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Phytoremediation of Metal Component of Oil Spill Site Using Common Vegetables

J.O. Oti Wilberforce

Department of Industrial Chemistry, Ebonyi State University,
Private Mail Bag 053, Abakaliki, Ebonyi State Nigeria

Abstract: Seven days old water leaf (*talinum triangular*) and pig weed (*amaranthus viridis*) were employed to clean up an oil spill simulated site. Samples of the crude oil, soil and plants were analysed for heavy metals before, during and after the experiments using X-ray Fluorescence techniques. Different parts of the plants such as roots, stems and leaves were harvested and analysed fortnightly till the end of the ten weeks experiment. The results obtained revealed mean concentration (mg/Kg) of heavy metals were of the range: Pb (0.01-1.42); As (0.02-2.02); Cd (0.01-3.42) Cr (0.03-6.14); Ni (3.21-6.42); Mn (4.12-8.46) and V (10.04- 1.22). High values of Bioaccumulation Factor and Translocation Factors evaluated for different metals showed that the *amaranthus viridis* is an efficient hyperaccumulator. Phytoremediation technique offers the best panacea to remediate vast oil spill lands especially in Niger Delta of Nigeria.

Key words: *Amaranthus viridis* · Oil Spill · Phytoremediation · *Talinum triangular and* X-ray Fluorescence

INTRODUCTION

Petroleum is in constant transit around the globe and as a result oil spill is encountered during exploration, extraction, transportation and utilization [1]. Some of the major historic oil spills include the wreck of Torrey Canyon of the cost of England in 1967, Exxon Valdez spill in Prince William South Alaska, United States in 1989, recently the Shell spill Bonga field of Nigeria in 2011 and even most recently the MV Marathassa spill, British Columbia, Vancouver, Canada in 2015 [2]. The largest oil spill in history was BP's Deepwater Horizon oil-drilling platform explosion which killed 11 workers and spilled 210 million US gallons on April 20, 2010 [3 and 4]. Causes of oil spill include natural causes, equipment failure, human error, blow out among others. Oil spills devastates the ecosystem and introduced toxic substances into the environment. Petroleum is composed of various proportions of hydrocarbon such as alkanes, aromatics and polycyclic aromatic hydrocarbons (PAHs) and non-hydrocarbon including compounds of sulphur, nitrogen, oxygen and metals. Metallic compounds do not occur in free states in petroleum rather they occur as stable organometallic which distil above 500°C. All metals through atomic number of 42 (molybdenum) have been found in petroleum. Nickel and vanadium are present in virtually all the crude and usually at concentration far higher than any other metal [5]. Phytoremediation is a plant based bioremediation technologies which employs the engineered use of green plants for the in-situ treatment of polluted soil [6]. It is an environmentally friendly process which takes advantage of the unique and selective uptake abilities of plant roots, in addition to the translocation, bioaccumulation and degradation abilities of the entire plant body. Phytoremediation is cost effective and aesthetically pleasing because the plants can be easily monitored and metals absorbed by the plants may be extracted from harvested plant biomass and then recycled. However a major disadvantage of phytoremediation is that it is limited to the surface area and depth occupied by the roots. Moreover it relies on natural cycle of plants and therefore takes time [7]. *Talinum triangulare,* known as waterleaf, is a perennial herbaceous plant widely grown in Nigerian and other tropical regions as a leaf vegetable. It is mainly consumed as a vegetable or used as softener of other vegetable species and in addition it has been used medically in the management of cardiovascular diseases such as stroke, obesity [8]. *Amaranthus hybridus,* known as pigweed, is an annual flowering plant whose leaves combined with condiments is used to prepare soup. It is also used medicinally to treat a variety of ailments such as curing diarrhea and controlling bleeding from ulcers [9]. In addition to their nutritional and medicinal values, a recent study has implicated both *Talinum triangulare* and *Amaranthus hybridus* as potential phytoremediation agents because of their ability to absorb and retain petroleum pollutants

Corresponding Author: J.O. Oti Wilberforce, Department of Industrial Chemistry,
Ebonyi State University, Private Mail Bag 053, Abakaliki, Ebonyi State, Nigeria.

Single Pollution Index (PI) which compares metal concentration to Maximum Allowable Limit such as USEPA or WHO was used to determine the level of the pollutant. Values of PI < 1, PI =1 and PI>1 indicate that the soil is unpolluted, critical and unpolluted respectively [10]. Mathematically, PI is expressed as PI = C_{soil} / $C_{USEPA\text{-}standard}$
Where PI is the individual pollution index of study metal; C_{soil} is the Concentration of the metal in soil or plant. $C_{USEPA\text{-}standard}$ is the value of the regulatory limit of the heavy metal by USEPA [11].

Bioaccumulation factor is defined as a ratio of metal concentration in plant shoot to extractable concentration of metal in the soil [12] for a plant to be an efficient phytoremediation agent, the BAF > 1.

Mathematically, BAF is expressed as BAF = C_{root} / C_{soil}

Translocation factor is the plant's ability to translocate heavy metal from root to harvestable aerial part [13]. When TF >1 is obtained, it indicates a preferential partitioning of metals from soil to root and from root to shoot respectively. Mathematically,

TF is expressed as TF = C_{shoot} / C_{root}

Where C_{shoot} and C_{root} is the concentration of metal in shoot and root respectively [14].

This study focused on investigating the uptake and bioaccumulation of Pb, As, Cd, Cr, Ni, Mn and V by water leaf (*talinum triangular*) and pig weed (*amaranthus viridis*) planted in a simulated oil spill environment.

MATERIALS AND METHODS

Before simulation of oil spill, samples of soil and crude oil used in this study were collected and analysed for heavy metals using X-ray Fluorescence. Oil spill scenario was designed by spiking variable oil concentrations of 250 mg crude oil/g to the selected soil samples in 25 litre buckets. Seven days old *talinum triangular* and *amaranthus viridis* were transplanted to the buckets and the concentration of the studied metals was obtained by harvesting and analyzing the roots, stem and leaves of the investigated plants on day 0, 14, 28, 42, 56 and 70.

Collection and Preparation of Soil and Plant Samples

Crude Oil: The petroleum crude oil used in this work was obtained from Mobil terminal of Nigerian National Petroleum Corporation, Port-Harcourt, Rivers State Nigeria. XRF liquid analysis requires minimal sample preparation. Samples of crude oil are simply loaded into a standard Premier cup and attached with a thin film sample support material. Rings and/or Sleeves are applied over the cup body to secure and complete the sample preparation cup assembly.

Soil: Composite samples of the soil were collected, air-dried and mechanically grounded with stainless steel soil grinder and sieved to obtain < 2 mm fraction. 30 g sub-sample was drawn from the bulk and reground with laboratory mortar and pestle to obtain < 200 μm fraction. The sample was further dried in an open inert vessel of a muffle furnace at 105 ºC for 2 hours which removes soil moisture and the samples were cooled in desiccators [12].

Plant: The roots, stems and leaves of *Talinum triangulare* and *Amaranthus hybridus* were separated in each case and the components were cut into pieces. Dust, soil and other particles were removed by putting them through a three step washing sequence which involves washing with water, then with P-free detergent and followed by de-ionized water [15]. The samples were air dried and placed in a dehydrator at approximately 80°C for 48 hours so as to stop enzymatic activity. The samples were grounded mechanically into fine powdery using an agate mortar and afterwards were dried at 65ºC in an oven to obtain a constant weight.

X-Ray Fluorescence (XRF) Analyses of Metals in Plant and Soil Samples: Using a pressure of 6 - 8 torr from CAVER model manual palletizing machine, a 13mm pellet of the each sample was formed and metal concentration was analyzed using XRF procedure [16]. This involves the use of a voltage of 25KV and current of 50µA produced from X-ray tube to bombard the sample in XRF system for 18 minutes at 1000 counts and the characteristic X-ray of the metals and their corresponding concentrations were computed in the read out device using Si-Li detector.

For soil sample, the pH, percentage of Organic Matter and percentages of sand, silt and clay were also determined using Orion 920A pH meter; Walkley and Black method and Hydrometer method respectively [17].

Statistical Analysis: The samples were assayed in triplicates and the XRF generated data were reported as Mean $\pm$ Standard Error. One way analysis of variance (ANOVA) and Fisher's Least Square Difference (LSD) were used to determine significant difference within and between groups, considering a level of significance of less than 5% (P<0.05) and from the generated data, PI, BAF and TF were calculated.

DISCUSSION

Table 1 shows the soil characteristics by their pH, organic matter, sand, silt and clay contents and their values shows that the soil would supports agronomical activities. The percentage of organic matter content

Table 1: Properties of Soil

Properties	(n = 3)
Sand (%)	60.22 ± 5.2
Silt (%)	8.02 ± 0.7
Clay (%)	34.60 ± 4.1
Organic Matter (%)	1.48 ± 0.6
pH	6.68± 0.6

Table 2: Metal Concentration in Crude oil and Soil Samples and PI on Metals in the Soil

Metals Conc (ppm)	Pb	As	Cd	Cr	Ni	Mn	V
Crude oil	1.48±0.1	2.12 ± 0.1	3.44±0.2	6.64±0.5	7.62±0.8	8.82±1.0	10.46±1.2
Soil (after)	10.82±0.8	0.81 ± 0.9	54.11±5.6	0.48±0.5	6.48±0.6	120.1±10.1	0.22±0.2
Soil (before)	18.81±1.6	2.68 ± 0.2	68.4±0.8	1.86±0.2	26.2±3.1	126.2±8.8	0.82±0.2
US-EPA ML	420	75	85	-	75	-	-
Pollution Index	0.00	0.01	0.08	-	0.35	-	-

Table 3: Concentration of metals in plants exposed to oil spill simulation of 250 mg crude oil/g soil

Metal	Plant	Plant parts	Day 0	Day 14	Day 28	Day 42	Day 56	Day 70
Pb	*talinum triangular*	root	0.42±0.01	0.68±0.01	0.72±0.01	0.77±0.01	0.82±0.01	0.98±0.01
		stem	0.26±0.01	0.56±0.01	0.58±0.01	0.60±0.01	0.60±0.01	0.66±0.01
		leaves	0.01±0.01	0.16±0.01	0.18±0.01	0.20±0.01	0.22±0.01	0.22±0.01
	amaranthus viridis	root	1.11±0.01	1.18±0.02	1.18±0.02	1.20±0.02	1.22±0.02	1.42±0.03
		stem	1.02±0.01	1.12±0.02	1.14±0.02	1.18±0.02	1.20±0.02	1.34±0.03
		leaves	1.00±0.01	1.10±0.02	1.12±0.02	1.16±0.02	1.20±0.02	1.22±0.02
As	*talinum triangular*	root	1.18±0.02	1.28±0.02	1.40±0.02	1.56±0.02	1.60±0.02	1.88±0.04
		stem	1.04±0.01	1.08±0.02	1.11±0.01	1.20±0.01	1.20±0.01	1.20±0.03
		leaves	0.02±0.01	0.88±0.01	0.98±0.02	1.00±0.01	1.00±0.01	0.92±0.02
	amaranthus viridis	root	1.52±0.02	1.60±0.03	1.62±0.03	1.78±0.03	1.88±0.03	2.02±0.06
		stem	1.18±0.02	1.40±0.03	1.44±0.03	1.76±0.03	1.80±0.03	2.14±0.05
		leaves	0.91±0.01	1.10±0.02	1.12±0.02	1.28±0.02	1.80±0.03	1.88±0.04
Cd	*talinum triangular*	root	1.18±0.02	1.20±0.03	1.22±0.02	1.38±0.02	1.40±0.02	1.44±0.03
		stem	0.88±0.01	1.00±0.02	1.10±0.02	1.18±0.01	1.20±0.01	1.22±0.03
		leaves	0.01±0.00	1.04±0.02	0.06±0.00	0.06±0.00	0.06±0.00	0.08±0.00
	amaranthus viridis	root	2.12±0.03	2.22±0.03	2.44±0.04	2.68±0.04	2.88±0.04	3.42±0.08
		stem	2.28±0.03	2,32±0.03	2.34±0.04	2.56±0.04	2.86±0.04	3.60±0.07
		leaves	1.58±0.02	1.78±0.03	2.00±0.03	2.10±0.04	2.10±0.04	2.12±0.05
Cr	*talinum triangular*	root	1.88±0.02	2.00±0.03	2.12±0.04	2.22±0.04	2.24±0.04	2.34±0.06
		stem	1.00±0.01	1.12±0.03	1.18±0.03	1.20±0.01	1.20±0.01	1.24±0.03
		leaves	0.03±0.01	1.10±0.02	1.11±0.02	1.18±0.02	1.20±0.01	2.08±0.03
	amaranthus viridis	root	4.28±0.07	4.66±0.07	5.42±0.09	5.88±0.09	6.00±0.90	6.14±0.10
		stem	3.00±0.05	3.20±0.05	3.60±0.07	3.66±0.05	3.88±0.07	4.11±0.09
		leaves	2.42±0.04	2.78±0.03	2.88±0.06	2.98±0.04	3.00±0.06	3.22±0.06
Ni	*talinum triangular*	root	4.44±0.07	4.64±0.08	4.80±0.08	4.88±0.08	5.00±0.08	5.12±0.09
		stem	3.31±0.06	3.88±0.06	3.98±0.07	4.00±0.07	4.10±0.07	4.12±0.07
		leaves	3.21±0.06	3.64±0.03	3.76±0.06	3.80±0.06	3.98±0.06	4.01±0.07
	amaranthus viridis	root	4.02±0.07	4.42±0.08	4.80±0.07	4.81±0.07	4.94±0.08	6.42±0.12
		stem	3.41±0.04	3.78±0.04	3.80±0.06	3.86±0.07	3.88±0.07	4.41±0.08
		leaves	3.00±0.04	3.02±0.04	3.18±0.06	3.20±0.07	3.20±0.06	3.22±0.06
Mn	*talinum triangular*	root	4.14±0.07	4.44±0.07	4.60±0.08	4.62±0.09	4.88±0.08	6.82±0.18
		stem	5.02±0.07	5.00±0.08	5.12±0.08	5.20±0.09	5.20±0.09	5.22±0.09
		leaves	4.12±0.07	4.80±0.07	4.92±0.07	5.00±0.07	5.10±0.08	5.12±0.08
	amaranthus viridis	root	4.46±0.08	5.68±0.09	5.98±0.12	6.82±0.15	7.81±0.18	8.46±0.20
		stem	4.40±0.07	4.66±0.08	4.78±0.08	4.98±0.08	5.61±0.09	6.40±0.18
		leaves	4.42±0.07	5.08±0.07	5.10±0.07	5.12±0.08	5.20±0.08	5.42±0.09
V	*talinum triangular*	root	3.24±0.05	3.90±0.07	4.00±0.07	4.10±0.07	4.12±0.08	4.24±0.08
		stem	2.02±0.03	2.84±0.04	2.66±0.04	2.50±0.04	2.42±0.04	2.38±0.04
		leaves	1.22±0.01	1.66±0.02	1.80±0.02	1.90±0.02	2.01±0.03	2.22±0.05
	amaranthus viridis	root	6.94±0.11	7.22±0.14	8.22±0.19	8.62±0.18	8.88±0.20	10.04±0.21
		stem	6.02±0.10	7.12±0.12	7.60±0.16	7.66±0.17	7.80±0.16	8.02±0.18
		leaves	4.21±0.07	4.82±0.09	5.00±0.08	5.02±0.08	5.12±0.09	6.21±0.10

Table 4: Maximum Bioaccumulation Factors and Translocation Factors of Studied Metals

METALS	PLANTS	BAF ($C_{root}/C_{soil\ -after}$)	TF (C_{stem}/C_{root})
Pb	*Talinum triangular*	0.1	0.7
	Amaranthus viridis	0.1	0.9
As	*Talinum triangular*	2.3	0.7
	Amaranthus viridis	2.6	1.1
Cd	*Talinum triangular*	0.0	0.9
	Amaranthus viridis	0.0	1.1
Cr	*Talinum triangular*	4.9	0.5
	Amaranthus viridis	12.8	0.7
Ni	*Talinum triangular*	0.8	0.8
	Amaranthus viridis	1.0	0.7
Mn	*Talinum triangular*	0.1	0.8
	Amaranthus viridis	0.1	0.9
V	*Talinum triangular*	19	0.6
	Amaranthus viridis	46	0.8

was 1.48 % which indicates the extent of soil pollution with heavy metals and their subsequent uptake by crops. Organic matter content is known to increases with decrease in pH and an increase in metal concentrations and more importantly, it serves as an index for soil fertility. Table 2 shows the concentration of metlas in the crude oil used in this work in the order of Pb < As < Cd < Cr < Ni < Mn < V. Vanadium and lead had the highest and the lowest concentration in the soil respectively. The pollution Indices of metals in the soil before the experiment reveal PI < 1 in all cases which means that the soil used was not polluted. Table 3 shows that the concentrations of the metals were higher in the root than in the stem and leaves with in order of root > stem > leaves except in arsenic and cadmium. This is caused by a concentration gradient created between the root and the leaves as plant absorb water and nutrient from the soil.

Table 4 shows that bioaccumulation factors were significantly high for arsenic, chromium and vanadium in both plants. Greatest values of BAF (19 and 46) were observed for vanadium in *Talinum triangulare* and *Amaranthus hybrids* respectively. Bioaccumulation factor is known to decrease with increasing metal concentration in the soil [18 and 19]. Conversely low values of BAF of cadmuim in the studied plants (BAF <1) suggests that the plants could be cadmium excluders and hence they may not be employed for remediation purposes. A key trait of metal hyperaccumulators is the efficient metal transport from roots to shoots, characterized by the TF being greater than one [20]. Table 4 shows the maximum BAF and TF of metals in the plants. Elevated translocation factors (TF > 1) was observed for As and Cd in *Amaranthus viridis* suggesting that the plant has the potential to hyperaccumulate arsenic and cadmium (Table 4). However cadmium BAF value (BAF = 0.0) contradicts the TF value (TF=1.1) in *Amaranthus viridis*, meaning that though the plant has low propensity to absorb cadmium in the soil but once absorbed it has a high propensity to transport it from root to the shoot. For a scenario as this, soil amendment, involving the use of chemicals such as the metal sulphate, is highly recommended that will make cadmium to be available for absorption via plant roots.

CONCLUSION

Based on high values of BAF, both *Talinum triangular* and *Amaranthus viridis* has been identified as potential hyperaccumulator of arsenic, chromium and vanadium while based on TF only *Amaranthus viridis* roots showed the ability to absorb arsenic and cadmium. In view of deteriorating state of Niger Delta where oil spills is devastating the ecological resources and the environment generally, the two studied plants have the potential to remediate the metal component of the oil spill.

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