & Ahern, K. (2002). Physical and emotional effects of whistle blowing. *Journal of*  
325 *Psychosocial Nursing and Mental Health Services*, 40(1), 14-27.

326 McGrath, S. (1990). Chromium and nickel. *Heavy metals in soils*, 125-150.

327 Members, A. T. F., Poldermans, D., Bax, J. J., Boersma, E., De Hert, S., Eeckhout, E., . . . lung, B.  
328 (2009). Guidelines for pre-operative cardiac risk assessment and perioperative cardiac  
329 management in non-cardiac surgery: the Task Force for Preoperative Cardiac Risk  
330 Assessment and Perioperative Cardiac Management in Non-cardiac Surgery of the  
331 European Society of Cardiology (ESC) and endorsed by the European Society of  
332 Anaesthesiology (ESA). *European*

333 Ogbefun, D.E.,Archibong,U.D.,Chiedu,I.E.,Ikpe, E.E.(2019)Assessment of water quality of  
334 boreholes in selected areas in Benin city, Edo state , Nigeria. *Chemical science*  
335 *international journal*.

336 *Heart Journal*, 30(22), 2769-2812.

337 Numbere, A. O. (2019). Bioaccumulation of total hydrocarbon and heavy metals in body parts of  
338 the West African red mangrove crab (*Goniopsis pelii*) in the Niger Delta, Nigeria.  
339 *International Letters of Natural Sciences*, 75, 1-12.

340

341 Okop, I. J., & Ekpo, S. C. (2012). *Determination of total hydrocarbon content in soil after*  
342 *petroleum spillage*. Paper presented at the Proceedings of the world congress on  
343 engineering.

344 Opaluwa, O. D., Aremu, M. O., Ogbo, L. O., Abiola, K. A., Odiba, I. E., Abubakar, M. M., & Nweze,  
345 N. O. (2012). Heavy metal concentrations in soils, plant leaves and crops grown around  
346 dump sites in Lafia Metropolis, Nasarawa State, Nigeria. *Advances in Applied Science*  
347 *Research*, 3(2), 780-784.

348 Organization., W. H. (1996). Permissible limits of heavy metals in soil and plants. *Geneva,*  
349 *Switzerland*.

350 Osam, M. U., Wegwu, M. O., & Uwakwe, A. A. (2011). The Omoku old pipeline oil spill: Total  
351 hydrocarbon content of affected soils and the impact on the nutritive value of food  
352 crops. *Arch Appl Sci Res*, 3, 514-521.

353 Osuji, L. C., & Onojake, C. M. (2004). Trace heavy metals associated with crude oil: A case study  
354 of Ebocha-8 Oil-spill-polluted site in Niger Delta, Nigeria. *Chemistry & Biodiversity*, 1(11),  
355 1708-1715.

356 Ribes, A., Grimalt, J. O., Garcia, C. T., & Cuevas, E. (2003). Polycyclic aromatic hydrocarbons in  
357 mountain soils of the subtropical Atlantic. *Journal of Environmental Quality*, 32(3), 977-  
358 987.

359 Schwab, A., Su, J., Wetzel, S., Pekarek, S., & Banks, M. K. (1999). Extraction of petroleum  
360 hydrocarbons from soil by mechanical shaking. *Environmental Science & Technology*,  
361 33(11), 1940-1945.

- 362 Ubong, U. U., Ekwere, I. O., & Ikpe, E. E. (2020a). Risk and Toxicity Assessments of Heavy Metals  
363 in *Tympanotonus fuscatus* and Sediments from Iko River, Akwa Ibom State, Nigeria.  
364 *International Journal of Environment and Climate Change*, 10(3), 38-47.
- 365 Ubong, U. U., Ekwere, I. O., & Ikpe, E. E. (2020b). Risk and Toxicity Assessments of Heavy Metals  
366 in *Tympanotonus fuscatus* and Sediments from Iko River, Akwa Ibom State, Nigeria.
- 367 Ubong, U. U., Horsfall, M., & Ubong, I. U. (2011). Distribution of heavy metals in tissues of  
368 *Callinectes latimanus* from the New Calabar River Nigeria. *Afr. J. Environ. Pollut. Health* 9  
369 (1): 7-13.
- 370 Ubong, U. U., Ubong, I. U., Ubong, E. U., & Etukudoh, O. U. (2015). Assessment of Quality of  
371 Potable Water Sources in Eket Local Government Area of Akwa Ibom State, Nigeria.  
372 *International Journal of Advanced and Innovative Research*, 4(8), (2278-7844).
- 373 Udoetok, I., Akpanudo, N., Uwanta, E., & Ukpogon, E. (2011). Associated petroleum  
374 hydrocarbons and heavy metals of an oil spilled site in the Niger Delta, Nigeria. *Global*  
375 *Journal of Pure and Applied Sciences*, 17(3), 261-265.

376

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