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Ecological risk assessment of heavy metals and microbiological diversity of soil samples from some automobile workshops in Benin City, Nigeria

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Article info:
Received: 30 January 2025
Accepted: 19 September 2025

ABSTRACT

The indiscriminate discharge of waste engine oil arising from the preponderance of auto-mechanic workshops is known to compromise the soil health and environmental quality. This study investigated the pollution status of heavy metals and microbiological diversity of waste engine oil contaminated soils in Benin City. Standard laboratory methods were used in the study. The physicochemical parameters recorded were sandy loam, pH (6.8 - 7.9), electrical conductivity (1.9 - 5.4 uS/cm), sulphate (42.51 - 68.45 mg/kg), Nitrate (1.08-2.10mg/kg), Phosphate (2.60- 4.92 mg/kg), Calcium (0.32 -0.64mg/kg), Magnesium (0.17- 0.28mg/kg), Sodium (0.11-0.14 mg/kg) Potassium (0.10-0.18mg/kg), Zinc (31.92- 68.40mg/kg), Copper (9.21-13.65mg/kg), Chromium (0.23 -0.40mg/kg), Lead (2.40 - 6.20mg/kg), Manganese (32.92-51.70mg/kg), Iron (826.24 — 1012.4mg/kg), Nickel (2.11 — 3.10mg/kg) and Cadmium (1.98-3.10mg/kg). The total heterotrophic bacterial counts of soils samples from location A had the highest of $8.61 x 10^4$cfu/g while location E had the least of $1.12 x 10^4$cfu/g. location B had the highest fungal count of $8.04 x 10^3$cfu/g while location C had the least of $1.11 x 10^3$cfu/g. The bacterial and fungi isolated were *Bacillus*, *Pseudomonas*, *Micrococcus*, *Clostridium*, *Corynebacterium*, *Penicillium*, *Fusarium*, *Aspergillus*, *Trichoderma* and *Geotrichum* species. The pollution indices indicated that iron had the highest contamination factor of 8.88 while Chromium had the least of 0.87. The geo-accumulation index showed that cadmium had the highest of 0.76 while iron had the least. The Quantification of concentration revealed that chromium had the highest of 90.32 while nickel had the least of 57.25. The waste engine oil polluted soil revealed unpolluted to moderately polluted status. This study recommends proper disposal of waste engine oil thereby safeguarding soil health and environmental quality.

Key words: soil contamination, heavy metals, waste engine oil, public health, soil health

Introduction

Engine oil being one of the products from crude oil refining is utilized in automobile for running of engines, powering plants and in preventing corrosion of metal parts and equipment. It is made up of straight and branched chain hydrocarbons like polycyclic aromatic hydrocarbons (PAHs) which is toxigenic, mutagenic, and carcinogenic (Guarino *et al.*, 2017). The preponderance of automobile workshops and the replacement of waste engine oil at such workshops are known to contribute hugely to waste engine oil contamination in the soil environment (Enerijofi *et al.*, 2020). Internal combustion engines (ICE) employ lubricating oil, which when occasionally discharged from the engine during vehicle maintenance is referred to as waste engine oil (Jain & Singh, 2011). The breakdown of additives and pollutants from combustion byproducts, contaminants from metal addition (wear and tear of engines), and byproducts of combustion causes lubricating oils chemical composition to change throughout usage in ICE. As a result, wasted engine oil contains a combination of heavy metals, additives, chlorinated biphenyls, chlorodibenzofurans, and petroleum hydrocarbons, particularly polyaromatic hydrocarbons (PAH) including benz(a)anthracene, benz(a)pyrene, and fluoranthene (Adebusoye *et al.*,2007).

One of nature's most valuable resources is soil which support plant growth, and all forms of life (Alsabhan *et al.*, 2022). Soil is known to be the storehouse for nutrients and moisture which support the growth and proliferation of different microorganisms and plants (Enerijiofi & Ekhaise,

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2019; Enerijiofi & Ikhajiagbe, 2021). Waste engine oil also contains metals like arsenic, zinc, cadmium and other possibly noxious substances that can leach via various soil layers to pollute ground water, air, and soil thereby attracting public attention. (Rahman & Singh, 2019). The pollutants do not disappear during the refinement or reprocessing of used crude oil; instead, they bio accumulate as waste sludge. These impurities make the greasy sludge extremely poisonous. In Nigeria, according to Enerijiofi et al. (2020), it is almost a norm to release waste engine oil indiscriminately into channels, water drains, open unoccupied areas and farmlands. A wide variety of microorganisms, in particular the indigenous bacteria found in soil, aid in the microbial breakdown of hydrocarbon contaminated sites. *Pseudomonas* strains capable of breaking down poly aromatic hydrocarbons have been found in considerable numbers in soil and aquifers. *Bacillus, Micrococcus, Alcaligenes*, *Flavobacterium, Corynebacterium* and *Streptococcus* species are other bacteria that break down petroleum hydrocarbons (Ajuzie, *et al.,* 2015). Fungi and other creatures are also somewhat capable of decomposing the hydrocarbons in waste motor oil. Shittu and Ogor, (2018) posited that the addition of nutrients lacking in the environment such as nitrogen and phosphorus, can speed up the natural biodegradation process. Also, bio-augmentation which involves the introduction of potent microorganisms with high biodegradable capacities to oxidize petroleum products for cell growth is also a useful technique that ensures a sustainable environment. However, some factors such as the site's microbial concentration, soil type, oxygen, nutrient availability, temperature, physical & chemical properties and size of the oil surface play significant roles in biodegradation. Therefore, the capacity to create, sustain and optimize environmental factors that can improve waste engine oil breakdown is crucial in the realization of waste engine oil biodegradation knowhow. When used, engine oil can pick up a number of toxins that are dangerous to both the surrounding and public health (Enerijiofi *et al.,* 2020). The discriminate and indiscriminate disposal methods of waste engine oil, from an ecological and economic standpoint, are undesirable because of the public health implications. Therefore, this work is aimed at assessing the ecological risk assessment of heavy metals and microbial diversity of waste engine oil tainted soil samples from some automobile workshops in Benin City, Nigeria.

Materials and Methods

Area of study and sample collection

Waste engine oil samples were collected from (5) different automobile workshops in Benin City. The locations were Lucky Igbinedion Way, Ikpoba-Okha Local Government Area (6.37558° N, 566020° E); Ramat Park, Ikpoba-Okha Local Government Area (6.37586° N, 5.65998° E); First East Circular, Oredo Local Government Area (6.34096° N, 5.63501° E); New Benin Market, Oredo Local Government Area (6.34970° N, 5.63105° E) and opposite the University of Benin, Ovia North East (6.40376° N, 5.60803° E). The metal scraps and other visible particles were removed from the surface of the soil. Thereafter, Auger was used to collect soil samples at a depth of between 0 - 15cm and emptied into well labelled polythene bags. The soil samples were transferred to the laboratory for physicochemical, bacterial and fungal analyses using standard laboratory methods.

Determination of physiochemical parameters

The polluted soils were air dried in the laboratory at room temperature (25^{o}C), creased in a porcelain mortar, and sieved using a 2mm mesh steel sieve. Ten grams (10g) of the soil samples were measured and emptied into polythene bags for analyses according to protocols (AOAC, 2005; Enerijiofi & Ekhaise, 2019).

The pH and electrical conductivity

They were determined by measuring 20 grams of the air-dried soil samples and emptying them into 20 milliliters of distilled water in a beaker. The mixture was swirled continuously for 30 minutes with a glass stirrer. Thereafter, the pH and Electrical conductivity meter electrode was introduced into the mixture and recorded when the reading was constant.

Determination of metals and heavy metals

Two grams of the soil samples were placed into a conical flask and broken down prior to analysis. The digestate were processed using a freshly made solution of HNO_3: HCL (3:1) and allowed to cool. Twenty-five milliliters (25mls) of distilled water was added to the mixture, placed in a water bath and heated for 20 minutes till a milky colour developed. Thereafter, they were removed from heat and left to cool. The digested solutions were filtered through a 110 mm Whatman filter paper into 50ml sample bottles and distilled water was added to bring the volume to 25mls. The concentrations of metals, sodium, potassium, calcium and magnesium were determined directly using flame photometer, Jenway, model PFP7 while the concentrations of the heavy metals were also determined directly using Atomic Absorption Spectrophotometer (AAS), model PG 550 model buck scientific, 210VGP with the use of air acetylene flame as the carrier gas (Akpoveta *et al.,* 2010).

Assessment of the impact of waste engine oil on the receiving soil

Three indices as explained below were used to access the impact of heavy metals in waste engine oil contaminated soil on the receiving soil environment (Enerijiofi & Obade, 2018).

(i) Contamination factor (Cf)

$$C_f = \frac{C^i_{0-1}}{C^i_n}$$

C^i_{0-1}: mean metals concentration from a minimum of 5 polluted samples and C^i_n is the metal concentration in the control soil.

(ii) Geo-accumulation index (I-geo)

$$I - geo = \log\frac{C_n}{1.5 \times B_n}$$

Cn: concentration of heavy metals in polluted soil samples and Bn: heavy metals concentrations in the unpolluted (control) sample. The factor1.5 is introduced to reduce the consequence of likely deviations in the control

(iii) Quantification of Concentration (QoC)

$$Q_oC = \frac{X - X_c}{X} \times 100\%$$

X: metal concentration in the polluted soil sample and X_c: heavy metal concentrations in the control.

Bacterial and Fungal analyses

One gram (1g) of waste engine oil contaminated soil was measured and emptied into a sterile beaker containing 9 mls of distilled water. Thereafter, 1ml of 10^{-2}, 10^{-5} and10^{-8} diluents were inoculated into nutrient and potato dextrose agars plates respectively for bacterial and fungal isolation. Nystatin was added to the nutrient agar plates to prevent fungal growth while streptomycin was added to the potato dextrose agar plates to inhibit bacterial growth (Enerijiofi *et al*., 2022). The inoculated plates were incubated at 37^0C for 24 hours and at room temperature of 25^oC for 72hours for bacterial and fungal enumeration respectively. The distinct bacterial and fungal isolates were identified phenotypically following the standard methods of Holt *et al.* (1994) and Barnett and Hunter (1972) respectively.

Results

Soil samples from locations A, B, C and E were sandy while location D was Loamy. The concentration of physiochemical parameters of the waste engine oil contaminated soil samples recorded had pH (6.8 - 7.9), Total organic carbon (3.4 - 8.2%), electrical conductivity (1.9 - 5.4 uS/cm), total hydrocarbon content (1362.7 - 1912.5), SO_4^{2-} (42.51 - 68.45 mg/kg), PO_4^{2-} (2.60 - 4.92 mg/kg) NO_3^- (1.08 - 2.10 mg/kg (Table 1). The concentration of macro elements revealed sodium (0.11 - 0.14 mg/kg), potassium (0.10 - 0.18 mg/kg), calcium (0.32 - 0.64 mg/kg) and magnesium (0.17 - 0.28 mg/kg). The heavy metals concentrations recorded had copper (9.21 - 13.65 mg/kg), zinc (31.92 - 68.40 mg/kg), iron (826.24 - 1012.4 mg/kg), manganese (32.92 - 51.70 mg/kg), cadmium (1.98 - 3.10 mg/kg), chromium (0.23 - 0.40 mg/kg), nickel (2.11 - 3.10 mg/kg), lead (2.40 - 6.20 mg/kg) (Table 2). The pollution indices indicate that iron had the highest contamination factor of 8.88 while chromium had the least of 0.87. The geo-accumulation index showed that cadmium had the highest of 0.76 while Iron had the least. The quantification of concentration revealed that chromium had the highest of 90.32% while nickel had the least of 57.25% (Tables 3 and 4). The bacterial and fungal diversity analyses of soil samples showed the total heterotrophic bacterial and fungi counts. Soils from location A had the highest of 8.61 x 10^4cfu/g while location E had the least of 1.12x 10^4cfu/g. Location B had the highest fungi count of 8.04 x 10^3 cfu/g, while location C had the least fungal count of 1.11x10^3cfu/g. *Bacillus* sp. and *Pseudomonas* sp. were present in all the contaminated soil while *Pseudomonas* sp. was only isolated in the control soil sample. *Micrococcus* sp. was isolated in soil samples from locations A, C, D and E. *Corynebacterium* sp. was isolated in soil samples from locations A, D and E while *Clostridium* was isolated only in samples from location E. *Penicillium* sp. and *Fusarium* sp. were isolated in soil samples from all contaminated soils. *Aspergillus* sp. was isolated form locations B, C, D and E while *Trichoderma* sp. and *Geotrichum* sp. were isolated in soil samples from location E (Table 5).

Discussion

This study documented the ecological effect of indiscriminate waste engine oil disposal on the soils physiochemical & heavy metals concentration as well as the bacterial and fungal diversities. The waste engine oil polluted soils embody a mystifying habitat due to its nature and the presence of some heavy metals. The soils studied showed to be predominantly sand except samples from New Benin which was loamy. Sandy soils are light soils that are rapidly permeable, with reduced cation exchange capacity, low concentration of nutrient and poor potential to hold water. These aforementioned characteristics of sandy soils are key issues that need to be addressed. According to Patnaik et al. (2013), clay soil has a great capacity for cation exchange and pH buffering. The result revealed different concentrations of physicochemical parameters. The pH range was basic. This basic range had earlier been reported in previous studies to favour bacterial and fungi growth and aid in the easy movement of heavy metals in soils (Akpoveta et al., 2010 and Enerijiofi et al., 2020). The micronutrient solubility is highest at low pH values, while micronutrient availability to plants is lowest at high pH levels. The pH of the soil often increases with depth. The soils electrical conductivity is directly proportional to the concentrations of ions in soil. The concentrations of sulphate were far higher than phosphate and nitrate which corroborates with earlier report (Pande et al., 2022). According to Yennawar et al. (2013), the particle size, clay minerals, organic matter, and ground water quality have direct impact on the water content of soil.

http://www.jbb.uni-plovdiv.bg DOI: 10.69085/jbb20252137

Table 1. *Physicochemical parameters in soil samples from different locations.*

Heavy metals (mg/kg)	Location A	Location B	Location C	Location D	Location E	Control	FEPA limit (1991)
pH	7.6	7.3	7.6	6.8	7.9	6.9	50
TOC (%)	7.4	8.2	8.0	7.8	3.4	3.2	0.2
EC uS/cm	3.9	3.5	1.9	5.4	2.1	1.1	100
THC	1526.4	1912.5	1889.6	1698.1	1362.7	238.3	100
SO_4^{2-}(mg/kg)	56.70	42.51	48.31	51.90	68.45	23.47	1.5
PO_4^{2-}(mg/kg)	3.13	2.60	2.68	3.10	4.92	1.2	1.0
NO_3^-(mg/kg)	1.23	1.08	1.14	1.14	2.10	0.98	20
Sand (%)	80	80	75	50	80		
Silt (%)	10	10	20	40	15		
Clay (%)	10	10	5	10	5		
Soil class	Sandy loam	Sandy loam	Sandy loam	Loam	Sandy loam		

Legend: A = Igun Street, B = Lucky way, C = Ramat park, D = New Benin, E = Ugbowo

Table 2. *Heavy metals concentrations (mg/kg) in soil samples from different locations*.

Heavy metals	Location A	Location B	Location C	Location D	Location E	Control	FEPA limit (1991)
Na	0.11	0.09	0.10	0.10	0.14	0.07	50
K	0.12	0.10	0.11	0.12	0.18	0.04	0.2
Ca	0.40	0.32	0.36	0.39	0.64	0.11	100
Mg	0.19	0.17	0.18	0.18	0.28	0.14	100
Cu	9.81	13.65	12.80	12.10	9.21	4.57	1.5
Zn	48.90	68.40	62.62	59.14	31.92	22.41	1.0
Fe	830.51	1012.4	982.90	976.12	826.24	104.20	20
Mn	32.92	51.70	50.80	44.63	35.90	12.38	0.5
Cd	2.14	3.10	2.81	2.40	1.98	0.35	-
Cr	0.28	0.31	0.40	0.31	0.23	0.03	0.5
Ni	2.66	2.94	3.10	2.68	2.11	1.15	1.0
Pb	4.61	6.20	6.15	5.82	2.40	1.02	-

Legend: A = Igun Street, B = Lucky way, C = Ramat park, D = New Benin, E = Ugbowo

The heavy metals concentrations; chromium, iron, copper, zinc, nickel, cadmium, manganese and lead reported in this study were generally low. This implies that the soils receiving waste engine oils have some levels of heavy metal enrichments with iron and zinc playing essential role in microbial growth. This corresponds with the submission of Mgbemena *et al*. (2012) where they reported low heavy metals concentrations and also posited that low concentrations of heavy metals act as catalysts in speeding up chemical reactions. This means that zinc and iron are not as toxic as lead to microbial growth and development. The presence of lead principally from the deposition of incomplete combustion engines of automobiles and during repairs at the automobile workshops also contributed to its build-up. However, Momodu and Anyakora (2010) stated that lead poisoning could cause loss of appetite, severe kidney pain, constipation, insomnia as well as damage the brain due to exposure for a long time. It is far more worrisome that in children, some of the heavy metals reported could take the place of calcium during bone formation which leads to abnormalities during skeletal build-up (Enerijiofi and Ajuzie, 2012). The heavy metals pollution status showed unpolluted to moderately polluted status. The pollution status in soils expressed in terms L-geo index showed unpolluted to moderately polluted in the order cadmium > lead > nickel > copper > manganese > zinc > iron. All the values recorded for L-geo were within 0.02 to 0.76 and according to the standard in table 3 for pollution grade of geo-accumulation, the pollution status of the waste engine oil contaminated soils fell between unpolluted to moderately polluted status. The QoC trend followed the trend chromium > iron > cadmium > lead > manganese > copper > zinc > nickel, while the CF trend was iron > cadmium > lead > manganese > copper > zinc > nickel > chromium. However, despite the different impact assessment indices, the results revealed that the heavy metals contamination indicated anthropogenic inputs in the contaminated soils. There were some similarities in the trend of heavy metals; as cadmium and lead were among the top four heavy metals reported using the three methods of ecological risks assessments in the study. However, there were some inconsistencies despite the control which ensured data quality, reduce uncertainty and increased accuracy. These variations in the patterns of heavy metals contamination

Table 3. *Ecological risks assessment of heavy metals in contaminated soils.*

Soil parameters	CF	L-geo	QoC (%)	BC
Cu	2.51	0.16	60.29	4.57
Zn	2.42	0.05	58.65	22.41
Fe	8.88	0.02	88.74	104.20
Mn	3.45	0.09	71.34	12.38
Cd	7.10	0.76	85.94	0.35
Cr	0.87	-	90.32	0.03
Ni	2.35	0.25	57.25	1.15
Pb	4.94	0.46	79.76	1.02

Legend: CF = average contamination factors, L-geo = geo-accumulation index,
QoC = Quantification of concentration, BC = Background values

Table 4. *Pollution grade of geo-accumulation index.*

L-geo class	L-geo value	Pollution status
0	L-geo < 0	Unpolluted
1	0 < L-geo < 1	Unpolluted to moderately polluted
2	1 < L-geo < 2	Moderately polluted
3	2 < L-geo < 3	Moderately to heavily polluted
4	3 < L-geo < 4	Heavily polluted
5	4 < L-geo < 5	Heavily to extremely polluted
6	L-geo < 5	Extremely polluted

could be due to the different methods of assessment and variability in the soil sampled locations. It could also result from characteristics like pH, electrical conductivity and class of the different soil sampled in the study. The aforementioned are known to be responsible for influencing the mobility of heavy metals in soil environment as corroborated in earlier studies by Pande *et al.*, (2022) and Yennawar *et al.* (2013).

The heterotrophic bacterial counts ranged from 1.12x10^4 to 8.6x10^{4}cfu/g while the fungi counts ranged from 1.1x10^3 to 8.0x10^3 cfu/g. These counts could probably be due to the different nutrients arising from different organic matter. It could also be due to the low concentrations of heavy metals and other ecological factors that affect bacterial growth and proliferation. However, the relatively low counts recorded in waste engine oil contaminated soil samples could be due to the lethal or adverse effect of the waste engine oil contamination (Enerijiofi *et al.*, 2020, Pande *et al.*, 2022). In auto-mechanic workshops, there are always change in the microbial communities, bio diversities, colour and texture which arise from the discriminate and indiscriminate release of waste engine oil. The bacterial loads reported were far higher than the fungal loads in all sampled locations in the study except location B; Lucky way which the fungal count was higher than the bacterial. However, this study reported more of culturable gram-positive microorganisms like *Bacillus*, *Corynebacteria*, and *Micrococcus*. The bacterial *Bacillus*, *Pseudomonas*, *Corynebacteria* and *Aspergillus* species could also be used as bioremediation potentials to clean up waste engine oil contaminated environment. The data recorded revealed contamination due to anthropogenic activities of humans.

Conclusion and Recommendation

The result revealed that the waste engine oil contaminated soils contained some levels physicochemical and heavy metals concentration as well as varied bacterial and fungal counts and diversities above the control. Although, the heavy metals pollution status showed unpolluted to moderately polluted status, this may cause serious environmental and public health risks to the teeming citizens if the contamination continues unabated with time. However, there is need for educational campaigns on waste disposal and recycling by concerned authorities with the active

Table 5. *Bacterial and fungal diversity analyses of soil samples.*

Sampled locations	THBC (x10^4 cfu/g)	THFC (x10^3 cfu/g)	Bacterial and fungal diversity
A	8.61	1.33	*Bacillus* sp., *Pseudomonas* sp., *Micrococcus* sp., *Corynebacterium* sp., *Penicillium* sp., *Fusarium* sp.
B	6.83	8.04	*Bacillus* sp., *Pseudomonas* sp., *Penicillium* sp., *Fusarium* sp., *Aspergillus niger*.
C	7.24	1.11	*Bacillus* sp., *Pseudomonas* sp., *Micrococcus* sp., *Penicillium* sp., *Fusarium* sp., *Aspergillus niger*
D	8.11	1.16	*Bacillus* sp., *Pseudomonas* sp., *Micrococcus* sp., *Corynebacterium* sp., *Penicillium* sp., *Fusarium* sp., *Aspergillus niger*
E	6.12	5.62	*Bacillus* sp., *Pseudomonas* sp., *Micrococcus* sp., *Clostridium* sp., *Corynebacterium* sp., *Penicillium* sp., *Fusarium* sp., *Aspergillus niger*, *Trichoderma* sp., *Geotrichum* sp.
Control	3.17	3.22	*Pseudomonas* sp., *Aspergillus niger*, *Penicillium* sp.

Legend: THBC: Total heterotrophic bacterial count, THFC: Total heterotrophic fungal count, A = Igun, B = Lucky way, C = Ramat park, D = New Benin, E = Ugbowo participation of individuals and local communities.

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