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Effect of crude oil spillage on plant species and diversity

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Abstract

This study assessed the impact of crude oil spill in Amukpe, Niger Delta of Nigeria after two years of recorded incidence. Field reconnaissance, physicochemical parameters and heavy metal concentration in plant samples were used to assess the adverse effects of the spill on the vegetation. In this study, a total of 1437 plant species distributed into 14 families were recorded at the study site. Family poaceae had the highest no of species (749) with a frequency of 52% followed by family cyperaceae with 320 no of species with a frequency of 22% while family euphorbiaceae and malvaceae had 98 and 94 number of species and 6.8% and 6.5% frequencies respectively taking the 3rd and 4th position respectively. Shannon**-**Wiener's index showed that vegetation in control site was more diverse and heterogeneous than vegetation in impacted sites with mean species diversity index of 9.5 and 5.6 respectively. Individual Shannon-Weiner's index for the polluted site which reveals the location with the most species diversity showed that site 8 is the most diverse with a value of 6.29. Species richness analysis shows that site 5 is the richest location in the polluted site. Generally, there was a significant difference between the metal concentration in food crop (cassava) recorded in the polluted site and that from the control site. The result also showed that the concentration of most heavy metal in all the medicinal plant samples from polluted site were higher than the permissible limit set by WHO. The result showed that there is no significant difference between heavy metal concentrations in spice and WHO limit and as such the consumption of spice from this study area possesses no health risk caused by heavy metal accumulation. The lower presence of flora in the crude oil spilled site shows that the detrimental effects of crude oil pollution on soils can linger for years.

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Introduction

Crude Oil exploration and exploitation are the most apparent activities associated with increasing oil pollution problems in the Niger Delta region of Nigeria (Ogri, 2001). These activities have resulted in spills and other environmental in the region impacting on the fundamental rights of occupants in this region because their source of livelihood, farming and fishery are repeatedly being upset (Agbogidi *et al.*, 2009).

Crude oil is a mixture of hydrocarbons although it may contain Sulphur, Nitrogen, and sulphur. Hydrocarbon molecules when present in the soil penetrate plant tissues leading to damage of cell membrane, resulting in leakage of cell contents seeping into and blocking intercellular spaces thereby reducing metabolic processes of respiration and transport (Odjuvwederhie *et al*., 2006). Nwadinigwe and Onwumere (2003) reports that crude oil substantially impeded germination, growth, development, and pod production of soyabean. According to Oluyemi *et al*. (2008), crude oil contamination reduced seed germination and plant growth by coating plant roots and this adversely affected water and nutrient absorption. They further reported changes in growth and anatomical features of *Chromolaena odorata* including distortion of cells of the epidermal and cortical regions of the root, stem, and petiole (Gill *et al*., 1996). Nkwocha and Duru (2010) reported that younger plants are more susceptible to oil pollution than older plants and they showed partial defoliation and dieback.

Plant species react differently to disturbances and alteration in their environment hence the knowledge of species diversity is helpful for ascertaining the impact of biotic disturbances, the rate of succession and environmental stability (Giliba *et al.,* 2011; Edwin-Wosu and Edu, 2013). According to Omosun *et al*. (2008) responses of plant metabolism to oil impact are complex, depending on the type of exposure, type of oil, time of spill, density of spilled oil and sensitivity of the plant species to oil. Crude oil pollution is reported to be bring about the death of more than a few hectares of mangrove forest and swamps (Agbogidi *et al.,* 2009; Asaolu and Asaolu, 2010). Crude oil with high Heavy metal component is reported to affect the development of plants by disrupting their natural habitat, both in the aquatic and terrestrial ecosystems. Exposure of plants to elevated levels of heavy metals including Cadmium (Cd) results in reduced photosynthesis and nutrient and water uptake. Mohanpuria *et al.* (2007) reports that plants growing in soils containing an elevated level of Cd showed visible indications of injury, which was reflected by growth inhibition, browning of root tips, chlorosis, and in cases of acute toxicity, death.

Plants vary in their ability to accumulate and/ or remediate heavy metal contamination (Zurayk *et al*., 2001). The ability to whether accumulate heavy metal in the roots of translocate them to the shoot also varies among plant species (Zurayk *et al*., 2001; Bada and Raji, 2010). Zurayk *et al*. (2001) however reported that some metals have characteristic physiological fates; Lead (Pb) for example, accumulates in roots, Nickel (Ni) and Copper (Cu) accumulates both in shoots and in roots. Zurayk *et al*. (2001) concludes that the plants functions as 'diffuse samplers', accumulating more of the heavy metals into themselves, resulting in a higher concentration in the plants when compared to the surrounding environment, a product of longer time of exposure to the polluted environment. Bada and Raji, (2010) reported that the uptake of metals by plants is dependent on the plant type, the age of the plant, the soil type, soil pH, redox potential, cation exchange capacity of soil, surface area and texture of soil particles, organic matter content of the soil, the presence and concentration of foreign ions, growth rate and growth conditions.

The ability of plant species to tolerate heavy metal concentration is dependent on metal exclusion and metal accumulation strategies (Baker *et al*., 2000). The Metal exclusion strategy encompasses evading metal uptake and restricting transport of the metals to shoots (Ayari *et al*., 2010). The Pseudometallophytes are examples of such plants that use the metal exclusion strategy, as they are mainly plants that tolerate high

metal content but can thrive under normal conditions. They are used in re-vegetation of bare soil areas, especially in areas of excessively high metal pollution. The accumulation strategy encompasses the accumulation of the metal in high concentrations, metals in the plant tissues. This study therefore aims to determine the effect of crude oil spill on plant species diversity in Amukpe, Delta State.

Materials and methods

Description of Study Area

The study area is Amukpe community in Sapele Local Government Area of Delta State located on longitude $0.5^{\circ}44^{\prime}$ E and latitude $0.5^{\circ}51^{\prime}$ N, south of River Ethiope (Atakpo and Akpoborie, 2008). This area is operated by Shell Petroleum Development Company, having two flow stations, a gas compressor and many oil wells in Amukpe. In April 2011, a spill occurred, resulting from a high-pressure pipeline leakage, spilling several thousands of barrels of crude oil into the environment covering about 20 hectares. A geographically similar unaffected area located 5 km adjacent to the polluted site against the direction of drainage with no recorded evidence of oil spillage prior to this investigation served as the control site. The rich silty soil coupled with salt- and freshwater bodies provided the necessary incentives for the people who are predominantly farmers and fishers. Cultivated crops in this area include*, Dioscorea* spp*., Manihot esculenta, Musa paradisiaca, Saccharum officinarum, Gnetum africana, Capsicum annum, Elaeis guineensis, Cocos nucifera, Ricinus communis, Telfaria occidentalis, Zea mays, Amaranthus hybridus, Arachis hypogea, Sorghum vulgare, Lycopersicum esculentum* and fodders like *Pennisetum purpureum* (Atakpo and Akpoborie, 2008).

Sample Design and Data Collection

The stratified random sampling method was adopted for this study. It was based on the standard procedure for ecological assessment. In each location two hectares of land were mapped out in each farming zone. Eight 200m line transects separated by 50m line transect were laid out and divided into 10 grid plots measuring 25m x 20m. During sampling, plant

species inside the quadrat as well as those at the quadrat edge, but with about 75% of their branches inside the quadrat were counted (Phil-Eze and Okoro, 2009; Osuji *et al.*, 2004). The vegetation was sampled in 2013 first sampling year during the wet season. Representative plant species sampled were identified using flora keys such as (Joyce and Stanfield, 1974; Hutchinson and Dalziel 1972; Ivens *et al*., 1972; Keay 1989; Joyce, 1989).

Estimation of Flora

Density and percentage frequency of plant species were used to determine the distribution of the plant population. Density was used to estimate the composition and species abundance of polluted and unpolluted sites. Density can be used to detect changes in vegetation caused by pollution, global warming, climate change or any aspects of man's impact on the environment (Nwadinigwe, 2013). To determine density, the average number of individuals per quadrat was divided by the area of the quadrat. The result obtained is expressed as the number of individuals of a particular species per square meter.

Density =
$$
\frac{\text{average number of plants}}{\text{Area of quadrant}}
$$

Percentage Frequency

This term refers to the degree of dispersion of individual species in an area and is usually expressed in terms of percentage occurrence. Sampling was done randomly at different points within the study area, and the number of species observed recorded for each sampling unit. The percentage frequency was collated using the equation:

Percentage Frequency

$$
= \frac{\text{Number of quadrats in which the species occurred}}{\text{Total number of quadrats studied}} \times 100
$$

Species diversity

A diversity index is a mathematical measure of species diversity in a community. Diversity indices provide information on community composition rather than species richness (i.e., the total number of distinct species present). Species diversity considers

the relative abundance of distinct species in a particular area. Diversity indices provide vital information on the rarity and commonness of species in a community. The capacity to compute species diversity is a valuable tool for biologist in their quest to understand community structure (Kaplunovsky, 2004). Species diversity in the study area at Amukpe community was estimated using Margalef index and Shannon-Wiener index (1949). Margalef index shows the richest locations in the study area while Shannon-Wiener index (1949) shows the site with the most species diversity.

Using the formula

$$
H = -\sum_{i=1}^{s} Pi \log_e Pi
$$

Where $Pi =$ Proportion of the ith species

 log_e = natural logarithm of Pi

S = number of species in the community

 $\Sigma =$ Summation

 H = Value of Shannon- Wiener diversity index $H = -\sum$ Pilnpi (i) Shannon

$$
\angle
$$
 - *Weinerindex*

Where

H = Shannon Weiner index

$$
pi = proportion of species = ni/N
$$

 $In =$ natural log

ni = Number of individuals of a species

 $N =$ total number of individuals in the population

 $R_1 = \frac{S-1}{I_p}$ $\overline{In}^{(N)}$ (ii) Margalefindex

Where

 R_1 = Margalef index

S = Total number of species

N = total number of individuals observed

Heavy metal analysis in selected plants from sampled site

Sample Preparation

Selected plant samples from the sampled quadrats were collected, held under running water to remove soil particles adhering to the roots and then rinsed with distilled water before splitting into shoots and tubers (for cassava). The components were cut into pieces, air dried for one week to remove residual

moisture and then oven dried (Gallen Kamp, England) at 80° C for 6hrs to constant mass. Ten grams of the dried materials of each component were powdered in a hammered mill and sieved to obtain particles less than 2mm and stored in an airtight glass bottle and stored at 4° C in a refrigerator for subsequent analysis (Nkwocha *et al*., 2011; Nwajei, *et al*., 2012; Mbong *et al.,* 2013).

Digestion of plant sample

Spices including Negro pepper, Black pepper, African nutmeg, and Grains of paradise were collected, taken to the laboratory and oven dried at 90 $\rm ^{o}C$ for 14hours. Dried spice samples were ground with the aid of a commercial blender (TSK – West Point, France) and kept in polythene bag. Following the Doherty *et al*. (2012) method of digestion, 2g of sieved dried sample of each spice was weighed into a digestion beaker (flask) and labelled for each spice sample and treated with 9ml of a cocktail of acid, 3ml of concentrated nitric acid (HNO₃), 3ml of hydrochloric acid (HCl) and 3ml of sulphuric acid (H₂SO₄) (i.e., 3ml $HNO₃ + 3ml$ $HCl + 3ml H₂SO₄$). A blank sample was prepared by using a cocktail of acids to produce 9ml of concentrated $HNO₃$, HCl and $H₂SO₄$ into an empty digestion flask and heated for 30 minutes an electric hot plate at 80 – 90^oC to bring to boil to obtain a clean solution. The solution was allowed to cool before filtering using Whatman filter paper. After which it was transferred quantitatively to a 100ml volumetric flask and 50ml of de-ionized water added to it and preserved in a universal bottle for other analysis.

Data Analysis

Collected data was analysed using analysis of Variance (ANOVA) and the means compared using Duncan's new multiple range test (DNMRT) at P< 0.05 levels of significance. Species diversity indices were computed for distribution of plant species in the sampled area using Shannon-Wiener index and Margalef index.

Results

Quantitative and qualitative vegetation analysis

In this study, a total of 1437 plant species distributed into 14 families were collected at the study site (Table 1). Family Poaceae had the highest number of species (749) with a frequency of 52% followed by family Cyperaceae with 320 number of species with a frequency of 22% while family Euphorbiaceae and Malvaceae had 98 and 94 number of species and 6.8% and 6.5% frequencies respectively taking the $3rd$ and $4th$ position respectively (Table 4).It is noteworthy to report the occurrence of *Paspalum vaginatum* and *Cynodon dactylon* in a clustered pattern in site 5 and 10.Shannon**-**Wiener's index showed that vegetation

in control site was more diverse and heterogeneous than vegetation in impacted sites with mean species diversity index of 9.48 and 5.69 respectively (Table 2). Individual Shannon-Weiner's index for the polluted site which reveals the location with the most species diversity showed that sites 10 and 9 with index values of 9.55 and 9.42 respectively were the most diverse followed by site 5 with a value of 6.80 while site 3 with an index value of 5.01 is less diversified. Species richness analysis shows that site 9 is the richest location in the sampled plots.

Note: Plots 1 – 8 are experimental while 9 and 10 are controls also labelled as Control 1 and 2

Table 3. Diversity Indices for the sample plots.

Note: Plots 1 – 8 are experimental while 9 and 10 are controls also labelled as Control 1 and 2

Metal concentration in food crop (Cassava) at sampled site

The results for the determination of metals in cassava plant found in the vicinity of crude oil impacted sites are presented in Table 4. The concentrations of the metals in the cassava varied significantly ($P \le 0.05$) with respect to the different plant parts as well as sites. The concentration of metals in the control sites were lower than those found at the crude oil impacted sites. The pattern of distribution of metals in the cassava plant followed the order Fe> Zn > Cu> Mn > Pb> Ni > V > Cr> Cd> As. In study plant, the shoot of the cassava plants had higher concentration than the tubers.

Table 4. Metal Profile (mg kg⁻¹) in Cassava Plant at Different sites of the Study Area.

Plot	Samples	As	$_{\rm Cd}$	Pb	Сr	Ni	Cu	v	Mn	Zn	Fe
$\mathbf{1}$	Shoot	BDL	1.96 ^b	6.93 ^a	1.78 ^b	5.61 ^d	83.65°	2.06 ^a	74.63 ^a	96.41 ^a	$102.62^{\rm b}$
	Tuber	BDL	0.83^e	4.21 ^e	2.01 ^a	6.78c	74.93^e	1.79 ^c	66.05^e	64.94°	97.01^e
3	Shoot	BDL	1.02 ^c	5.34 ^d	1.31 ^d	7.92 ^b	94.12 ^a	1.83 ^b	67.86 ^d	69.5 ^b	109.3 ^a
	Tuber	0.002 ^b	0.94^d	4.06 ^f	1.53 ^c	8.36 ^a	86.94^{b}	0.93	$70.72^{\rm c}$	61.74^e	99.08c
5	Shoot	0.007 ^a	1.36 ^a	5.46 ^c	1.26 ^e	4.69 ^e	79.51 ^d	1.56 ^d	71.49 ^b	63.21^d	98.41^d
	Tuber	0.003^{b}	0.9 ^t	5.2^{b}	1.01^t	$3.03^{\rm t}$	74.94^e	0.78 ^e	71.86 ^b	59.68 ^f	93.02 ^t
Control 1	Shoot	BDL	0.04 ^h	1.07 ⁸	0.06 ^g	0.01 ^g	11.46 ^f	0.06 ^f	11.03 ^f	12.97 ^h	28.63^8
	Tuber	BDL	0.01 ¹	0.94 ^h	0.01 ¹	0.004 ^h	9.39 ^h	BDL	11.16 ^f	10.08^{i}	20.17 ¹
Control 2	Shoot	BDL	0.07 ^g	0.96 ^h	0.07 ^g	0.03 ^g	10.3 ^g	0.09 ^t	10.46 ^g	13.968	24.92 ^h
	Tuber	BDL	0.03 ^h	$0.78^{\rm i}$	0.14 ^h	0.001 ¹	7.08^{i}	0.01 ^g	8.93 ^h	8.64^{j}	18.41°
LSD		0.20	0.86	1.94	1.26	2.84	3.17	1.01	3.57	2.46	4.87

Means with different alphabets within the same column are significantly different at $P \le 0.05$ using Duncan's Multiple Range Tests

Distribution of metals in selected medicinal plants

The result for the determination of heavy metal concentration in dominant medicinal plants within the study area is presented in Table 5. There were significant ($P \le 0.05$) differences in the concentrations of metals among the selected medicinal plants. *Anthocleista djalonensis* had significant higher concentrations ($P \le 0.05$) of the investigated metals than those found in *Tamarinds indica* from the same site. *Khaya grandifolia* and *Anthocleista* sp*.* were better accumulator of metals than the other species of medicinal plants found in the crude oil impacted sites. Above all, the metal concentration in the plant species study area were significantly ($P \le 0.05$) higher that their corresponding control sites. concentration in all the sampled medicinal plants was higher than the WHO limits for Fe in medicinal plants

(20mg / kg). The concentration of Zn in *Khaya grandifolia* and *Anthocleista djalonensis* in polluted site were also higher than WHO limit for Zn in medicinal plant (50mg / kg) while the concentration of Mn in all the sampled medicinal plants were lower than WHO (2007) limit for Mn in medicinal plants (200mg/ kg).

Distribution of metals in selected spices

The results obtained from analysing the heavy metals concentration in spices are presented in Table 6. The highest concentration of Pb (12.63mg kg-1) was recorded in *Tetrapleura tetraptera,* followed by *Piper guineense* (8.24mgkg-1) while the lowest (3.84mg kg-1) was recorded in *Monodora myristica* in polluted sites. Pb value in *Tetrapleura tetraptera* were above the WHO limit which is 10mg kg-1. Chromium was below detection limit in spices at the control site while the highest concentration (0.06mg kg-1) was recorded in *Xylopia aethiopica* (Table 28). The values of Chromium in the four spices were below the WHO limit which is 30mg kg-1.

***Means with alphabets in a particular column shows that the means in Duncan's Multiple Range Tests are significantly different at P≤ 0.05

Nickel had highest concentration with value up to 3.09mg/kg in *Xylopia aethiopia* and the lowest of (1.28mg/kg) in *Aframomum sceptrum*. The highest concentration of Copper (1.46mg/kg) was recorded in *Tetrapleura tetraptera* followed by *Piper guineense* (1.12mgkg) while the lowest (0.01mg/kg) was recorded in *Piper guineense* in the control site. The range of Copper in the sampled spices (0.01- 1.46mg/kg) was less than the WHO limit of Cu for spice which was 50mg/kg. The concentration of Manganese in the spices shows that *Tetrapleura tetraptera* have the highest concentration of (1.94mg/kg) while *Xylopia aethiopica* had the lowest concentration of (0.06mg/kg). The values of Manganese in various spice were below the WHO limit of 100mg/kg. Level of zinc in the spices

showed that *Tetrapleura tetraptera* had the highest concentration of $(18.30mg \text{ kg}^{-1})$ while *Xylopia aethiopica* had the lowest concentration of (2.85mg kg-1) from polluted sites. Concentrations of Zn were also below WHO limit (100mg kg-1) in all the spices. Fe in the spices showed that the highest concentration of (25.60mg/kg) which was recorded in *Tetrapleura tetraptera* while the least (2.96mg/kg) was obtained in *Xylopia aethiopica* in control plot. Concentration of iron in various samples was below the WHO permissible limit which is 300mg/kg. The transfer coefficient from soil to selected spices is displayed in Table 10. The entire investigated metal had transfer coefficient less than 1 indicating low metal uptake from soil by spices.

Table 6. Metal concentration (mg kg⁻¹) in selected spices in the study area.

Plant	Plant parts analysed	Location	Pb	Сr	Ni	Cu	Мn	Zn	Fe
Aframomum sceptrum	Seed	5	4.17 ^d	0.01 ^a	1.28c	1.06 ^b	1.14 ^c	7.03°	12.88^e
Monodora myristica	Seed	8	3.84^e	0.01 ^a	1.64 ^c	0.98 ^c	1.02 ^c	6.82 ^d	$13.15^{\rm d}$
Xylopia aethiopica	Hull	5	5.25°	0.06 ^c	3.09 ^a	1.04^{b}	1.08 ^c	7.64c	14.75°
	Hull	Control 1	1.73^{\dagger}	ND	0.18 ^d	0.09 ^d	0.06 ^d	$2.85^{\rm f}$	2.96 ^g
Piper guineense	Seed	5	8.24^{b}	0.01 ^a	2.41^{b}	1.12^{b}	1.27^{b}	10.57^{b}	17.02^b
	Seed	Control 1	1.01 ^t	ND	ND	0.01 ^d	0.03 ^d	3.09 ^e	4.34 ^f
Tetrapleura tetraptera	Seed	5	$12.63^{\rm a}$	0.03^{b}	$2.65^{\rm b}$	$1.46^{\rm a}$	$1.94^{\rm a}$	18.3°	$25.6^{\rm a}$
LSD			2.16	0.02	1.32	0.97	0.88	2.14	3.11

***Means with alphabets in a particular column shows that the means in Duncan's Multiple Range Tests are significantly different at P≤ 0.05

Discussion

Plant species react differently to disturbances and alteration in their environment hence the knowledge of species diversity is helpful for ascertaining the

impact of biotic disturbances, the rate of succession and environmental stability (Giliba *et al.,* 2011; Edwin-Wosu and Edu, 2013). The Niger Delta Environmental Survey (NDES, 1999) acknowledged that plants are the greatest indicators of changes in any habitat. They surmised that the decline in the number and diversity of species can be a precise indicator of deterioration of an ecosystem. From the study, a total of 1437 plant individuals distributed into 14 families were recorded of which Family Poaceae had the highest number of individuals (749) family Cyperaceae with 320 number of species. Family Euphorbiaceae and Malvaceae had 98 and 94 number of species, respectively. These families with high species diversity indicate that they may serve as heavy metal hyper accumulators and Prasad and Feitas (2003) reported about 400 plant species that are hyper accumulators of heavy metals.

Members of Family Poaceae and Cyperaceae were most abundant . This is due to their tolerance to the components of crude oil spill and probably due to their regenerating abilities. This is explained further from studies by Edema *et al.* (2009) and Prasda and Feitas (2003). They also reported that Poaceae was tolerant to heavy metal. Consequently, species like *Paspalum vaginatum* and *Cynodon dactylon* were seen in a clustered pattern. Shannon**-**Wiener's index showed that vegetation in the control site was more diverse and heterogeneous than the vegetation in impacted sites with mean species Diversity Index of 9.5 and 5.6, respectively. Individual Shannon-Weiner's Index for the polluted site revealed that the location with the most species diversity was Site 5 is the most diverse with a value of 6.80.Species richness analysis showed that Sites 5 and 8 are the richest location in the polluted site. Shannon index is used to evaluate species evenness (distribution) and richness (number of species). The greater the species diversity, the larger the value of Shannon index (Gilliba *et al.,* 2011).

Cassava is a major food crop in Niger Delta, and they tend to bioaccumulate heavy metals from soil (Osuji *et al*., 2004). Variations were recorded in the metal status of plants. The study showed high Copper toxicity, reflected in Cu level saw to be 7 times higher than the control. This results in Cu been bound and made unavailable to plants through formation of complexes.

Pb concentration in this study varied between 0.78- 6.93mg kg-1 the shoot accumulating more than the tubers which corroborates with Pb values reported by Idodo- Umeh and Ogbeibu, (2010) in cassava grown in soil affected by crude oil products. Arsenic was below detection limit for most cassava plant sample while Fe had the higher concentration in the cassava (shoot) samples compared to the others.

The assessment of heavy metals in spices from this study shows that the values obtained were within WHO (2007) limit for spice which are 50, 100, 300, 10, 100, 30mg kg-1 for copper, nickel, manganese, iron, lead, zinc, and chromium, respectively. Lower values of heavy metal in spices were reported by Iwegbue *et al.* (2011) for spices in Nigeria while higher value of heavy metal in spices has been reported by Nkansah and Opoku (2010) for spices in Kumasi markets in Ghana. They observed elevated amounts of Cd and Pb in Cinnamon (0.20mg kg-1 and 6.24mg kg⁻¹, respectively), Basil (2.25mg kg⁻¹ and 0.47mg kg-1, respectively) and Savory (1.29mg kg-1 and 0.40mg kg-1, respectively.

Conclusion

The result showed significant difference between heavy metal concentration in sample spices and WHO limit. However, given time, and the increased bioaccumulation, a lethal level is envisaged which is an inherent health risk. This study supports the argument that the Niger Delta environment is facing enormous environmental degradation due to the frequent oil spillage. Plants recorded in this study can be used to revegetate crude oil polluted land. Data from this plant diversity studies can be applied for conservation and environmental management.

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