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Full Length Research Paper

Physical and ionic characteristics in water soluble fraction (WSF) of Olomoro well-head crude oil before and after exposure to *Azolla africana* Desv.

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The values of ionic and physical characteristics at 25, 50 and 100% water soluble fraction (WSF) of Olomoro well-head crude oil before and after exposure to *Azolla africana* were investigated. The WSF values before and after exposure to the plants showed that more ions were available after the introduction of the test plant. The increases in ionic and physical characteristics following introduction to *A. africana* to WSF decreased the population of the macrophyte. These changes are signs of leakage of ions from the plant cells as a result of ionic stress and thus degradation of the plants. Statistical analysis showed significant difference in heavy metal contents between WSF concentrations of Olomoro crude oil at 0.05% probability. Reduction in NO_3^-, Mg^{++} (100%), Cl^- (100%) and V ions in the growth medium (WSF) after exposure to the macrophyte was observed. This indicates uptake of these ions. The results also showed accumulation of Mg at 25 and 100% WSF in the plant tissue after exposure.

Key words: Physical and ionic characteristics, heavy metals, water soluble fraction, crude oil and *Azolla africana*.

INTRODUCTION

The incidence of oil spills will continue to be anticipated as long as exploration and exportation continue in Nigeria. The water environment experience many dynamic changes induced by various events such as the spillage of toxic chemical that may have significant impact on aquatic life (Camougis, 1981). Water and oil are usually considered to be non-miscible. However, crude oil contains a very small soluble fraction (Kavanu, 1964). The water soluble fraction constituents are dispersed particulate oil, dissolved hydrocarbon and soluble contaminants such as metallic ions (Kauss and Hutchinson, 1975). The components of crude oil that go into solution make up the WSF. They are taken up by living cells and are metabolized (Ali and Mai, 2007). This is ecologically important because in event of an oil spill into aquatic habitant, this is absorbed by living organisms with serious effects on the ecosystem. The contamination of the aquatic system with heavy metals has been on the increase since the last century due to industrial activities (Ali and Mai, 2007).

Azolla Africana or water velvet belongs to the family Azollaceae and the class Pteridophyta (Suvarna and Charya, 1995). It is a common weed of ponds, dams, lakes and rice paddies. It is an indigenous species in Africa in which it is widespread (Akobundu and Agyakwa, 1998). It is a small free-floating aquatic fern, reproducing from fertile spores. The plant is greenish at early stages of its life cycle but turns pink or dull reddish brown with age. The stem is a small fragile rhizome that floats and reproduces numerous small rootlets in water. The leaves are tender, dissected and overlap in two rows with the upper and a lower lobe that are banded together in a scale-like manner. The upper lobe is the photosynthetic leaf with minutes hairs that make the leaf surface difficult to wet. The lower lobe is thin, submerged and bears the female and male spores. *A. africana* is an obligate water plant. The ideal habitat for its growth is calm sheltered water.

A. africana Desv. has been used to remove heavy metals from solution (Tel-or and Sela, 1992). Harmful

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metallic ion present in the WSF may inhibit root growth (Winter et al., 1976). *Azolla* species as an aquatic macrophyte is a source of food, effluent indicator, fish capture, shelter for marine organisms, oxygenator and habitats diversification (Akobundu, 1987; Petri, 1987; Brix and Shierup, 1989; Anonymous, 2002; Agbogidi and Edema, 2003).

The main objective of this study is to determine the differences in the values of physical, ionic and some heavy metals in WSF of Olomoro crude oil before and after exposure to *A. africana* and how these differences may affect the plant species.

MATERIALS AND METHODS

Sample areas

The crude oil sample was collected from Olomoro flow station (with well No. 40085 owned by Shell Development Company of Nigeria Limited) in Isoko Local Government Area, while the experimental plant was collected from River Ethiope in Sapele Local Government Area, both from Delta State. The plant species is common in the area and enough quantity was obtained for the study. Olomoro and Sapele are situated between longitude 5^o 5^1 and 6^o 30^1 N, latitude 5^o 5^1 and 6^o 15^1 of the equator.

Plant collection

The plants were collected by hand. They were gathered into and carried in plastic bags to the Laboratory (Wood, 1975). They were transferred to an aquarium for 7 days for culturing to acclimatize before further use. Before use, the plant samples were washed with tap water to remove debris and then rinsed in deionized water (Harris and Davidson, 2002).

Preparation of the water soluble fraction (WSF)

The WSF was prepared according to the method of Anderson et al. (1974). A sample of crude oil (500 ml) was slowly mixed in equal volume of deionized water in a 2 litre screw-cap conical flask. A Gallenkamp table top magnetic bar was used for mixing. Stirring was done for 20 h and after mixing the oil-water mixture was allowed to stand overnight in a separating funnel. The lower phase was collected and used as the 100% WSF (stock solution) and diluted with water to give 50 and 25% strength WSF which were stored in screw-cap bottles prior to use. The water samples were applied at three levels 25, 50 and 100%. The water used for the preparation of WSF samples was deionized water.

Physical observations

Daily physical observations were carried out for 3 days, while elemental analysis of growth medium was carried out after 5 days. The number of plants was counted before and after exposure to WSF. Five replicates were used for the number of plants, while three replicates were used for the plant and growth medium analyses.

Growth medium analysis

The pH values of the growth medium (WSF) were measured by using a pH meter. Electrical conductivity (EC) values were measured with a conductivity meter (Model DDS – 307). Titration method described by Ademoroti (1996) was employed for the chemical oxygen demand (COD). The total dissolved solids (TDS) is half of electrical conductivity value as described by Ademoroti (1996). The growth medium was analysed for heavy metals and ionic contents in the WSF using the atomic absorption spectrophotometer (Model UNICAM 969) and standard methods by APHA (1998). Three replicates were analysed.

Elemental analysis of plant samples

Elemental analysis of plant samples were carried out after 5 days. Standard methods described by APHA (1998) were used after digestion of plant materials. The following elements were analysed Na, Ca, Mg, P and K.

Statistical analysis

Statistical analysis employed include percentage, standard error, ANOVA and error bars.

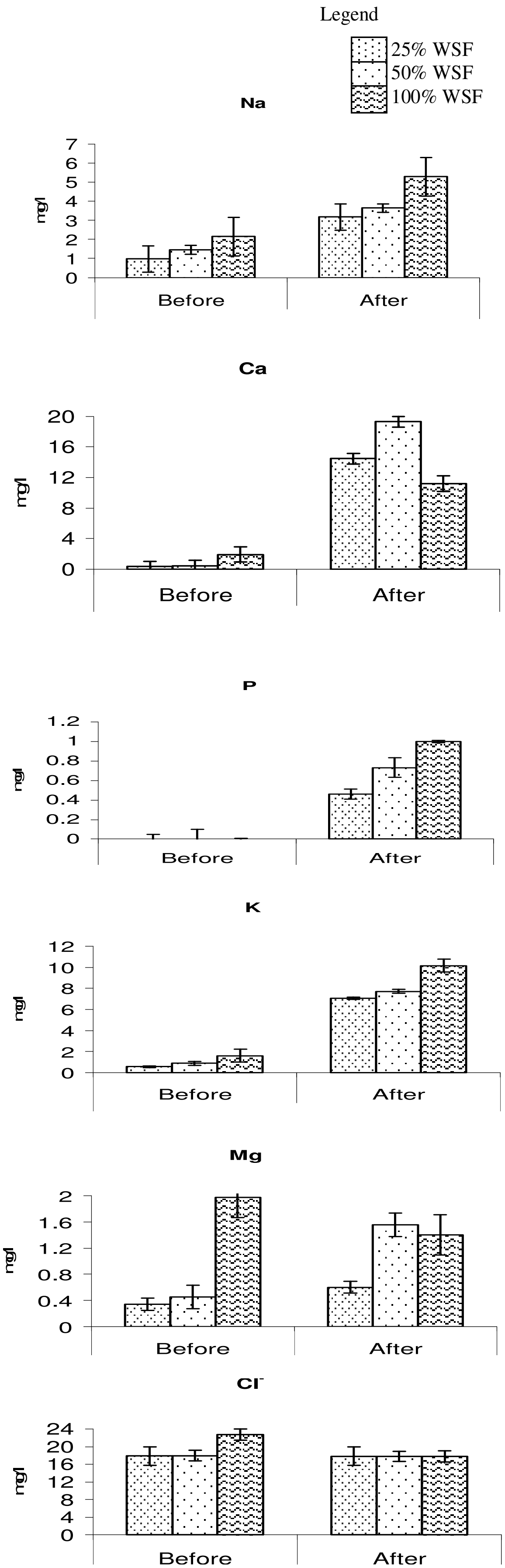
RESULTS AND DISCUSSION

The water soluble fraction obtained from Olomoro well-head crude oil was used at 25, 50 and 100% concentrations. It was found to contain the cation Ca^{++}, Mg^{++}, Na^+, K^+, Fe^{++}, NH_4^+ and phosphorus and anions Cl^-, SO_4^{2-} and NO_3^-. Also, present were the heavy metals Cr, Pb, Cu, Zn, Mn and V. This is in agreement with the statement of Kauss and Hutchinson (1975) that the WSF of crude oil contains metallic ions among other soluble contaminants.

Figure 1 shows the ionic values of Olomoro WSF before and after exposure to *A. africana*. There was increases in sodium, sulphate, iron, ammonium, phosphorus, potassium, magnesium and calcium, except at 100% WSF content of Olomoro crude oil where reduction in values were recorded for Cl^- and Mg^{++} after it was exposed to *A. africana*. The increase in ionic contents could be due to leakage of the cells brought about by salt (ionic) stress and associated oxidative damage. Hopkins (1999) explains that salt stress refers to an excess of ions and is not limited to Na^+ and Cl^- ions. According to Hernandez et al. (1995), oxidative stress is influenced by environmental factors including metal ion deficiency and toxicity.

The values of Mg ions in WSF before and after exposure were lower than the desirable levels of 50 ppm specified (WHO, 1995). The results also showed that magnesium in WSF before and after exposure were lower than the concentration of magnesium in river waters of Africa (Wetzel, 2001). Magnesium may not account for the toxic effects of WSF. However magnesium ions can combine with carbonate and sulphate ions to give suspended solids (Freeze and Cherry, 1987).

Water soluble fraction treatment affected the number of plants and elemental composition of the plant tissue of *Azolla* species as shown in Tables 1 and 2. *A. africana* showed reduction in elemental components of the test



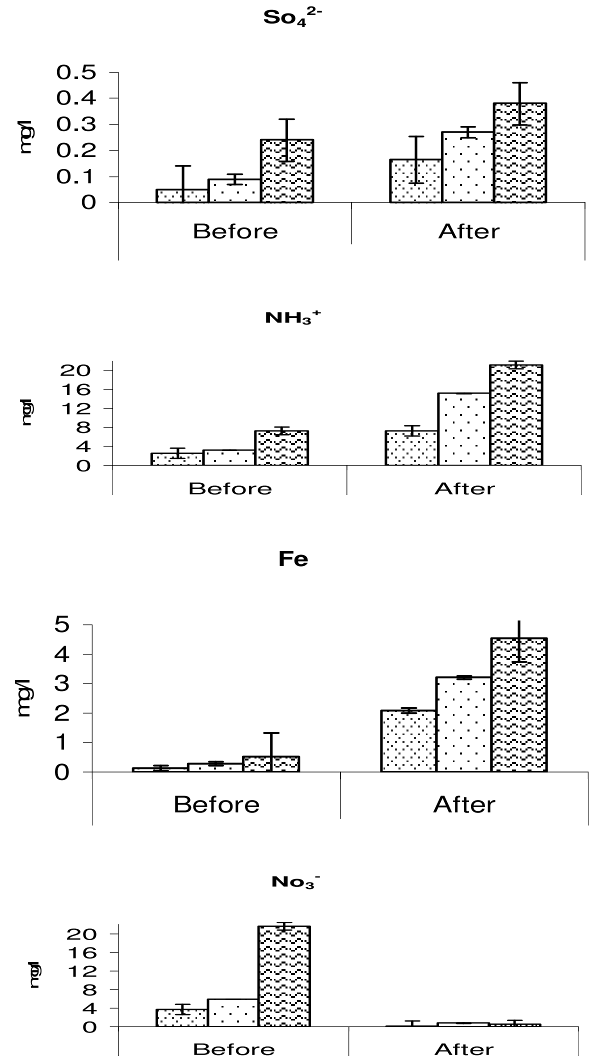


Figure 1. Ionic values of Olomoro WSF before and after exposure to *A. africana.*

plants over the control, except at 25 and 100% WSF concentrations of magnesium content which showed enhancement (Table 2). The reduction in elemental plant tissue indicates increase in the concentration of the different ions of the growth medium (WSF) after exposure to the plant. The enhancement of Mg in the plant tissue indicates absorption of magnesium by the plant.

The results in Figure 1 show reduction in values of nitrate in the WSF after exposure to the aquatic macrophyte. Nitrate levels in fresh WSF are reduced to near undetectable values upon exposure to *Azolla* species. Macrophytes take up elements in their ionic form (Harris and Davidson, 2002). Nitrate levels in Olomoro WSF are concentration related. Reduced values could be attributed to absorption of nitrate by *A. africana*. Inorganic nutrients e.g. nitrate, phosphate and sulphate are known to excessively enrich aquatic ecosystem causing eutro-phication (Odiete, 1987). Nutrients supplied in the form of

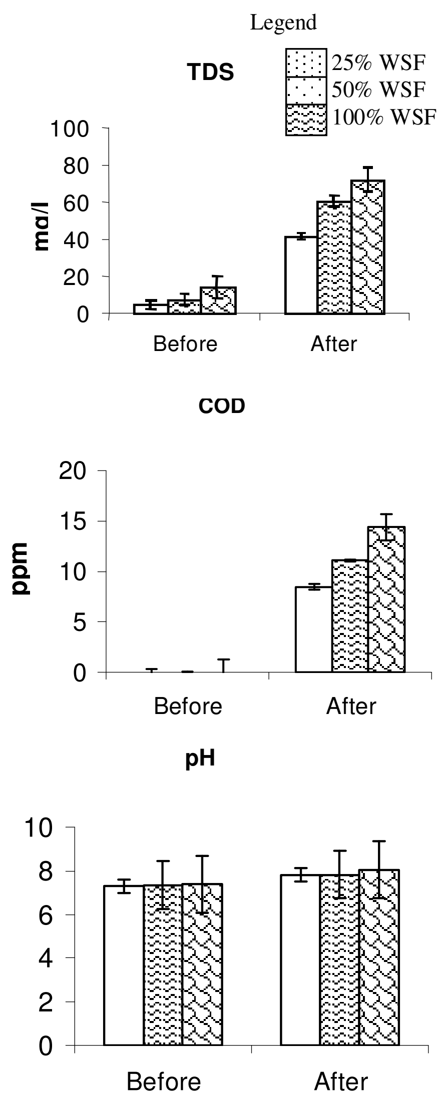
Table 1. Mean number of *A. africana* before and after exposure to Olomoro WSF.

WSF (%)	Day 0	Day 3	Difference *	% Difference
0 (Control)	6.00	6.8 ± 0.00	+0.8	113.33
25	6.00	6.2 ± 0.25	+0.2	103.33
50	6.00	5.4 ± 0.58	-0.6	90.00
100	6.00	5.6 ± 0.00	-0.8	93.33

Table 2. Elemental components of *A. africana* exposed to Olomoro WSF.

WSF (%)	Elemental composition (%)				
	Na	Ca	Mg	P	K
0 (Control)	100	100	100	100	100
25	85.95	76.12	155.55	93.00	74.41
50	66.74	54.15	100.00	87.95	77.80
100	75.15	96.84	115.42	75.31	32.60

Significant difference at P = 0.05



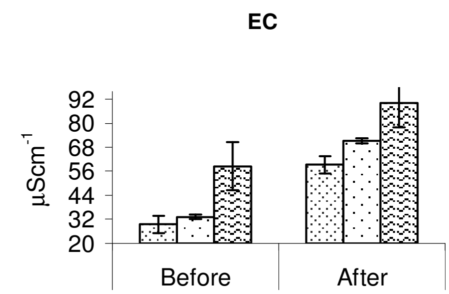


Figure 2. Physical characteristics of Olomoro WSF before and after exposure to *A. africana.*

anions in particular nitrogen (NO_3^-) must be provided in large quantity to ensure sufficient uptake by the plant (Hopkins, 1999).

Nitrogen which is a component of chlorophyll is essential for photosynthesis. Protein contains approximately 18 percent nitrogen, which is absorbed mainly as nitrate (Hoagland, 1972), is reduced and incorporated into organic components.

Figure 2 shows the physical characteristics of Olomoro WSF before and after exposure to *Azolla* sp. Exposure of *A. africana* to Olomoro WSF produced high elevation of pH values (from 7.30, 7.35 and 7.40 to 7.83, 7.82 and 8.03) at 25, 50 and 100% WSF levels respectively. Higher pH recorded after the introduction of the plant suggested the increased synthesis of organic acid and the corresponding excretion of protons into (WSF) solution. All the pH values recorded were within the maximum permissible level (pH 6.5 – 9.8 values) (WHO, 1995). The values for electrical conductivity, total dissolved solids

Table 3. Standard limit for toxic substances in drinking water (H_2O).

Parameter	Acceptable drinking water limit
Fe	0.05 mg/l
Cu	1.50 mg/l
Mn	1.00 mg/l
Cr	0.3 mg/l
Pb	0.05 mg/l
V	-
Zn	15.00 mg/l
Cl^-	200 mg/l
SO_4^{2-}	200 mg/l
NO_3^-	11.3 mg/l
TDS	<500 mg/l
Conductivity	760 µohm/cm
pH	7.0 – 8.5

Sources: WHO (1995), Freeze and Cherry (1987) Botkin and Keller (1998).

Table 4. Mean values# (SE) of heavy metal contents of Olomoro WSF before and after exposure to *A. africana*.

WSF (%)	Heavy metal contents of growth medium (ppm)													
	Fe		Cu		Mn		Cr		Pb		V		Zn	
	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2
25	0.12 (0.01)	2.14 (0.09)	0.02 (0.00)	0.15 (0.01)	nd	0.12 (0.02)	0.01 (0.00)	0.03 (0.00)	nd	0.05	nd	nd	0.02 (0.00)	0.32 (0.02)
50	0.25 (0.01)	3.02 (0.02)	0.03 (0.00)	0.27 (0.01)	0.01 (0.01)	0.13 (0.02)	0.01 (0.00)	0.04 (0.00)	nd	0.05	nd	nd	0.01 (0.00)	0.28 (0.01)
100	0.51 (0.01)	4.52 (0.01)	0.04 (0.02)	0.28 (0.02)	0.01 (0.00)	0.12 (0.02)	0.02 (0.00)	0.06 (0.00)	0.01 (0.00)	0.15	0.01	nd	0.02 (0.00)	0.43 (0.01)

#Obtained from triplicate exposure.
nd = Not detected.
T_1 = initial value.
T_2 = Final value.

and chemical oxygen demand were significantly increased. High EC, TDS and COD are signs of pollution. The results showed that more ions were available after the introduction of the test macrophyte. Increase in EC indicates that most inorganic elements exist in abundance (Kadiri, 2006). The total dissolved solids (TDS) values before and after exposure to the plants were all within the highest desirable limit of WHO (500 mg/l, Table 3) and Talling and Talling (1965) classification scheme of African water with conductivities of between 6,000 – 16,000 μScm^{-1}. With continuous spillage the values may rise and exceed the WHO values.

Table 4 presents the values of heavy metals in Olomoro WSF before and after exposure to *A. africana*. Heavy metals were detected in 25, 50 and 100% WSF prepared from Olomoro well-head crude oil, except Pb, V and Mn at 25 and 50% WSF before exposure. Levels of heavy metals were raised in all concentrations after exposure to the test plant. The results revealed leaching of heavy metals from *Azolla* species. Thus, the introduction of WSF of crude oil into the aquatic system can increase the ionic, heavy metals and physical characteristics of aquatic ecosystem. And with continuous spillage there would be a build up of these ions in the aquatic environment.

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