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## Toxicity Assessment of Trace Metals in Bille Oil Impacted Site in Rivers State, Niger Delta, Nigeria

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**Abstract** Surface (0-15cm) and subsurface (15-30cm) soil samples from Billeoil spill impacted site in Niger Delta, Nigeria were sampled after a field reconnaissance survey. Heavy metals such as Fe, Cd, Co, Cr, Cu, Mn, Ni, Pb, V and Zn were determined using Atomic absorption spectroscopy. Metals analysis showed an expected high concentration of Fe which ranged between 9.609 to 31.136 mg/kg. The concentration of Cd was least which ranged from 0.28 to 0.67 mg/kg. The V/Ni ratios which varied from 0.66 to 1.24 and enrichment factors (greater than unity for all the metals) suggested that the lingering input of crude oil was from a single source. A one-way analysis of variance (ANOVA) test was conducted at 95 % confidence limit to test the outcome of the sampling locations on the individual means. The test result highlights the insignificance between the sampling points as the F ratio (0.02) from the test was found less than F critical (2.58) at 95% confidence limit.

**Keywords** Field reconnaissance, Atomic absorption spectroscopy, Enrichment factor, Analysis of variance (ANOVA)

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### Introduction

Oil spillage can be referred to as the release of liquid petroleum into the environment by either anthropogenic or natural process [1]. Oil spillage became globally contemporary issue with the exploration, exploitation and processing of crude oil [2]. Ifunanya [3] defined oil spillages as a type of industrial pollution associated with the uncontrolled discharge of crude oil due to exploration and transportation of the product. Oil spill contributes to damage of aquatic and terrestrial ecosystem in addition to health and depletion of natural assets [4]. Since the discovery of commercial quantity of crude oil in the Niger Delta, the environment has had its own fair share of some challenges ranging from soil pollution, air pollution, water pollution, and damage of the entire ecosystem, damage to biodiversity and loss of fertility.

The overall features of spilled crude in the environment depend on the physicochemical and biological factors which include evaporation, dissolution, microbial degradation, photooxidation, and interaction among oil and sediments. The mixture of these different factors is called weathering and it accounts for reduction of hydrocarbon concentrations in sediment and water and changes the chemical characteristics of spilled oils [5]. Spillages usually occur by accidents involving oil tanks, refineries, barges, pipelines and oil storage facilities. These accidents can be caused by human errors or carelessness and sometimes by natural phenomenon such as earthquakes, intentional acts of terrorists, militants or vandals. The presence of heavy metals in crude oil contributes significant toxicity to plants and animals, especially cadmium and lead. Some plants exposed to contaminated farmlands do not only accumulate



these toxic metals in their roots but also translocate from roots to the leaves and fruits [6]. Khan *et al.* [7] reported the existence of heavy metals in crude oil contaminated soils and when absorbed by plants is potent to make the plant leaves potentially toxic and harmful to man and livestock if consumed as food. Study conducted by [8] established that crude oil contamination leads to gradual heavy metal build-up in plants germinating in such soils signifying that such environment poses potential perils to pasturing animals, human beings and the entire food chain. Crude oil comprises of a convoluted mixture of organic mixtures like the paraffins, naphthenes, aromatics, etc occurring with inorganic compounds and complexes. Metals like nickel, vanadium, chromium, iron, copper forms a group of organic and inorganic compounds [9]. Trace metals are metals whose occurrences are at 1000mg/Kg or less in the earth crust. Their densities account for their being light or heavy. Metals with densities greater than  $5\text{g/cm}^3$  and less than  $5\text{g/cm}^3$  are heavy and light metals respectively [10]. In trace metal geochemistry, nickel, vanadium and cobalt are invaluable because their concentration and ratios usually remain unchanged regardless of diagenetic alterations in oil reservoirs [11]. Crude oil whose source is lacustrine and terrestrial shows V/Ni ratio between 1-10 which is expected for marine source rocks where the input of porphyrin-precursor chlorophylls to organic material derived from algae and bacteria is high while minimum amount of Ni and V is expected from land plant derived oil [9].

Atomic Absorption Spectroscopy (AAS) is an analytical tool considered powerful and reliable for quantitative and qualitative analysis of trace metals present in samples. The quantity of a particular atom present in a sample can be determined by measuring the quantity of light absorbed. This technique involves the measurement of light energy of specific wavelength absorbed by uncharged atoms present in their ground level and becomes excited after absorbing energy.

## Materials and Methods

### Description of Sampling Site

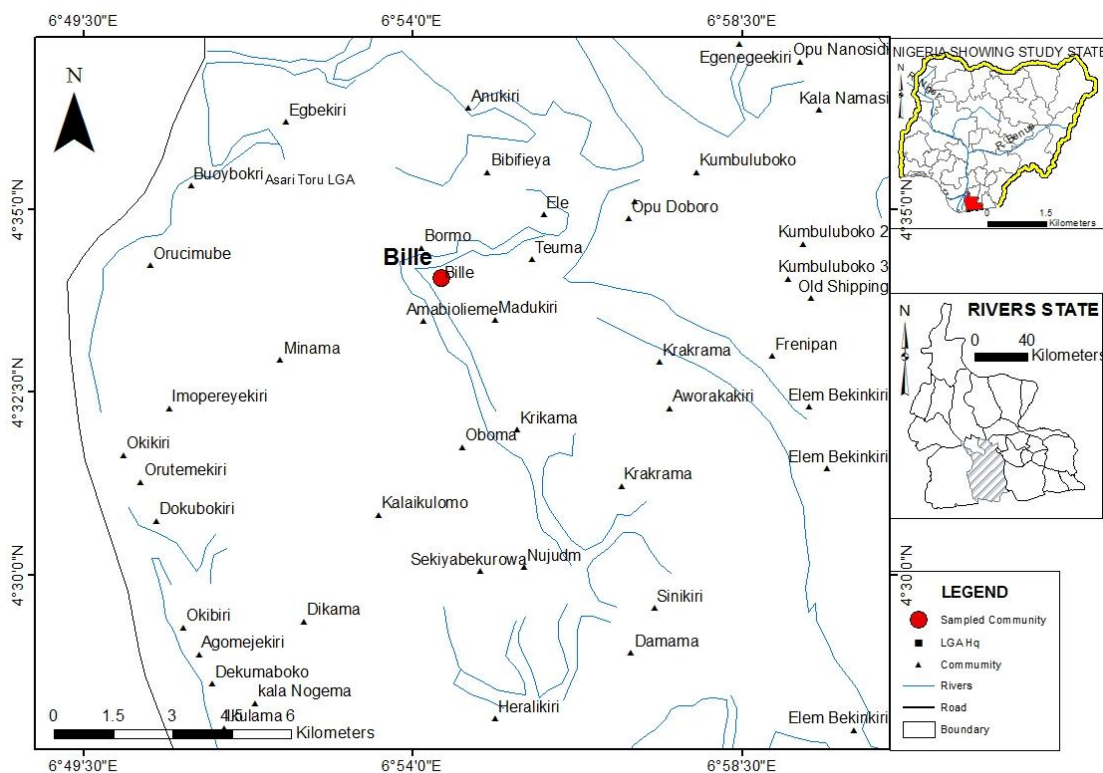


Figure 1: Map of study area showing sampling location

The geographical coordinates of the study area are  $4^{\circ} 34' 37''$  North,  $6^{\circ} 53' 19''$  East. Bille, the study area is in Degema Local Government Area (LGA) of Rivers State and it is a major oil producing region in the Niger Delta and



this particular LGA contributes majorly to the economic activities of the State and invariably, the country. Bille like other coastline communities, is an island on the bank of Bille Creek and a tributary of the Sombrero River and only a few feet above the sea level with low-lying land in the vast mangrove forest region of the Niger Delta and it is situated in the south-eastern province of the present Degeme LGA of River State and about 20-25km away from the metropolis of Port Harcourt, the capital of Rivers state, The study area, Bille as displayed in Fig. 1. is a community susceptible to frequent crude oil spillages due to incessant vandalization of pipelines. The volume of spilt crude is unknown at this time of sampling.

### Field Reconnaissance and Sampling

Field reconnaissance was carried out to delimit the area to be sampled. Sampling was done using the grid method reported by Osuji and Onojake [10]. The impacted area was delimited by reconnaissance with the area of heaviest spill as the epicenter. The sampling area of dimension  $100 \times 100\text{m}^2$  was divided into 100 grid plots with the center representing the epicenter of the spill, with each cell denoting a sampling station. Sixty samples were taken with auger at two depths of 0 – 15 cm and 15 – 30 cm from 30 cells selected randomly representing surface and subsurface soils respectively indicating 30% of the impacted area. About one kilometer away from the impacted site, one soil sample was taken which represented the uncontaminated site. After collection, the samples were transferred into sealed polyethylene bags that were previously washed and the samples were preserved for laboratory analysis.

### Sample preparation for Atomic Absorption Spectroscopic Analysis

The soil sample was crushed to facilitate weighing after drying and 4g of dried sample was weighed into a 250mL beaker. 100ml of acidified deionized water, 1ml of concentrated  $\text{HNO}_3$  (sp.gr 1.42) and 10mL concentrated HCl (sp.gr 1.19) were added slowly to prevent any foaming reaction. The beaker was then covered with watch glasses and heated at  $95\text{--}105^\circ\text{C}$  on a hot plate without allowing it to boil. The beaker was removed from the hot plate and allowed to cool to ambient temperature when the volume of the solution was about 15ml. Finally, the content of the beaker was filtered and quantitatively transferred into a 50ml volumetric flask and diluted to volume with acidified deionized water.

The analysis of the metals was done using Perkin Elmer 700 series AAS consisting of a hollow cathode lamp (HCL) for radiation source, a nebulizer-burner system for sample aspiration, a monochromator made of diffraction grating for selecting light of one wavelength, a photomultiplier detector and an amplifier and recorder. All the components are aligned in a fashion that allows the radiation from the HCL to pass directly through the flame, monochromator and the detector. The instrument measures the amount of absorption of a precise wavelength of radiation by uncharged atoms present in their ground state and becomes excited. The chemical compounds present in the sample are dissociated into atoms by enough energy supplied from the flame necessary for the atomic absorption measurement. Hollow cathode lamp helps in the generation of specific wavelength of radiation required for the specific metal analysis.

### Results and Discussion

**Table 1:** Trace metal concentration in mg/Kg for surface, subsurface and control at Bille oil spill impacted site

Element	A1	A2	A3	B1	B2	Control	Mean $\pm$ SD
Fe	23,214.47	21,239.40	31,136.37	24,434.74	25,810.97	9,609.50	$25,167.19 \pm 3,735.73$
Cd	0.46	0.30	0.41	0.67	0.40	0.28	$0.45 \pm 0.14$
Co	30.64	28.39	29.11	37.76	32.33	26.16	$31.64 \pm 3.74$
Cr	37.22	28.58	30.22	47.07	32.31	21.62	$35.08 \pm 7.45$
Cu	14.91	8.95	21.14	15.93	13.22	10.53	$14.83 \pm 4.42$
Mn	105.69	90.91	88.60	108.97	85.66	28.32	$95.97 \pm 10.61$
Ni	32.20	20.28	25.98	46.20	27.91	5.12	$30.51 \pm 9.76$
Pb	20.53	25.66	18.69	17.36	18.30	0.44	$20.11 \pm 3.31$
V	25.68	25.18	23.24	30.43	27.01	7.98	$26.31 \pm 2.67$



Zn	63.71	48.93	83.13	54.52	59.22	24.20	61.90 ± 13.08
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Sample Keys: A1 = First surface soil; A2 = Second surface soil; A3 = Third surface soil; B1 = First subsurface soil; B2 = Second subsurface soil

**Table 2:** Enrichment factor of heavy metals of Bille oil spill impacted site

Metals	Fe	Cd	Co	Cr	Cu	Mn	Ni	Pb	V	Zn
Mean Concentration	25,167.19	0.45	31.64	35.08	14.83	95.97	30.51	20.11	26.3	61.90
Control	9,609.50	0.28	26.16	21.62	10.53	28.32	5.12	0.44	8.00	24.20
EF	2.62	1.61	1.21	1.62	1.41	3.39	5.96	45.70	3.29	2.56

EF = Enrichment factor

**Table 3:** V-to-Ni ratio of the spilled oil of Bille oil-impacted site

Samples	V	Ni	V/Ni
A1	25.68	32.20	0.80
A2	25.18	20.28	1.24
A3	23.24	25.98	0.89
B1	30.43	46.20	0.66
B2	27.01	27.91	0.97
Mean ± SD	26.31 ± 2.67	30.51 ± 9.76	0.91 ± 0.22

**Table 4:** One-Way Analysis of Variance (ANOVA) for the samples

Source of Variation	DF	SS	MS	F	F <sub>crit</sub>
Factor	4	5608911	1402228	0.02	2.58
Error	45	2892523847	64278308		
Total	49	2898132758			

DF, degree of freedom; F, factor mean square; SS, sum of squares; MS, mean square; p<0.05

Fe was observed to have the highest concentration with an average of 25,167mg/Kg. This value is greater than those reported by Ekweozor *et al.*, [12]; Udo *et al.*, [13]; Oluwole *et al.*, [14]. The high amount of iron is likely due to the association of the metal with crude having an abundant input of porphyrin-precursor chlorophylls to organic substance [15].

### Monitoring of the Contamination of Soil with Heavy Metals

In minor concentrations, heavy metals are not hazardous to plants and animals except lead, cadmium and mercury; they are toxic even in low concentrations [16]. Therefore, evaluation of contaminated soils using heavy metals is important considering the negative effects they have on surface water, ground water, plants and animals [17]. A known approach is using the enrichment factor (EF) to evaluate the extent of contamination (naturally or anthropogenically) of the contaminated soils by comparing the concentrations of the metals in the contaminated soils to that of the control site [18]. The enrichment factor can be evaluated by the formula below: [18].

$$EF = \frac{\text{Metal concentration in spill impacted site}}{\text{Metal concentration in control site}}$$

Table 2 shows the enrichment factor (EF) calculated from the metal concentrations and as EF increases, the contribution of anthropogenic source of the pollution also increases. EF values of Cd (1.61), Co (1.21), Cr (1.62) and Cu (1.41) are lower than 2 indicating minimal enrichment while Fe (2.62), Mn (3.39), V (3.29) and Zn (2.56) values are between 2-5 indicating moderate enrichment. Ni (5.96) and Pb (45.70) show significantly and extremely high enrichment respectively. Therefore, EF values (greater than unity) describes the input of an anthropogenic source of pollution attributable to the spillage.



### V/Ni Ratio

In chemical fingerprinting, V/Ni ratio is an important parameter because the ratio does not change with the biodegradation or weathering of spilled oil; this implies that this ratio allows the correlation of the crude from different depths of the soil [19]. The ratio of V/Ni ranged from 0.66 to 1.24 with an average of  $0.91 \pm 0.22$  as presented in Table 3 which clearly depicts less variability. This ratio of V/Ni shows a high degree of some homogeneity among the spill samples, reflective of lingering input of spilled crude from a single source over time. V/Ni as an excellent diagnostic ratio can be used to trace the diagenetic pathway of the crude oil where the magnesium ions of the petroporphyrins are substituted by vanadium and nickel. The application of V/Ni as a diagnostic marker is acceptable because the chemical complexes in which they are present are importantly changed by weathering or microbial degradation [19].

Again, since the V/Ni parameter is a relative ratio, it is susceptible to little variations due to changes in absolute amounts of the metals that might occur when the crude weathers by evaporation. Hence, V/Ni ratio can precisely show differences in V/Ni distributions between samples and relative to suspect sources even among variably weathered crude [20]. Wang *et al.* [5] reported that V/Ni diagnostic ratios complement petroleum hydrocarbon-based methods of oil characterization.

### Environmental and Ecological Effects of Heavy Metals

Heavy metals and other pollutants such as PAHs are the foremost constituents of petroleum hydrocarbons. From the view point of environmental pollution, metals may be grouped into non-toxic but accessible, toxic but non-accessible and toxic and accessible. Metals under the category of toxic and accessible have attracted more attention because of environmental pollution and public health concerns [21]. Entrance of toxic heavy metals into the ecosystem can lead to geo-accumulation, bio-accumulation and bio-magnifications. They get accumulated quickly in soils and plants and would stimulate an undesirable effect on the functional activities of plants (e.g. photosynthesis, gaseous exchange and nutrient absorption) which eventually contribute to the reductions in plant growth, dry matter accumulation and yield [17, 22]. Iron is involved in substantial pathways in plant such as physiological role and biochemical activities. Fe is required by plants for the formation of chlorophyll and vital for synthesizing chloroplast structure and its function. Fe also plays important role in transport chain process, photosynthesis, respiration and complex biological functions and it is a major part of sensitive enzymes like cytochromes. An increased Fe content in soils can hinder the flow of water and air penetration mostly in silting soil pores, it can also lead to hard concretions and disruption of plant [23].

Cd contaminated soils higher than 0.05 to 0.12 $\mu\text{g/g}$  exhibit chlorosis and reduced growth. When grown in Cd contaminated soils, the roots of corn, oats, soybeans, tomatoes and alfalfa accumulate highest levels of Cd, whereas the aerial parts of carrot, potato, lettuce and tobacco accumulate highest levels of Cd [21]. From the result, Cd is seen to be present in average concentration of 0.451mg/kg and this is unaccepted for plant growth.

Co was discovered as 31.64mg/Kg higher than the control of 26.16mg/Kg, suggesting that anthropogenic source was responsible for the presence of Co in the soil samples. Chronic exposures of Co have been reported by Monni *et al.*, [22] to be liable for hypoxia and polycythemia.

Chromium (Cr) can exist as Cr (III) and Cr (VI) based on toxicities and chemical properties. Cr (III) is 10 to 100 times less hazardous than Cr (VI) and a known micronutrient whereas Cr (VI) is an oxidizing agent known for its high toxicity and can alter the population of microbes in the soil with negative effects on cell metabolism [24]. Cr (III) showed that LD<sub>50</sub> value is about 1mg per 100g body weight. Onojake and Frank [25] reported that Cr as a micronutrient is carcinogenic, mutagenic and teratogenic. Oral median lethal dose (LD<sub>50</sub>) values in rats within 20-250mg of Cr (VI) per kg, at the ppb level, it is lethal to aerobic microbes and soils containing 0.2-0.4% of Cr are regarded unproductive for agrarian activities [16]. Again, the existence of Cr in the contaminated samples greater than the control sample points to anthropogenic inputs.

Cu is essential to plants for synthesizing chlorophyll and for enzymatic activities. However, at higher levels about 0.1mg/kg Cu can inhibit plant growth [21]. Ingestion of Cu at a concentration of 648-972mg is poisonous and



considered toxic to some aerobic microbes and Cu inhibits lettuce growth and germination even at ppb levels. The average amount of Cu from this research was 14.83mg/Kg more than the control of 10.53mg/kg. Mn is among the least toxic metals to plant. Higher concentrations of Mn may be toxic, particularly in acidic soils. Accumulation of Mn in leaves leads to chlorosis and necrosis of leaves [21].

At low concentrations, Mn is a desirable trace element which contributes to well-being of the cells because it acts as a co-factor in some enzymatic reactions such as those involved in phosphorylation and synthesis of cholesterol. However, when exposed to levels higher than 2.5-5mg of daily intake, it gets accumulated in kidney, liver, and bones and cause “Manganese psychosis” which is an irreversible brain disease known by uncontrollable laughter, euphoria, impulsiveness, sexual excitement followed by impotency [26].

The accepted limit of Ni in plants recommended by WHO is 10mg/kg. From this research, Ni (30.51mg/Kg) showed that Ni toxicity to plants arising from the spill is attainable. Ni is beneficial in low concentrations [27]. Ni is a main constituent in nine metalloenzymes like the urease, this implies that a deficiency of Ni leads to a reduction of the action of urease and disturbs nitrogen assimilation [28]. Ni as a cofactor, enables urease to catalyze the transformation of urea into ammonium ions which plants must use as nitrogen source, therefore without Ni this conversion process is impossible [28].

Pb contaminated soils are usually characterized by reasonable decrease in crop production and growth resulting in poor agricultural yields [29]. This study showed that the existence of Pb in the samples resulted from the occurrence of the oil spill.

V is a redox-sensitive metal that is released to soils by weathering and anthropogenic emissions and well retained in soils [30-31]. A study reported by Onojake and Frank [25], stated that rats and mice have shown LD<sub>50</sub> results varying between 10.0-160mg/Kg. V occurs rarely in crust and its presence is usually from petroleum origin. V addition inhibits nitrification and nitrogen mineralization [32-33]. Soil microorganisms are identified to be sensitive to metals but knowledge of V toxicity is scarce [34].

Soils contain about 50mg/kg Zn since this metal is essential to plant nutrition and plant growth [35-36]. Zn becomes toxic at higher concentrations than 50mg/Kg [37]. Excess of Zn prompts negative interferences with other nutrients uptake and enzyme activities which elicits wilting, necrosis of old leaves, biomass decline and stoppage of cell growth and splitting [38-40]. Moreover, the level of toxicity sharply varies among plant species and duration of exposure to Zn stress.

### Analysis of Variance (ANOVA)

ANOVA is a statistical tool available to a scientist which is traditionally used to test significant differences between means of several observations. The superiority of this tool over the “t-test distribution” lies in its ability to deal with multiple observations. A one-way analysis of variance (ANOVA) test was conducted as presented in Table 4 at 95% confidence limit to test the outcome of the sampling locations on the individual means. The test result highlights the insignificance between the sampling points as the F ratio (0.02) from the test was found less than F critical (2.58) at 95% confidence limit.

### Conclusion

Since 1956 when crude was discovered in commercial quantity in Oloibiri, present-day Bayelsa, Nigeria, the country has been bedeviled with magnitudes of harmful environmental consequences of oil exploration, exploitation, refining and transportation. Sabotage remains a chief root of spillage cases in the study area and Niger Delta and has devastated coastal vegetation, polluted drinkable water, especially contributed to soil pollution by introducing some dangerous substances like TPH, PAHs and heavy metals into the environment. Soil samples from Bille oil spill impacted site were analyzed for the determination heavy metals using AAS. The research revealed heavy metals at the impacted site in concentrations greater than the concentration found in the control site, suggesting that the distribution pattern of the metals in the impacted area were greatly influenced by oil spillage. The high concentration of Fe as revealed by the research likely suggests that there is an abundant input of porphyrin-precursor chlorophyll to the carbon-based matter in terrestrial origin in oxic environment.



The distribution of vanadium and nickel and their ratios are important parameters in chemical fingerprinting as they remain unchanged during weathering or biodegradation of crude and they are important in tracing the source and extent of spill. From this research, V/Ni ratio of 0.91 identified continuous input of crude oil as the single cause of the existence and abundance of metals and organic compounds in the study region.

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