American-Eurasian J. Agric. & Environ. Sci., 2 (6): 633-638, 2007
ISSN 1818-6769

Physico-Chemical Characteristics of the Water-Soluble Fraction of Ogini Well-Head Crude Oil and the Effects on *Pistia stratiotes* Linn (Water Lettuces)

Noyo E. Edema, George E. Okoloko and Mary O. Agbogidi

Department of Botany and Microbiology, Delta State University, P.M.B. 1, Abraka, Nigeria

Abstract: Physico-chemical characteristics of different concentrations of the water-soluble fraction (WSF) of Ogini well-head crude oil were studied in relation to the effects on *Pistia stratiotes* Linn. Water-soluble fraction of Ogini was found to contain metallic ions and other physical characteristics. Following exposure of *P. stratiotes* to Ogini WSF the levels of ions, heavy metals and physical characteristics were raised, except for NH^{+}_{4}, NO^{-}_{3} and V ions which were reduced. The results showed increases in values of chlorotic, necrotic and number of *Pistia* leaves with increase in concentrations of Ogini WSF. The results also indicated accumulation of lipid and chlorophyll b at 25, 50 and 100% concentrations, while reduction in values were observed for total chlorophyll and chlorophyll a with enhancement at 50% concentration for total chlorophyll and chlorophyll a. Uptake of nitrate, ammonium and vanadium by plant tissue may play an important role in the accumulation of % lipid, chlorophyll b (25, 50 and 100%) and chlorophyll a and total chlorophyll (at 50% concentration). While increase in ionic contents of the WSF after exposure could be due to leakage of cell brought about by ionic stress and associated oxidative damage.

Key words: Physicochemical characteristics · *Pistia stratiotes* · crude oil · water soluble fraction

INTRODUCTION

Crude oil is a mixture of compounds with different quantities of individual components present in the crude oil. Contamination of the aquatic environment by crude oil and petroleum products constitutes a source of stress to aquatic organisms [1]. This is of important to the wetland environment because during spillage, water supply becomes critical and unsuitable for the growth of aquatic organisms.

The Water Soluble Fraction (WSF) of crude oil is that small fraction of oil containing components fully or sparingly soluble in water [2]. Organisms exposed to WSF of crude oil take up the dissolved hydrocarbon and react to their effects. The severity of the effects depends on the organisms exposure, the concentration of the components and mode of exposure (Overton *et al*., 1994). Toxicity of crude oil is the fraction of the presence of toxic components like xylene, naphthalene, benzene and toluene [3].

The water-soluble fraction of crude oil has been found to reduce the growth rate and biomass turnover of some aquatic macrophytes. [5], Kauss and Hutchinson [6] found that aquatic macrophytes population was reduced in the presence of water soluble petroleum components. Winter *et al.* [7] also reported that harmful metallic ions present in WSF may inhibit root growth. *Pistia* species has been similarly employed for the removal of nutrients from polluted bodies [8]. The plants absorb and incorporate the dissolved materials into their structures.

Water lettuce (*Pistia stratiotes*) belongs to the family Araceae and order, Arales [9, 10]. It is like lettuce and is commonly found floating in conglomerates on stagnant or slow floating water [11]. The young plant is attached to the parent by a connecting stem which grows out of the stem. It may grow up as the young plant grows larger, to render it independent. *Pistia stratiotes* a free floating macrophyte is separated by numerous spongy cells in the lower parts of the leaves, stem and roots. It is characterized by excessive proliferation which renders it a threat to water users.

Pistia stratiotes is used in waste treatment because of its ability to extract mineral elements from water which is later harvested as a source of manure.

This study presents the physico-chemical characteristics of water-soluble fraction of Ogini well-head crude oil and the effects on *Pistia stratiotes*.

Corresponding Author: Dr. Noyo E. Edema, Department of Botany and Microbiology, Delta State University, P.M.B. 1, Abraka, Nigeria

MATERIALS AND METHODS

Plant collection: The plants were collected by hand. They were gathered into and carried in plastic bags to the laboratory, they were transferred to an aquarium for 7 days for culturing to acclimatize before use.

Physical observation: Daily physical observations were carried out for 3 days, while elemental analysis of growth medium and plant samples were carried out after 5 days. The number of plants was done by counting before and after exposure to WSF.

Growth medium analysis: The method for growth medium (WSF) and elemental analysis used were those described by Thomas *et al*. [12, 13] and Perkin-Elmer (1968). The pH values were measured by using a pH meter. Electrical Conductivity (EC) values were measured with a conductivity meter (Model DDS-30). Titration method described by Ademoroti [14] was employed for the Chemical Oxygen Demand (COD). Total Dissolved Solids (TDS) is half of electrical conductivity value as described by Ademoroti [14].

Preparation of Water Soluble Fraction (WSF): The WSF was prepared according to the method of [15]. A sample of crude oil (500 ml) was slowly mixed in equal volumes of deionized water in a 2 litre screw-cap conical flask. A Gallenkam 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 WSF and diluted with water to give 50 and 25% strength WSF which were stored in screw-cap bottles prior to use. The WSF sample was applied in three levels 25, 50 and 100%.

The water used for the preparation of WSF samples was deionized water prepared as described by Ademoroti [14].

Determination of lipid content: Known weights of samples were taken from each treatment and samples were extracted continuously for 8 h in a soxhlet apparatus using n-hexane as solvent. After extraction the hexane extraction was allowed to cool and filtered under vaccum and then evaporated to dryness at 40°C in the Rotary-evaporator. After cooling the weight of the lipid extract was determined according to the method described by Bleak *et al*. [16]. The replicate samples were prepared for each experiment.

Determination of chlorophyll: One gramme of fresh leaf was placed in a ceramic mortar and 80% acetone was added. Grinding was done until the chlorophyll was released. The extract was filtered through Whatman No 1 filter paper and read with spectrophotometer at 645 and 663 nm. The method is that described by Holden [17].

The analyses employed include percentage, standard error and error bars.

RESULTS AND DISCUSSION

The water-soluble fraction obtained from Ogini well-head crude oil in Delta State of Nigeria was used at 25, 50 and 100% (full strength) concentrations. It was found to contain metallic ions. This is in agreement with the earlier report of Kauss and Hutchinson [6] who reported that WSF contains metallic ions among other soluble contaminants.

Following exposure of *Pistia stratiotes* to Ogini WSF Na^+, Ca^{++}, Mg^{++}, P and Cl-levels were raised, while NH_4+and NO^-_3 levels were reduced upon WSF exposure (the results as presented in Fig. 1). The increases in WSF ions after exposure could be due to leakage of cells brought about by salt (ionic) stress and associated oxidative damage [18]. Hopkins [19] explains that salt stress refers to an excess of ions and is not limited to Na+and Cl-ions. According to Hernandez *et al*. [20], oxi dative stress is influenced by environmental factors including metal ion deficiency and toxicity.

Accumulation of ions can result in several stress problems. Ions like Na+and Ca+may combine with Cl-in solution to form NaCl and $CaCl_2$. These ions may destroy plant cell wall and membrane [20], causing leakage of cell contents and eventual death of the plants. Increase in ionic particles could lead to pollution of the water environment. Also, the continued import of ions into the leaf, coupled with diminished moisture content may result in a steep rise in the concentration of ions in leaf sap [21].

The results of heavy metals in Fig. 2 show increase in the levels of heavy metals upon exposure of *Pistia* plant to Ogini WSF, except vanadium which was no longer detected under *Pistia* species. The rise in values after plant exposure may be attributed to leaching of ions into the aqueous environment (WSF) as a result of destruction of the cell walls and membranes by compounds such as KCl and NaCl. The values of chromium for 25 and 100% before and after plant exposure were higher than the desirable limit given by World Health Organization [22] (0.3 mg L^{-1}). This may account for the observed

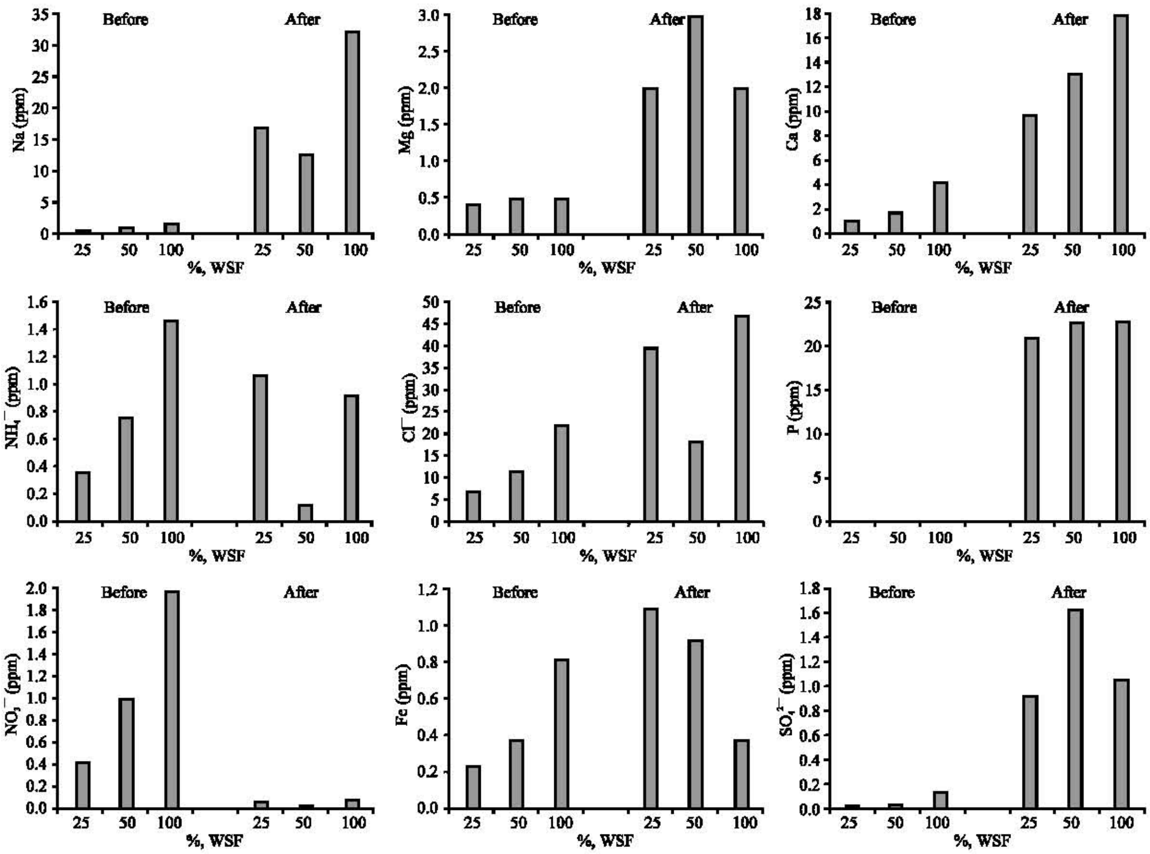

Fig. 1: Ionic Contents of ogini WSF before and after exposure

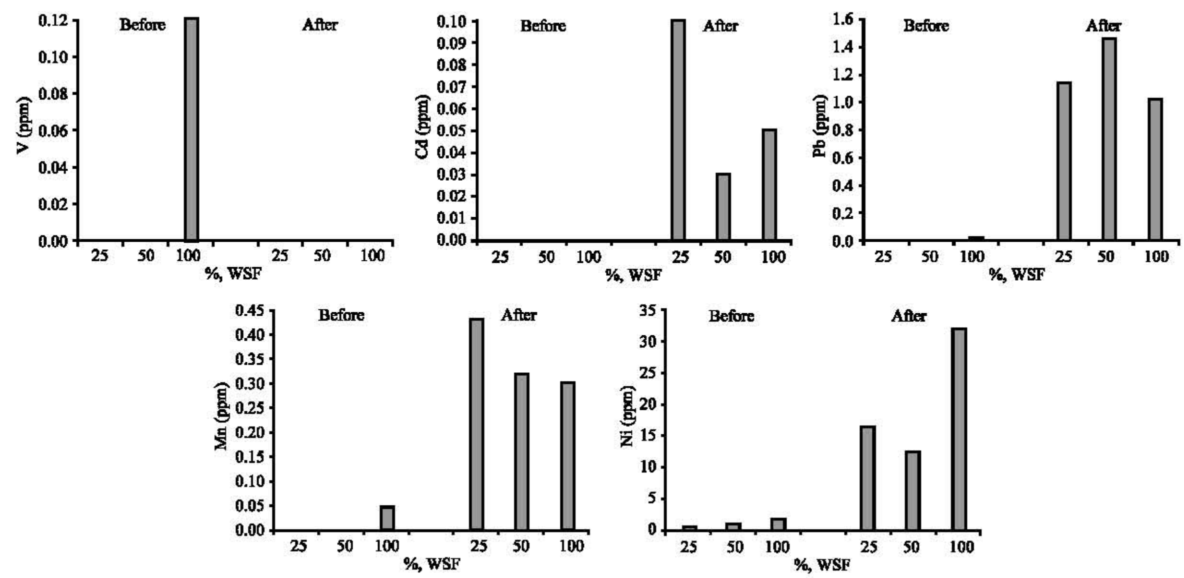

Fig. 2: Heavy metal contents of ogini WSF before and after exposure to *pistia sp.*

effects of Ogini WSF. Chromium can cause chromosome abnormality and membrane destruction [23].

Following exposure of *Pistia* species to Ogini WSF the values of physical characteristics (Fig. 3) were elevated. Electrical conductivity values are raised as much as 16-fold in 100% WSF-following exposure to *Pistia sp.* The results of chemical oxygen demand in the WSF showed positive values with increase in WSF

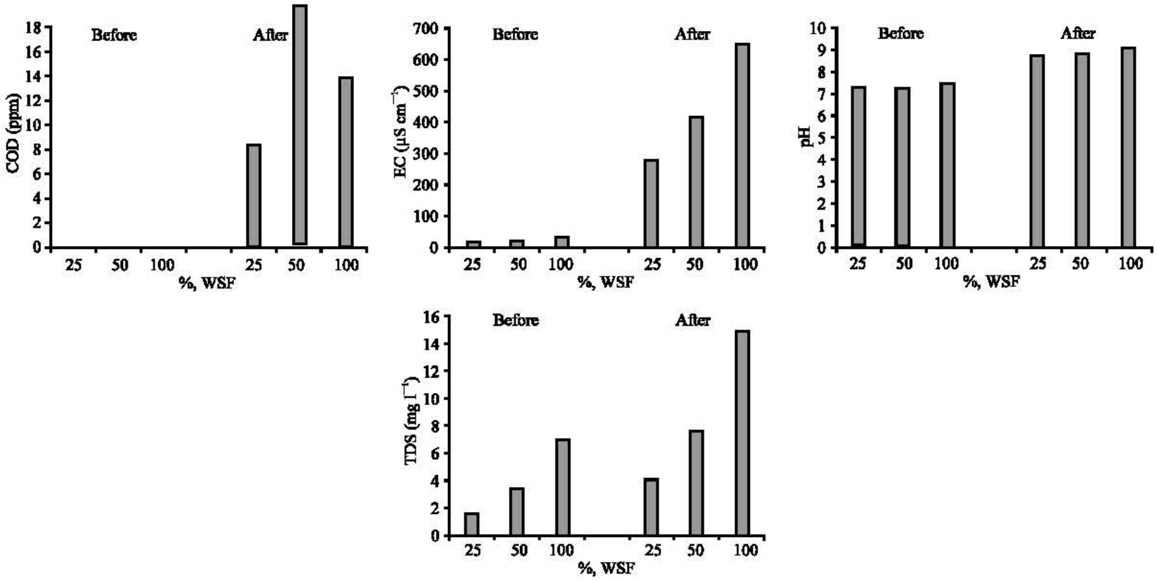

Fig. 3: Physical characteristics of ogini WSF before and after exposure to *Pistia sp.*

Table 1: Number of *Pistia* leaves affected by chlorosis

%, WSF	Day 0	Day 1	Day 2	Day 3	Diff.	% control
Control	0.00	0.00	0.00	0.20	0.20	1.89
25	0.00	0.20	0.20	0.82	0.82	6.95
50	0.00	0.20	0.40	1.00	1.00	8.93
100	0.00	0.40	0.40	1.20	1.20	12.24

Table 2: Number of *Pistia* leaves affected by necrosis

%, WSF	Day 0	Day 1	Day 2	Day 3	Diff.	% control
Control	10.00	1.00	1.00	1.00	+1.0	9.40
25	00.00	4.20	4.60	4.60	+4.60	38.93
50	00.00	3.00	3.60	3.60	+3.60	32.14
100	00.00	1.60	5.20	5.20	+5.20	53.04

Table 3: Number of *Pistia* leaves before and after exposure to WSF

%, WSF	Before	After	% mean
Control	10.00	10.60	94.43
25	11.80	10.20	86.44
50	11.20	9.00	80.36
100	9.80	5.20	53.06

concentration after the introduction of test macrophyte. This means that there was no organic matter ordinarily in WSF and that organic matter was introduced into the WSF from experimental plant. Aquatic ecosystems with inadequate oxygen supply are considered polluted for organisms that require dissolved oxygen above the existing level [24]. The higher the COD value are the higher the organic matter is.??????

Uptake of excess anions raises soil pH [25]. High pH was recorded after the introduction of the plant suggested that increasing synthesis of organic acids and the corresponding excretion of protons into the (WSF) solution. The values recorded within the maximum permissible level (pH 6.5-9.8 values) according to World Health Organization [22].

The results showed that more ions were available after the introduction of *Pistia* plant. The WSF values before and after exposure to the plants were all within the highest desirable limit of World Health Organization [22] (500 mg L^{-1}), but with continuous spillage the values may rise and exceed of the World Health Organization [22] values.

Table 1-3 indicated that the values of chlorotic; necrotic and number of *Pistia* leaves respectively were increased with increasing in concentrations of Ogini WSF. Chlorosis, necrosis and reduced number of leaves were signs of chlorophyll degradation and abnormal growth Bowan and Lockshir [26] reported that, if nutrients are limiting growth may stop or become abnormal. Ions can limit or affect photosynthesis as the ions accumulate in the chloroplast thereby damaging the thylakoid ???? membrane.

Table 4 shows the accumulation of lipid content. According to Anderson *et al.* [15] the effect of WSF on lipid composition depends on the concentration and the plant species involved. Increase in % lipid may be caused by synthesis of chlorophyll pigment. Chlorophyll pigment is an example of lipid under the steroids [19].

Table 4: Effects of Ogini WSF on % lipid of *Pistia Sp.*

%, WSF	% lipid
Control	72.696
25	88.993
50	77.05
100	89.423

Table 5: Effects of Ogini WSF on total chlorophyll, chlorophyll a and chlorophyll b contents of *Pistia. Sp.*

%, WSF	Total Chl.	Chl. A	Chl. B
Control	0.736"0.027	0.203"0.01	0.0033"0.0013
25	0.388"0.11	0.122"0.034	0.097"0.025
50	1.316"0.51	1.186"0.013	0.136"0.12
100	0.250"0.11	0.186"0.017	0.010"0.012

Table 5 indicates accumulation of chlorophyll b and reduces synthesis of total chlorophyll and chlorophyll a. The strong uptake of nitrate by plant cell may account for the improvement in chlorophyll b content (25, 50 and 100%) chlorophyll a and total chlorophyll at 50% concentration. Protein contains approximately 18 percent nitrogen, which is absorbed mainly as nitrate [21]. Nitrogen is a component of chlorophyll is essential for photosynthesis. When nitrogen is deficient leaves will contain relatively little chlorophyll and thus tend to be chlorotic and pale green in colour [27].

REFERENCES

1. Omoregie, E.B.C., O. Ufodike and C.O.E. Onwuliri, 1997. Effects of water soluble fractions of oil on carbohydrate reserves of *Orceochronis nilolicus* (L). J. Aquat. Sci., 12: 1-7.
2. Kavanu, J.L., 1964. Water and Water Soluble Interactions. Holden Day Publisher. San Francisca, pp: 101.
3. Overton, E.B., W.D. Sharp and P. Roberts, 1994. Toxicity of petroleum. In: Basic Environmental Toxicology. Cockerham, L.G. and B.S. Shama (Eds.). First Edition CRC Press Inc. New York, pp: 133-156.
4. Nelson-Smith, A., 1972. Oil Pollution and Marine Ecology. Paul Clack Scientific Brok Ltd., London, pp: 420.
5. Gunlacks, E.R. and M.O. Hayas, 1977. The Urguiola oil spill: case history and discussion of methods of control and clean up. Marine Pollution Bulletin, 8: 132-136.
6. Kauss, P.B. and T.C. Hutchinson, 1975. The effects of water soluble oil components on the growth of *Chlorella vulgaris* Beizerinck. Environ. Pollu., 9: 157-174.
7. Winter, K., R.O. Dannel, J.C. Batterlon and C. Van Ballon, 1976. Water soluble components of four fine oils chemical characterization and effects on growth of water plants. Marine Biol., 36: 269-276.
8. Aoi, T., T. Hayashi and D. Balley, 1996. Nutrient removal by water lettuce (*Pistia stratiotes*). Water Sci. Technol., 34: 7-8.
9. Zhou, J. and J.K. Chen, 1996. Phtocoencological studies on floating leaves anchored aquatic plants in Futeuhu Lake, Huba Province. Acta Hydrobiologica Sinica, 20: 49-56.
10. Akobundu, O. and C.W. Agyakwa, 1998. A Handbook West African Weeds. 2nd Edn. International Institute of Tropical Agriculture. Ibadan, Nigeria, pp: 1-22.
11. Morton, J.K., 1961. West African Lilies and Orchids. Green and Company Limited, pp: 51-52.
12. Thomas, G.O. D.O. Ataga and V.O. Ayala, 1987. Automated determination of Mg in plant materials by modified titan yellow method. Nig. J. Palms and Oil Seeds, 3: 22-33.
13. Perkin-Elmer, C., 1968. An Analytical Method for Atomic Absorption Spectrometry. In: Selected Methods for Soil and Plant Analysis. IITA, Ibadan, Nigeria, pp: 70.
14. Ademoroti, C.M.A., 1996. Standard Methods for Water and Effluents Analysis. 1st Edn. Foludec Press Ltd, Ibadan, pp: 44-154.
15. Anderson, J.N., J.M. Neff, B.A. Cox, H.E. Taten and G.M. Hightower, 1974. Characteristics of dispersions and water-soluble extracts of crude and refined oils and their toxicity to estuarine crustaceans and fish. Marine Biol, 27: 75-88.
16. Blaak, G., L.D. Sparnaaji and T. Menendez, 1963. Breeding and inheritance in oil palm. In: Method of bunch quality analysis. J. West Afr. Institute of Oil Palm Res., 14: 142-155.
17. Holden, M., 1976. Chlorophyll. In: Godwin T.W., (Ed.). Chemistry and Biochemistry of Plant Pigments. 2nd Edn., Academic Press, London, pp: 1-19.
18. Burdon, R.H., D. O'kane, N. Fadzillah, V. Gill, R.A. Boyd and R.R. Finch, 1996. Oxidative stress and responses in *Arabidopsis thallena* and *Oryza sativa* subjected to chilling salinity stress. Biochem. Soc. Trans., 24: 470-472.
19. Hopkins, W.G., 1999. Introduction to Plant Physiology. 2nd Edn., John Wiley and Sons, Inc. New York, pp: 512.

20. Hernandez, J.A., E. Olmos, T. Corpas, F. Sevilla and L.A. del Rio, 1995. Salt induced oxidative stress in chloroplast of pea plants. Plant Sci., 105: 151-167.
21. Hoagland, D.R., 1972. Mineral Nutrition of Plants. Principles and Practice. John Wiley and Sons. New York, pp: 412.
22. World Health Organization, 1995. Guidelines for Drinking Water Quality. World Health Organisation.
23. Odiete, W.O., 1999. Environmental Physiology of Animals and Pollution. Published by Diversified Resources Ltd. Nigeria, pp: 157-248.
24. Botkin, D.B. and E.A. Keller, 1998. Environmental Science. 2nd Edn. John Wiley and Sons. Inc. New York, pp: 639.
25. Wild, A., 1996. Soil and the Environment. 2nd Edn. Cambridge University Press. Britain, pp: 287.
26. Bowen, I.D. and R.A. Lockshir, 1981. Cell Death in Biology and Pathology. Champman and Hall, London, pp: 81-86.
27. Remison, S.U., 1997. Basic Principles of Crop Physiology. Sadoh Press Nigeria Limited. Benin City, pp: 158.