

IMPACT OF SPENT MUSHROOM SUBSTRATE ON SOIL MICROARTHROPODS IN SPENT AUTOMOBILE LUBRICANT HABITAT-TYPES AT UNIVERSITY FARM, UNIVERSITY OF PORT HARCOURT, RIVERS STATE, NIGERIA

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ABSTRACT: *This study aimed at investigating the impact of spent mushroom substrate on soil microarthropods in spent automobile lubricant habitat-types at the University of Port Harcourt farm (4°51'33.0N; 6°55'20.0E). Soil samples were collected from two depth ranges (0-10cm, 10-20cm) of contaminated (2L, 4L lubricant), remediated (2L + 1.7Kg, 4L + 1.7Kg substrate) and zero treatment (control) habitat-types. The samples were extracted in modified Berlese-Tullgren funnels. A total of 24 taxa of soil microarthropods were collected with 9 and 7 from 2L-and 4L-contaminated, 21 and 18 from 2L, 4L + substrate habitat types respectively. A total of 896 individual soil microarthropods were recorded with 285 or 31.6% (control), 76 or 8.4% (2L contaminated), 57 or 6.3% (4L-contaminated), 289 or 32.9% (2L + substrate) and 189 or 21% (4L + substrate) habitat-types. The THC values were 12.1mg/kg (2L-contaminated), 13.1mg/kg (4L-contaminated), 4.8mg/kg (2L+substrate) and 6.4mg/kg (4L+substrate). After 10weeks of post remediation, a reduction of 43.2% (7.3mg/kg) in THC was recorded in 2L-remediated habitat-types, which also had 12 species, out of 15 that were absent in the 2L-contaminated habitat-types. The data showed a significant difference in both abundance and THC between 2L contaminated and 2L+substrate amended habitat-types.*

KEYWORDS: Mushroom Substrate, Spent Automobile Lubricant, Soil Microarthropods, Post Remediation, Habitat-Types, Mean Abundance.

INTRODUCTION

Spent automobile lubricant is a brown-to-black liquid produced when new mineral-based crankcase oil is subjected to high temperature and high mechanical strain. They are lubricants that have been used to operate an automobile machine and considered not fit for initial purpose (Ameh *et al*, 2012).

Spent automobile lubricant also referred to as used or waste motor oil is a mixture of different chemicals, including low and high molecular weight (C₁₅ – C₂₀) aliphatic hydrocarbons, aromatic hydrocarbons, polychlorinated biphenyls, chlorodibenzofurans, lubricative additives, decomposition products, heavy metal contaminants such as aluminum, chromium, tin, lead, manganese, nickel and silicon that come from engine parts as they wear down (Achuba and Peretiemo-CLGarke, 2008, Wang *et al*, 2000; ATSDR, 1997).

Spent engine oil is a common and toxic environmental contaminant not naturally found in the environment (Dominguez-Rosado and Pichtel, 2004), but get into it when the motor oil is changed and disposed indiscriminately into the environment by motor and generator mechanics or artisans, and small scale engine oil sellers along the roads (Odjegba and Sadiq, 2002; Achuba and Perehemo-CLGarke, 2008). The waste oil also found its way into the environment during engine use and leaks released from the exhaust system (Osubor and Anoliefo, 2003).

Spent mushroom (*Pleurotus ostreatus*) substrate (SMS), is a composited organic material remaining after a mushroom crop has been harvested. The substrate contains essential nutrients such as nitrogen and phosphorus, micronutrients such as iron, manganese, copper and zinc which all occur in very low average that ranges between 0.01 and 0.2. It also contain salts such as sodium, calcium and magnesium, in which calcium and magnesium are always in larger amount than sodium and neutralizes sodium, thus preventing it from accumulating on the soil particles (Mike and David, 2013).

Soil microarthropods are arthropods that creep on and inside the soil (Iloba and Ekrakene, 2008) whose sizes are less than 2mm and do not permit the detailed study of their morphological features with the naked eye (Badejo, 1998). Soil microarthropods are the main components of soil inhabiting invertebrate fauna (Behan-Pelletier and Newton, 1999). They live in the soil and are mostly found in soil litres; litter-soil interphase and mostly top soil up to a depth of 10cm.

LITERATURE AND THEORITICAL UNDERPINNING

In Nigeria, more than 87 million litres of spent lubricant are disposed annually into the environment and no attention has been given to how it is been disposed (Osubor and Anoliefo, 2003). Agbogidi (2011) reported that spent oil is the commonest soil contaminant in the rural areas of Nigeria where agriculture farming forms the main stay of the rural inhabitants.

Spent engine oil when present in soil, creates an unsatisfactory condition for life by hardening the soil and changes its colour, which have untold health hazard on technicians and artisans (Udeani *et al.*, 2008).

It causes poor aeration in the soil, immobilization of soil nutrients and lowering of pH, alteration of soil biodiversity and availability of nutrients (Atuanya, 1987).

Spent mushroom (*Pleurotus ostreatus*) substrate (SMS), is a composited organic material remaining after a mushroom crop has been harvested. The substrate contains essential nutrients such as nitrogen and phosphorus. The essential nutrients contained in spent mushroom substrate perform the following functions; stimulation of microbial metabolism by making the hydrocarbon utilizing bacteria to carryout effective biodegradation in the soil (Okoh, 2006; Kin *et al.*, 2005; Fredick *et al.*, 2005), increase in soil organic waste which helps loosen the compactness of the soil, making sufficient aeration available for the indigenous bacteria present in the soil, thereby enhancing their metabolic activities in the contaminated soil (Abioye *et al.*, 2012). As organic wastes, the substrate also function in neutralizing the toxic effects of the oil on the microbial population by rapid improvement of the soil physico-chemical parameters (Jorgensen *et al.*, 2000), and decrease in total petroleum hydrocarbon in the soil amended with the substrate (Abioye, 2012). Following this development, spent mushroom substrate has been used as an organic soil amendment and fertilizer for crop production and other land management issues (Brady and Weil, 2000).

Soil microarthropods are arthropods that creep on and inside the soil (Iloba and Ekrakene, 2008) whose sizes are less than 2mm and do not permit the detailed study of their morphological features with the naked eye (Badejo, 1998). These organisms play certain roles in the soil such as decomposition and mineralization of dead organic matter, enhancement of soil fertility, regulation of microbial populations and as indicators of soil health.

These soil inhabiting microarthropods respond to indiscriminate disposal of spent automobile lubricant in the soil. Their response is an indication of the effects of the lubricant on the soil ecosystem. The presence of petroleum compounds in the lubricant interferes with the soil aeration and reduces the oxygen content of the soil and cause reduction in soil microarthropods diversity and abundance. This observation according to Vwioko and Anioleifo (2006) may be as a result of waxy texture of soil which could lead to blockage of soil pores, thus contributing to reduced oxygen content in the soil. Beside causing soil compactness and soil pore blockage which could also lead to reduction in water holding capacity of the soil, it has been reported that some soil organisms can tolerate lubricant impacted sites because they possess the ability to utilize lubricant as a source of energy and those that cannot tolerate the lubricant decrease in population (Olla *et al.*, 2013).

It has been argued that the decrease in population at petroleum impacted sites is influenced mostly by depletion in oxygen because of increase demand of oxygen by hydrocarbon degrading microbes for metabolic activities, and this cause them to migrate even below the 10cm depth, in order to avoid harsh condition (Iloba and Ekrakene, 2008). The current study was undertaken to determine the impact of spent mushroom substrate on species richness, abundance and distribution of soil microarthropods in spent automobile lubricant habitat-types.

METHODOLOGY

Study area/ Design

The study was carried out at the University farm behind University of Port Harcourt water bottling company, University Park, University of Port Harcourt located on latitude 4° 54' 33.0N and 6° 55' 20.0E from December to March, 2015.

The study area was divided into 4 plots; each into 4 sub-plots measuring 2m x 1.5m with 1m distance separating the plots in a Completely Randomized Design, where the sub-plots were the replicates. There were five treatments; zero, 2-litres, 4-litres, spent automobile lubricant and 2L+SMS and 4L+SMS which were assigned randomly to the four plots and its replicates, giving a total of 20 sub-plots measuring a total area of 160m². The zero treatment plots was the control and the replicates containing the same treatment were group together and referred to as habitat-types. Consequently, there were 5 habitat-types. Sixteen of these sub-plots were contaminated with 2 litres and 4 litres of spent automobile lubricant, and four sub-plots without any contamination.

Preparation of Study Plots

The total volume of lubricant used was 48 litres, which was applied by pouring the appropriate quantity into its plot and thoroughly mixed with a trowel to enhance uniformity.

The lubricant was obtained from an automobile mechanic workshop located in Port Harcourt, where cars came in for engine service after one month of regular driving as recommended by Osubor and Anoliefor (2003).

Fourteen days post-contamination, eight (8) of the contaminated plots containing 2 and 4-litres were treated with 1.7kg of spent mushroom substrate by mixing thoroughly with a trowel to ensure quick penetration. The spent mushroom substrate was applied as a remediating agent.

Soil Sampling/ Extraction

Soil samples were collected with an 8.5cm bucket-type soil auger at 14 days post-treatment from depths of 0-10cm and 10-20cm. Soil was taken randomly by pushing the auger sampler into the soil, rotated clockwise and anti-clockwise until the required depth was reached. Soil samples when collected are placed in labeled black polythene bags and taken to the Entomology Research Laboratory of the Department of Animal and Environmental Biology, University of Port Harcourt for a 3-stage analysis (extraction, sorting and identification (Gbarakoro *et al.*, 2010).

Extraction was carried out in modified Berlese-Tullgren funnels in the Entomology Research Laboratory of the Department of Animal and Environmental Biology, University of Port Harcourt. The extractor complex consisted of two rows of 8 units, each enclosed in an airtight aluminum cabinet with vertically sliding doors to ensure faster extraction. Description of the extractor unit and extraction procedure is documented (Badejo, 1990, 1995; Badejo and Olaifa, 1997, Gbarakoro *et al.*, 2010). Extraction lasted for 5 days. Sorting and identification was undertaken in the same laboratory and the method used for sorting has been described by Gbarakoro *et al* (2010). Identification key (Krantz, 1978; Norton 1990, Woolley, 1990) and type specimen were used. The identified soil microarthropods were counted and recorded according to the various habitat-types and respective depths.

The data obtained was subjected to analysis of variance, to ascertain the differences between treatments.

Total Hydrocarbon Content Analysis

Composite soil samples were collected from 10cm depth of each habitat and thoroughly mixed and put in an appropriately labeled black polythene bag and taken to the Laboratory where they were air- dried at room temperature. 20g of the dried samples were used to determine the total hydrocarbon concentration by Spectrophotometer method without silica gel.

RESULTS

Impact on Species Richness

At the end of the study, a total of 24 taxa of soil microarthropods that belong to three orders; Cryptostigmata, Mesostigmata and Collembola were collected from all the habitat-types. Nine and seven taxa out of the total were collected from 2-litre and 4-litre contaminated habitat-types while 21 and 18 taken from 2-litre and 4-litre remediated habitat-types respectively (Table 1).

Table 1: Species Richness of Soil Microarthropods at the End of the Study

	OL	2L	4L	2L+SMS		4L+SMS
CRYPTOSTIGMATA						
<i>Annecticarus</i> sp		+	+	+	+	+
<i>Archegozettes magnus</i> +		+	-		+	+
<i>Belba</i> sp		+	-		+	+
<i>Bicrythermannia nigeriana</i>			+	+	+	+
<i>Cephalid</i> sp	+	-	-	+		-
<i>Galumna</i> sp	+	+	+	+		+
<i>Mixacarus</i> sp	+	-	-	+		+
<i>Nothrus incavatus</i>		+	-	-	+	+
<i>Nothrus ifeansis</i>		+	-	-	+	-
<i>Oppia</i> sp		+	-	-	+	+
<i>Paranothrus nigeriensis</i>		+	-	-	+	+
<i>Scheloribates</i> sp		+	+	+	+	+
SUB-TOTAL	13	5	4		13	11
MESOSTIGMATA						
<i>Macrochelid</i> sp		+	-	-	+	+
<i>Parasitid</i> sp	+	-	-	+		+
<i>Pergamasus</i> sp		+	-		-	-
<i>Prodinichid</i> sp	+	-	-	-		-
<i>Rhodacarus</i> sp		+	+	+	+	+
<i>Uropoda</i> sp	+	+	-	-		-
<i>Trachyuropoda</i> sp		+	+	-	+	-
<i>Polyaspid</i> sp	+	-	-	+		+
<i>Gamisina</i> sp	+	-	-	+		+
SUB-TOTAL	8	2	1	5		4
COLLEMBOLA						
<i>Paronella</i> sp	+	+	+	+		+
<i>Hypogastura</i> sp		+	+	+	+	+
<i>Isotomurus palustris</i>		+	-	-	+	+
SUB-TOTAL	3	2	2	3		3
Grand total = 896		24	9	7	21	18

Legend: + Species present, - species absent

Impact on Species Abundance

A total 896 individual soil microarthropods were collected from all habitat-types at the end of the study. Out of these, 285 representing 31.6% was collected from the uncontaminated (control) habitat-type, 76 from 2-litre representing 8.4% contaminated, 57 representing 6.3% from 4-litre contaminated habitat-type. Individual soil microarthropods collected from the spent mushroom substrate remediated habitat-types were 289 representing 32.9% from 2-litre remediated, and 189 representing 21% from 4-litre remediated habitat-types (Table 2).

Table 2: Species Abundance of Soil Microarthropods at the End of Study

		OL	2L	4L	2L+SMS		4L+SMS
TOTAL							
CRYPTOSTIGMATA							
Annecticus sp		10	8	12	14	15	59
Archegozettes magnus		10	3	-	9	4	26
Belba sp		16	-	-	12	7	35
Bicrythermannia nigeriana		37	14	16	28	27	
Cephalid sp	14	-	-	2	-		16
Galumna sp	19	12	9	16	13		69
Mixacarus sp	20	-	-	12	13		45
Nothrus incarates		14	-	-	17	14	45
Nothrus ifeansis		5	-	-	6	-	11
Oppia sp		7	-	-	25	21	53
Paranothrus nigeriensis		7	-	-	14	8	29
Scheloribates sp		45	13	6	28	12	104
SUB-TOTAL	204	50	43	183	134		614
MESOSTIGMATA							
Macrochelid sp		11	-	-	10		16
Parasitid sp	13	-	-	29	16		58
Rhodacarus sp		11	8	5	22		9
Uropoda sp	11	6	-	8	-		25
SUB-TOTAL	46	14	5	69	41		175
COLLEMBOLA							
Paronella sp	22	9	5	16	2		54
Hypogastura sp		7	3	4	9	6	29
Isotomurus palustris	6	-	-	12	6		24
SUB-TOTAL	35	12	9	37	14		107

Grand total = 896

The result showed five species (taxa) whose individuals did not exceed five and as a result this low number of individuals were not included in the total population and not considered relevant in the statistical analyses. These species are *Perganasus* sp; *Prodinichid* sp, *Trachyuropoda* sp, *Polyaspid* sp, and *Gamisina* sp. However, other species collected in descending order of abundance were *Bicrythermannia nigeriana* (122) *Scheloribates yorubaensis* (104), *Galumna nigeriana* (69), *Annecticus* sp (59) and *Parasitid* sp (58).

The mean abundance of soil microarthropods collected from habitat-types showed that the total mean abundance of individuals' was 19.0 in 2-litre contaminated, 72.2 in 2-litre remediated, and 14.256 in 4-litre contaminated, and 47.25 in 4-litre remediated habitat-types (Table 3).

Table 3: Mean Abundance of Soil Microarthropods at each replicate of the Habitat-types at the end of the study.

Habitat-types	Replicates				TOTAL
	I	II	III	IV	
O-L (Control)	22.25	14.75	17	17.25	71.25
2-L contaminated	7.5	5	2.5	4	19
4-L contaminated	3.75	3.75	4.25	2.5	14.25
2L+SMS remediated	14.5	18.5	14	25.5	72.5
4L+SMS remediated	8	9.5	15.25	14.5	47.25
Total	56	51.5	53	63.75	224.25

L= Litre of spent automobile lubricant SMS= Spent mushroom substrate.

Impact on Vertical Distribution of Species

In this study, out of the 71.25 mean abundance individuals collected from the uncontaminated habitat-types, 53.75 were distributed at 0-10cm depth range. In the spent automobile lubricant contaminated habitat-types, a mean abundance of 12.0 and 7.25 were collected from 2-litre and 4-litre habitat-types respectively at that same depth range of 0-10cm. In the spent mushroom substrate remediated habitat-types, a mean abundance distribution of soil microarthropods showed that 42.0 was collected from 2-litre remediated habitat-types and 27.0 from 4-litre remediated habitat-types at the same depth range of 0-10cm (Table 4).

Table 4: Mean vertical distribution of soil microarthropods in contaminated and remediated habitat-types.

DEPTH (cm)	TREATMENTS				
	0L	2L	4L	2L+SMS	4L+SMS
0-10	53.75	12.0	7.25	42.0	27.0
10-20	17.5	7.0	7.0	30.25	20.25
TOTAL	71.25	19.0	14.25	72.25	47.25

Legend: L= volume of spent automobile lubricating oil in liters, SMS= Spent Mushroom Substrate

Impact on Total Hydrocarbon Content

The concentration of total hydrocarbon was 12.1mg/kg in the 2-litre contaminated habitat-types and 13.1 in the 4-litre contaminated habitat-types; 4.8mg/kg at 2L remediated habitat-types and 6.4mg/kg at 4L remediated habitat-types (Table 5).

Table 5: Concentration of Total Hydrocarbon in the habitat-types

Habitat-types	Total THC values (mg/kg)
Control	3.9
2L-contaminated	12.1
4L-contaminated	13.1
2L+SMS	4.8
4L+SMS	6.4

DISCUSSION

This result showed a decrease in richness of species from the control to the contaminated habitat-types indicating the severity of the spent automobile lubricant, but in the treated or remediated habitat-types (2-L and 4-L plus substrate) an increase in species richness occurred. The increase is an indication of the impact of spent mushroom substrate, which was more in the 2-litre – substrate habitat-types.

It decreased by 15 species representing 62.5% of the total habitat-type and increased by 12 species at the 2-litre-remediated habitat-type (Table 1). A similar observation was also recorded at the 4-litre contaminated and its remediated habitat-types. It decreased by 17 species at the 4-litre contaminated habitat-type.

Out of the 15 species that were absent in the 2-litre contaminated habitat-type, 12 species; *Paranollonothrus nigeriensis*, *Parasitid*, *Macrochelid sp*, *Polyaspid*, *Gamisina*, *Isotomurus palustris*, *Oppia*, *Belba*, *Nothrus incavatus*, *Cephalid*, *Mixacarus* and *Nothrus ifeansis* occurred in the 2-litre spent mushroom substrate remediated habitat-type representing 80% of the recovered species. A similar observation was made in the 4-litre spent automobile lubricant and 4-litre spent mushroom substrate habitat-types. The 15 species are very sensitive to the spent automobile lubricant. They were not able to tolerate the contaminant. Out of this 15 species, only 12 were able to recover in the remediated habitat-type indicating that 3 taxa were highly sensitive, and absent in the 4-litre spent mushroom habitat-types. The 3 taxa are *Cephalid*, *N. ifeansis*, and *Uropoda*. They are indicator species of the environment polluted with spent automobile lubricant. Seven other taxa; *Annectarus*, *G. nigeriensis*, *B. nigeriana*, *S.yorubaensis*, *Rhodacarus*, *Paronella* and *H. brevis* occurred in all habitat-types. These taxa are good candidates for monitoring changes in the ecosystem caused by spent automobile lubricant as they could tolerate the contaminant.

The mean abundance of soil microarthropods collected from all habitat-types showed that when the habitat-types were remediated there was an increase in the total mean abundance of individuals from contaminated to remediated habitat-types. The impact of the remediating potentials of spent mushroom substrate was observed in the reduction of total hydrocarbon concentration of the soil. This reduction enhances the recovery of the ecosystem and consequently increases species richness and abundance in the remediated habitat. The reduction in total hydrocarbon concentration decreased the toxicity effect of the spent automobile lubricant. After 10 weeks of post-remediation, for instance, a reduction of 43.2% (7.3mg/kg) in THC was recorded in 2-litre remediated habitat-types. The reduction in THC in this study is caused by both the activities of soil microarthropods and spent mushroom substrate. The soil microarthropods contain certain detoxification mechanism which breaks down hydrocarbon compounds contained in the spent automobile lubricant. These mechanisms confer some level of resistance to the microarthropods.

The soil microarthropods are indigenous to the impacted habitat-types and due to their resistance tolerated the contaminant. These species includes those that occurred in all the habitat-types of this study. They have the ability to naturally biodegrade spent automobile lubricant and withstand the toxicity of the contaminant. This ability may be adduced to the fact that some of the species have high levels of oxidases in their microsomes and could detoxify hydrocarbons by microsomal mixed function oxidases such as insect does. It could also be suggested that the hard cuticle of some species such as *Galumna* and *Scheloribates* probably decreased permeability of the cuticle and led to slower penetration of the contaminant. Some

of these species are referred to as monitor-species (Okiwelu, *et al*, 2011) as they can be used to monitor changes in the soil ecosystem and recovery of impacted ecosystem (Gbarakoro *et al*, 2010) from petroleum oil contaminant. These species are *Annecticarus*, *Galumna*, *Bicrythermannia nigeriana*, *Scheloribates yorubaensis*, *Rhodacarus*, *Paronella* and *Hypogastura*.

The abundance of these monitor species in the contaminated habitat-types was lower than their abundance in remediated habitat-types. It increased from 124 individuals in contaminated habitat-types to 217 in the remediated habitat-types.

The biodegrading activities of the monitor species are responsible for the abundance recorded at the contaminated habitat-types, indicating that they are participants in natural attenuation. In the remediated habitat-type, spent mushroom substrate is involved where the substrate biostimulated soil microarthropods and increased their efficacy in degrading hydrocarbon especially as they are naturally good bioremediators. This agrees with the report of Thieman and Palladino, (2009). This increase in abundance caused by the substrate was also recorded with the total mean abundance. It was observed that though the number of species collected from the control habitat-type was higher than those recorded in the 2-litre remediated habitat-type, total mean abundance in both habitat-types was nearly the same. The species whose abundance did not exceed five individuals is an indication that they are getting out of the ecosystem and could not be recovered by the mushroom substrate within the duration of study.

The higher abundance of soil microarthropods recorded at the upper 10.0cm depth (0-10cm) at the remediated habitat-types is an indication of the organisms' recovery of its normal soil profile. This is enhanced by the spent mushroom substrate.

Statistical analysis of the data obtained in the study showed that a significant difference existed between abundance in the habitat-types. There was a significant difference between 2l lubricant and 2l spent mushroom substrate habitat-types ($F= 0.05$; $P=0.008$), while no significant difference was recorded between the control and 2l spent mushroom substrate habitat-types.

IMPLICATIONS TO RESEARCH AND PRACTICE

This research implies that spent mushroom substrate when added to soil polluted with hydrocarbon petroleum products as a remediating agent could amend the soil and restore soil inhabiting organisms, especially, soil microarthropods. Furthermore, soil microarthropods are participants in bioremediation of hydrocarbon polluted soils, showing indicator species.

CONCLUSION

Spent mushroom substrates have impacts on hydrocarbon petroleum polluted soils, such as reduction in THC, increase in species richness and density and restoration of polluted ecosystems.

FUTURE RESEARCH

To investigate the remediation potentials of spent mushroom substrate on soil nutrients in hydrocarbon petroleum polluted soils.

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