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Changes in metals and polyaromatic hydrocarbon contents of a spent lubrication oil-polluted soil after exposure to sodium azide and hydroxlamine hydrochloride solutions: implications for intrinsic bioremediation

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SUMMARY. The study investigated the changes in heavy metals and polyaromatic hydrocarbon contents of an oil-polluted soil as a result of exposure to sodium azide and hydroxylamine hydrochloride. Measured 5kg of Oil-polluted soils (5%w/w), placed in experimental buckets, were saturated with solutions of sodium azide and hydroxylamine hydrochloride in 3 different concentrations (0.0625, 0.0312 and 0.0156 %v/w) respectively. The entire set up was observed in a well-ventilated Screen House for 3 months. Results showed that experimental concentrations of both mutagenic agents had no significant effect ($p>0.05$) on Fe concentration of soil (998.8 – 1106.2 mg/kg). Although soil levels of Fe exceeded permissible levels by over 5 times, concentrations of Mn, Cd, Ni, and V were below detection limits (<0.001 mg/kg) after application of chemical agents. Hydroxylamine HCl-moistened soil presented enhanced remediative capabilities for chromium (Cr = <0.001 mg/kg) than with sodium azide (Cr = 8.29 - 13.11 mg/kg). Sodium azide did not significantly enhance Cr remediation, compared to the control. Reductions in PAH fractions in the treated soils were better than in the control soils. Efficiency of PAH reduction in the control was 60.47%. application of mutagenic agents to polluted soils at lower to moderate concentrations significantly enhanced remediation efficiency to 80.95 -89.27%. Generally, however, hydroxylamine HCl showed better prospects in the enhancement of remediation (at lower to moderate levels) than did sodium azide.

Keywords: bioremediation, hydrocarbon, hydroxylamine, metals, mutation

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Introduction

Poly aromatic hydrocarbons (PAHs), as environmental pollutants, exist in air, water, sediments and soil. These compounds are also products of transportation, refuse burning, and gasification. Recently, indiscriminate disposal of spent lubrication oil oil has been identified as a significant source of PAHs in the environment (Anoliefo and Edegbai, 2001). PAHs are easily mixable with oil than water. They are toxic, mutagenic and carcinogenic in nature. Lower molecular weight PAH are acutely toxic; similarly, higher molecular PAH, apart from being genotoxic, are not easily biodegradable (Juhasz and Ravendra, 2000). Phenanthrene, for example, induces mutations by causing sister chromatids to interchange and obstructs gap junction intercellular communications. Phenanthrene can be bioaccumulated in aquatic organisms including fish, and then biomagnified up the food chain in humans (Mrozik *et al.*, 2003). In the soil, they hamper crop plant productivity; where in most cases they result in plant death (Ikhajiagbe, 2010)

There are several remediative measures to curtail and contain the spread of these PAHs, among which are those that rely on the primary mechanism of intrinsic remediation or natural attenuation – microbial biodegradation. During biodegradation of organic pollutants, available nutrients are converted by soil microorganisms into beneficial forms of energy and cell production. The biodegrading of contaminants, particularly PAHs, can be considerably boosted when the factors that directly or indirectly affect processes and mechanisms are improved upon. These factors may range from microbial and plant presence as well as other biological and abiotic factors. One of the methods of biological improvements for better performance has been reported to be by mutagenic enhancement (Mshembulla *et al.*, 2012).

Chemicals like sodium azide, hydroxylamine hydrochloride, oryzaline, as well as colchicine have been confirmed to cause mutagenesis. Sodium azide can bring about mutation plants and animals, including microorganisms (Asmahan and Nada, 2006). The ability of sodium azide to enhance crop development of chickpea (Mahesh and Vijay, 2009) and groundnut (Mensah *et al*., 2007) has been reported. Shittu and Ikhajiagbe (2013) reported enhancement of phytoaccumulation of heavy metals in an oil-spiked soil by *Vernonia amygdalina* after exposing the stem cuttings to sodium azide solutions before sowing. Ikhajiagbe and Chijioke-Osuji (2015) enhanced the capacity for Aspillia africana to remediate heavy metals in a spent lubrication oil-polluted soil after exposure to hydroxyl amine hydrochloride. The ability for these chemical agents to enhance plants' survivability in oil-polluted soils have been reported by Ikhajiagbe *et al*. (2013) in rice (*Oryza sativa,* var. FARO-57); Ikhajiagbe and Oshomoh (2014) in *Vigna unguiculata* (var. TVU-3541); Ikhajiagbe *et al*. (2014a) and Ikhajiagbe and Shittu (2015) in *Glycine max;* and Ikhajiagbe *et al.* (2014b) in fluted pumpkin *(Telfairia occidentalis)*.

The argument is whether simple mutagenic agents like these two, which have been reported to enhance plant capabilities by mere contact with plants and their propagules (Mshembula *et al.*, 2012; Omoregie *et al*., 2012; Ikhajiagbe *et al*., 2013) can also confer on soil microorganisms the ability to improve their bioremediation capabilities in oil-polluted soils. Although specific microorganisms are not directly targeted to achieve mutagenesis; it is however hoped that mere application of the chemical agent unto soil might in the long run affect soil microorganisms positively or negatively with regards to remediation rates. The research aim of the study therefore is to investigate to what extent Sodium azide and Hydroxylamine hydrochloride can affect intrinsic bioremediation of a spent lubrication oil-polluted soil.

Materials and methods

Soil contamination with oil

Top soil (0 - 10 cm), of predetermined physicochemical properties (Table 1), was collected from the Ugbowo Campus of the University of Benin, Nigeria. Measured quantity (mass of oil = 250g, specific gravity = 0.846) of spent lubrication oil was added to soil and mixed to get uniform concentration of 5% w/w.

Preparation of mutagenic solution

Measured quantities of sodium azide and hydroxylamine hydrochloride were dissolved in distilled water adjusted to pH 3 using a pH buffer. Sodium azide and hydroxylamine hydrochloride solutions were prepared at three concentrations (0.0625%w/v, 0.0312%w/v and 0.0156%w/v) respectively.

Saturation of polluted buckets with mutagenic solutions

Having previously determined the water-holding capacity of the soil to be 224ml/kg soil, the polluted soils were saturated with the mutagenic solutions using 1000ml of solution per bucket. Buckets were not perforated; the idea was to keep all content within the buckets under experimental condition. The set up was kept in a well ventilated Screen house (temp range = 29.21 ± 2.67 ^{o}C) for three months. Afterwards, soil was analyzed for polyaromatic hydrocarbon contents using the standard methods of Dean and Xiong (2000). Isolation and characterization of bacterial and fungal species was carried out using the methods of Sabba (1995) and Cheesebrough (1998). Heavy metal content of soil was also determined following the methods of SSSA (1971) and AOAC (2005). Soil physicochemical analyses were according the methods of Osuji and Nwoye (2007) and AOAC (2005). The results were subjected to statistical analysis using SPSS 16 ®.

Bioremediation efficiency

This is regarded as the proportion (%) of contaminant concentration that was bioremediated compared to a measured concentration at a start point. This is calculated according as;

$$\text{Efficiency (\%)} = \frac{(\text{c3MAPA} - \text{c1DAPA})}{\text{1DAPA}} \times 100$$

where c3MAPA = contaminant concentration at 3 months after pollution and amendment; c1DAPA = contaminant concentration at the first day following pollution and amendment.

Results and discussion

The physicochemical components of materials used in the study have been presented on Table 1. pH of both soil and spent lubricating oil (SLO) were both slightly acidic. Results as presented on Table 2 showed that the mutagenic agents had no significant effect on Fe concentration of soil (998.8 – 1106.2 mg/kg). These concentrations exceeded permissible levels in soil by over 5 times. Mn, Cd, Ni, and V were below detection limits (<0.001 mg/kg). ecological screening values for these metals were also not exceeded. Ecological screening valus were also not exceeded for Zn, Cu and Pb. However, there were significant reductions in these metals in the treated soils than in the control. Hydroxylamine HCl-treated soil showed better remediative capabilities for chromium (Cr = <0.001 mg/kg) than with sodium azide (Cr = 8.29 -13.11 mg/kg). Compared with the control, there were no significant differences in remediative capacities in the sodium azide-treated soil for chromium.

The effects of sodium azide and hydroxylamine HCl in the intrinsic bioremediation of heavy metals and PAH fractions on a spent lubricating oil-polluted soil has thus been reported. Although no significant changes in Fe concentration of soil was reported, remediation of other heavy metals in this study was significant as reduction was either total (100%) or significantly partial (50 - 98%). Remediation of heavy metals was comparatively better in the hydroxylamine hydrochloride-moistened soil.

Phytotoxicity screening limits for naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, and pyrene were exceeded at the first day when soil was polluted with oil (Table 3). However, after three months, concentrations of the polyaromatic fractions were below limits for naphthalene, acenaphthene, and fluorene. Ecological screening values for phytotoxicity of phenanthrene, anthracene, fluoranthene, and pyrene were still exceeded. Reductions in PAH fractions in the treated soils were better than in the control soils. Remediation efficiencies in the

sodium azide and hydrolyamine hydrochloride-treated soils comparable at lower and moderate concentrations (80.95 – 89.27%), compared to higher concentrations (69.99 – 77.93%). Efficiency of PAH reduction in the control was 60.47%.

Table 1.

Physical and chemical properties of soil and spent lubrication oil (SLO) contamination before commencement of experiment

Parameters	**Soil**	**SLO**		**Soil**	**SLO**
(Physicochemical parameters/heavy metals)			**(PAH content, mg/kg)**		
Ph	6.15	5.98	Naphthalene	<0.001	35.21
Electrical Conductivity (µs/cm)	309.00	NM	Acenaphthylene	<0.001	7.98
Total Org. Matter (%)	0.61	NM	2-bromonaphthalene	<0.001	35.24
Total Nitrogen (%)	0.16	NM	Acenaphthene	<0.001	36.32
Exchangeable Acidity (meq/100 g soil)	0.24	NM	Fluorene	<0.001	42.04
K (meq/100 g soil)	1.40	NM	Phenanthrene	0.85	16.54
Ca (meq/100 g soil)	12.20	NM	Anthracene	<0.001	78.20
Mg (meq/100 g soil)	9.95	NM	Fluoranthene	<0.001	26.41
P (mg/kg)	153.00	NM	Pyrene	<0.001	23.98
Copper, Cu (mg/kg)	<0.001	56.52	Benzo(a)anthracene	<0.001	42.05
Manganese, Mn (mg/kg)	<0.001	29.21	Chrysene	<0.001	115.27
Nickel, Ni (mg/kg)	<0.001	4.15	Benzo(b,j,k)fluoranthene	<0.001	41.68
Vanadium, V (mg/kg)	<0.001	3.95	Benzo(a)pyrene	40.28	129.87
Chromium, Cr (mg/kg)	0.08	14.29	Indeno(1,2,3-cd)pyrene	5.24	198.2
Lead, Pb (mg/kg)	<0.001	30.55	Dibenzo(a,h)anthracene	12.25	46.52
Iron, Fe (g/kg)	183.23	2123.21	Benzo(g,h,i)perylene	19.24	63.25
Zinc, Zn (mg/kg)	3.08	56.22			
Cadmium, Cd (mg/kg)	<0.001	3.65			

NM not measured.

Table 2.

Heavy metal contents of oil-polluted soil after amendment at 3 months after pollution and application of mutagenic agents

Treatments	**Fe**	**Mn**	**Zn**	**Cu**	**Cr**	**Cd**	**Pb**	**Ni**	**V**
					mg/kg				
Control	1106.2^{a}	18.2^{a}	42.2^{a}	38.11^{a}	10.08^{a}	0.97^{a}	22.24^{a}	2.53^{a}	3.91^{a}
					Sodium Azide-treated soil				
0.0156% w/v	1003.2^{a}	<0.001^{b}	3.65^{b}	31.52^{a}	13.11^{a}	<0.001^{b}	8.23^{c}	<0.001^{b}	<0.001^{b}
0.0312% w/v	1054.1^{a}	<0.001^{b}	5.98^{b}	22.03^{b}	9.06^{a}	<0.001^{b}	13.08^{bc}	<0.001^{b}	<0.001^{b}
0.0625% w/v	1035.1^{a}	<0.001^{b}	5.26^{b}	21.30^{b}	8.29^{a}	<0.001^{b}	16.82^{ab}	<0.001^{b}	<0.001^{b}
					Hydroxylamine HCl-treated soil				
0.0156% w/v	1100.6^{a}	<0.001^{b}	4.62^{b}	18.52^{b}	<0.001^{b}	<0.001^{b}	15.38^{b}	<0.001^{b}	<0.001^{b}
0.0312% w/v	1063.3^{a}	<0.001^{b}	4.92^{b}	21.04^{b}	<0.001^{b}	<0.001^{b}	13.26^{bc}	<0.001^{b}	<0.001^{b}
0.0625% w/v	998.8^{a}	<0.001^{b}	5.38^{b}	20.13^{b}	<0.001^{b}	<0.001^{b}	<0.001^{d}	<0.001^{b}	<0.001^{b}

Treatments	Fe	Mn	Zn	Cu	Cr	Cd	Pb	Ni	V
					mg/kg				
ESVp*	NA	100.00	50.00	40.00	1.00	4.00	50.00	30.00	2.00
ESVm*	200.00	100.00	100.00	100.00	10.00	20.00	900.00	90.00	20.00

***Benchmarks available at Efroymson *et al*. (1997); **NA** not available. **LSD** least significant difference; **ESVp** Ecological screening value for phytotoxicity of contaminant; **ESVm** Ecological screening value for toxicity of contaminant to soil microorganisms and soil microbial processes. Means on the same column with similar alphabetic superscripts do not differ from each other ($p>0.05$). $n = 3$.

A good number of polyaromatic hydrocarbon fractions were significantly remediated. Remediation was total (100%) for Acenaphthene, naphthalene, Acenaphthyelene, 2-bromonaphthalene, fluoranthene, chysene, benzo(b,j,k)flouranthene) and partial(60-90%) for phenanthrene, indo(1,2,3-cd)pyrene, dibenzo(a,h)anthracene in 0.0156%w/w sodium azide treatment as confirmed by Ikhajiagbe and Anoliefo (2011).

Table 3.

Polycyclic aromatic contents of oil-polluted soil after amendment at 3 months after pollution

	5 %w/w Oil-Polluted soil at first day of pollution	Control	**Sodium Azide-treated**			**Hydroxylamine HCl-treated**			**ESVp**
			0.0156 %w/w	0.0312 %w/w	0.625 %w/w	0.0156 %w/w	0.0312 %w/w	0.625 %w/w	
Naphthalene	29.25	<0.001	<0.001	<0.001	16.45	14.49	<0.001	15.29	0.10
Acenaphthylene	10.54	1.29	<0.001	<0.001	16.09	14.34	<0.001	<0.001	NA
2-bromonaphthalene	35.21	1.21	<0.001	16.91	17.65	15.30	16.76	16.04	NA
Acenaphthene	35.46	21.68	<0.001	16.25	16.52	14.51	16.06	15.32	20.00
Fluorene	45.22	32.59	14.22	17.02	17.40	15.10	16.67	16.26	30.00
Phenanthrene	5.62	44.84	0.49	0.81	1.76	0.94	0.71	1.03	0.10
Anthracene	29.24	23.25	15.04	18.03	18.53	15.78	17.41	16.99	0.10
Fluoranthene	42.52	17.34	<0.001	<0.001	16.49	<0.001	<0.001	15.82	0.10
Pyrene	36.20	12.20	14.45	17.16	18.06	15.07	16.68	17.06	0.10
benzo(a)anthracene	53.87	23.62	16.94	19.58	22.26	16.67	<0.001	20.36	NA
Chrysene	129.54	3.66	<0.001	<0.001	3.04	<0.001	<0.001	2.43	NA
benzo(b,j,k) fluoranthene	59.48	87.58	<0.001	<0.001	4.76	1.70	<0.001	8.78	NA
benzo(a)pyrene	209.16	40.58	24.30	31.80	60.47	40.49	22.42	42.49	NA
indeno(1,2,3-cd)pyrene	169.54	11.08	0.59	26.63	24.32	2.88	2.67	4.13	NA
dibenzo(a,h) anthracene	52.20	77.94	2.59	3.29	15.63	2.85	7.31	6.91	NA
benzo(g,h,i)perylene	72.65	2.65	20.38	24.83	35.37	23.38	26.14	25.27	NA
Total PAH	1015.70	401.51	109.00	192.31	304.8	193.5	142.83	224.18	1.00
Efficiency (%)	-	60.47	89.27	81.07	69.99	80.95	85.94	77.93	NA

***Benchmarks available at Efroymson *et al*. (1997); **NA** not available; **ESVp** Ecological screening value for phytotoxicity of contaminant.

Results showed microbial toxicity of higher concentrations of hydroxylamine HCl against *Achromobacter* sp. (Table 4). However, the presence of the chemical agent in soil encouraged the growth of *Micrococcus luteus* and *M. roseus.* Similarly, the absence of *Clostridium perfringens* and *Aspergillus flavus* may also suggest toxicity of hydroxylamine HCl to soil microorganism. The fact that the growth certain species of fungi and bacteria did not occur in some treated soil is not enough to prove toxicity of the chemical agents used in the study against the microorganisms. The possibility also exists that perhaps this change may vary from soil to soil as these chemical agents may involve in a number of possible chemical reactions to release toxic or even growth-enhancing substances in the soil. Hence, it is recommended that specific toxicity studies be conducted to further clarify the information provided herein. Further, the availability of heavy metals like Fe beyond ecological screening limits may also account for this change (see Table 2). There were significant reduction in heterotrophic bacteria count in the soils treated with higher concentrations of the chemical agents used in the study ($2.2 – 3.2 \times 10^5$cfu/g), compared to the control (4.3×10^5cfu/g). Percentage hydrocarbon degrading fungi ranged from 60% to absolute, compared to 34.88 – 90.91%.

The study reported differential effects of the chemical agents on microbial availability and processes in the soil; some were antimicrobial at higher concentrations, while others, at lower and moderate concentrations encouraged microbial proliferation. This is usually a precondition to intrinsic remediation of contaminants. However, environmental conditions like availability of nutrient and water also enhance activities of microorganisms in bioremediation. This study records many prevalent microorganisms like *Achromobacter* sp, *Bacillus pumilis*, *Clostridiumperfringens*, *Sarcina*sp, *Pseudomonasaeruginosa*, *Aspergillusniger*, A. *Flavus*, *Penicillium* spp., *P. notatum*, *Mucor* sp., *Fusarium* sp., and *F. solani*.

Table 4.

Microbial composition of treated and control soils at three months after pollution

		Sodium Azide-treated			**Hydroxylamine HCl-treated**		
	Control	0.0156 %w/v	0.0312 %w/v	0.625 %w/v	0.0156 %w/v	0.0312 %w/v	0.625 %w/v
Achromobacter sp.	+	+	+	+	+	+	-
Bacillus pumilis	+	+	+	+	+	+	+
Clostridium perfringens	+	+	+	-	-	-	-
Sarcina sp.	+	+	+	+	+	+	+
Micrococcus sp.	-	-	+	-	-	-	-
M. luteus	-	+		+	+	+	-
M. roseus	-	+	+	-	-	-	+
Pseudomonas aeruginosa	+	+	-	-	-	-	+
Heterotrophic (x 10^5cfu/g)	4.3^{a}	4.0^{a}	3.2^{ab}	2.7^{b}	4.1^{a}	4.0^{a}	2.2^{b}
Hyd. Deg. Bacteria (x 10^5cfu/g)	1.5^{bc}	2.5^{ab}	2.0^{abc}	2.0^{abc}	0.5^{c}	3.2^{a}	2.0^{abc}
% Hyd	34.88	62.50	62.50	74.07	12.19	80.00	90.91

		Sodium Azide-treated			Hydroxylamine HCl-treated		
	Control	0.0156 %w/v	0.0312 %w/v	0.625 %w/v	0.0156 %w/v	0.0312 %w/v	0.625 %w/v
Aspergillus niger	+	+	+	+	+	+	+
A. Flavus	+	+	+	+	-	-	-
A. fumigatus	-	-	-	-	+	-	-
Penicillium sp.	-	+	+	-		+	+
P. notatum	+	-	-	+	+	-	-
Fusarium sp.	-	-	-	+	-	-	-
F. solani	+	-	+	-	-	+	+
Mucor sp.	+	+	-	-	-	+	+
Heterotrophic Fungi (x 10^5cfu/g)	2.9^{ab}	3.0^{a}	2.0^{ab}	1.3^{b}	2.3^{ab}	2.2^{ab}	1.5^{ab}
Hyd. deg. Fungi (x 10^5cfu/g)	1.8^{a}	2.3^{a}	1.2^{a}	0.8^{a}	2.3^{a}	2.0^{a}	1.5^{a}
% Hyd	62.07	76.67	60.00	61.54	100	90.91	100

+ present, - absent, %Hyd Percentage hydrocarbon degraders. n = 3. Means on the same row with similar alphabetic superscripts are similar ($p>0.05$)

These microorganisms might have been involved in the remediation process, considering the fact that their prevalence, even in higher concentrations of SLO in soil, may signify tolerance to these pollutants. The microorganisms identified in this study have been previously reported to belong to the bioremediation microbial consortia by Cerniglia (1992), Ekundayo and Obuekwe (1997), Yogambal and Karegoudar (1997), Remero *et al.* (2001) and April *et al.* (2000). Some microorganisms like *Aspergillu niger* were present all though the experiment. The presence of the fungus was reported in both control and mutagen-treated soils. Also present was the bacterium *Bacillus pumilis*. *Aspergillus niger* metabolize PAHs (Yamazaki *et al.*, 1988). *A. fumigatus* also produces a cytochrome P450 that hydroxylatesbenzo[a]pyrene (Venkateswarlu *et al.*, 1996).

Conclusions

Chemicals agents abound that confer newer and more-improved capabilities to organisms. In the present study sodium azide and hydroxylamine hydrochloride were applied to petroleum hydrocarbon-polluted soil with a view to possibly improving the soil's biological capabilities in reclaiming the oil-degraded soil. Both agents differed in the enhancement remediation efficiencies. Further, lower to moderate levels of hydroxylamine hydrochloride presented better capability to enhance remediative capacities of oil-polluted soil than sodium azide..

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