
Microbial and Physicochemical Responses to Spent Engine Oil Contamination at Varying Chronological Intervals in Mechanic Workshops

Chigozie Nwagwu , [Emmanuel O. Fenibo](#)*, Juliana O. Pondei , Clement C. Ukanwa , Herbert O. Stanley

Posted Date: 27 February 2026

doi: 10.20944/preprints202602.1971.v1

Keywords: bacterial biomass shift; environmental safety; physicochemical parameters; public health; soil pollution



Preprints.org is a free multidisciplinary platform providing preprint service that is dedicated to making early versions of research outputs permanently available and citable. Preprints posted at Preprints.org appear in Web of Science, Crossref, Google Scholar, Scilit, Europe PMC.

Copyright: This open access article is published under a [Creative Commons CC BY 4.0 license](#), which permit the free download, distribution, and reuse, provided that the author and preprint are cited in any reuse.

Disclaimer/Publisher's Note: The statements, opinions, and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.

Article

Microbial and Physicochemical Responses to Spent engine Oil Contamination at varying Chronological Intervals in Mechanic Workshops

Chigozie Nwagwu¹; Emmanuel O. Fenibo^{2*}; Juliana O. Pondei³; Clement C. Ukanwa⁴; Herbert O. Stanley¹

¹ Department of Microbiology, Faculty of Science, University of Port Harcourt, Nigeria

² Department of Microbiology, Hensard University, Toru Orua, Sagbama, Bayelsa, State, Nigeria

³ Department of Microbiology, University of Abuja, Nigeria

⁴ Department of Microbiology, Madonna University, Elele, Rivers State, Nigeria

* Correspondence: fenibo1478@gmail.com

Abstract

Spent engine oil (SEO) contamination and its implications for soil ecosystems are a growing global concern. This study investigates the trend of SEO pollution across four mechanic workshops, aiming to understand the impact of pollutants on soil physicochemical and microbiological parameters. Standard cultural methods were employed to determine the biomass of total heterotrophic bacteria (THB) and hydrocarbon-utilising bacteria (HUB), while chromatographic (GC-MS) methods were used to assess total petroleum hydrocarbons (TPH) and polyaromatic hydrocarbons (PAH). Additionally, atomic absorption spectroscopy (AAS) was used to quantify heavy metal concentrations in the soil. The results indicated that SEO contamination increases levels of TPH, PAHs, total heavy metals, and organic carbon, while reducing soil pH and total organic nitrogen. A correlation study revealed that hydrocarbon pollution negatively impacted total heterotrophic bacteria but stimulated the growth of hydrocarbon-utilising bacteria (HUB). Furthermore, soils polluted for 20 years acted as a sink for heavy metals, PAHs, and TPH, contributing to their accumulation in the soil. This long-term accumulation poses a significant health risk, especially in environments where exposure to such pollutants is persistent. The findings highlight the need for active measures to monitor and remediate SEO-contaminated soils. Effective pollution management strategies are essential to mitigate potential health risks and restore soil health in affected areas, particularly in mechanic workshops that have experienced prolonged contamination.

Keywords: bacterial biomass shift; environmental safety; physicochemical parameters; public health; soil pollution

1. Introduction

Engine oil, essential for lubricating engines in vehicles, motorcycles, generators, and machinery, undergoes significant chemical changes during use. Over time, it degrades, accumulating combustion by-products, wear metals, and additives, resulting in spent engine oil, a complex mixture of paraffinic, naphthenic, and aromatic hydrocarbons (Sazzad et al., 2024). This waste also contains hazardous substances like carbon deposits, sludge, metallic salts, solvents, glycols, antifoaming agents, and water-in-oil emulsions. Particularly concerning are polycyclic aromatic hydrocarbons (PAHs), which are persistent, bioaccumulative, and toxic, posing serious ecological and health risks (Abdel-Shafy & Mansour, 2016). Sazzad et al. (2024) reported that globally, over 5.2 billion gallons of waste engine oil are generated annually, yet only 25–45% is properly recycled. The rest is often dumped or misused, leading to widespread pollution. Spent engine oil forms a hydrophobic layer on

the soil surface, significantly reducing water retention and aeration while creating a water-repellent barrier that impedes water infiltration (Armioni et al., 2024). This barrier reduces soil permeability and compacts the soil, damaging its physical structure and hindering its ability to support plant growth. The buildup of toxic substances from spent oil, including hydrocarbons, metal salts, and solvents, severely hampers root development and nutrient uptake, often resulting in stunted growth, yellowing leaves, and, in severe cases, plant death. Contamination also disrupts the availability of essential nutrients such as nitrogen, phosphorus, and potassium, mainly by inhibiting microbial activity responsible for nutrient cycling (Rashid et al., 2023). Toxic compounds such as polycyclic aromatic hydrocarbons (PAHs) and heavy metals can be absorbed by plants and soil organisms, enter the food chain, and bioaccumulate in animals and humans. PAHs, in particular, are carcinogenic and mutagenic, posing serious health risks such as increased cancer rates and genetic damage (Fenibo et al., 2024a). Additionally, spent engine oil adversely affects soil biodiversity.

Organisms such as earthworms, which are vital for aeration and decomposition of organic matter, are highly sensitive to oil contamination. Their decline further compromises soil health and ecosystem functions. Contaminants from spent oil can also leach into groundwater, creating a pathway for toxic exposure through drinking water sources and extending environmental and public health risks beyond the pollution site. Economically, improper disposal results in costly soil and water cleanup, regulatory fines, and resource loss. These impacts emphasise the urgent need for responsible management and recycling of spent engine oil to safeguard environmental and public health.

Spent engine oil (SEO) contamination significantly affects both the microbiological and physicochemical properties of soil. Microbiologically, SEO can stimulate the growth of hydrocarbon-degrading bacteria, such as *Pseudomonas* and *Bacillus*, which break down the oil (Monteiro, 2025). However, excessive contamination can overwhelm microbial populations, reducing microbial diversity and activity. High levels of SEO can create toxic conditions for beneficial microorganisms, thereby reducing soil fertility. Additionally, SEO contamination promotes the growth of fungi and actinomycetes capable of hydrocarbon degradation, but this alters the natural microbial balance, potentially destabilising the soil ecosystem (P. Liu et al., 2025). Physicochemically, SEO contamination can lead to soil acidification. The breakdown of hydrocarbons and the accumulation of heavy metals, such as lead, cadmium, and nickel, lower soil pH, making essential nutrients, such as phosphorus and nitrogen, less available to plants (Angon et al., 2024). SEO also disrupts the nitrogen cycle by inhibiting nitrification and impacting nitrogen-fixing bacteria, leading to nitrogen depletion (Z. Wang et al., 2025). Additionally, SEO can reduce phosphorus availability by promoting its conversion into insoluble compounds through soil acidification (Mohanta et al., 2024a). Prolonged contamination also depletes soil organic carbon, as microbial activity is hindered, negatively impacting soil fertility, moisture retention, and structure. The presence of heavy metals further harms soil quality, reducing microbial diversity and disrupting plant growth.

Although the existing public literature extensively examines the effects of spent engine oil (SEO) on soil physicochemical and biological characteristics, this study builds on that foundation by investigating the influence of contamination duration on those effects. The specific objectives of this research are to: (i) evaluate the effects of SEO on soil physicochemical properties; and (ii) examine its impact on soil bacterial biomass, with particular emphasis on (iia) total heterotrophic bacteria and (iib) hydrocarbon-utilising bacteria in contaminated soils. The outcomes of this study are anticipated to offer critical insights into the ecological risks posed by SEO pollution and its broader implications for public health and environmental safety.

2. Materials and Methods

2.1. Sample Collection

Soil samples contaminated with spent engine oil, along with corresponding control samples, were collected from selected automobile workshops in Choba, Rivers State, Nigeria (Fig. 1). Sampling was conducted using a sterile hand auger at two depths: 0–15 cm (topsoil) and 15–30 cm (subsurface), to capture both surface and subsurface layers potentially impacted by hydrocarbon infiltration. Three replicate samples were collected at each depth per site, then homogenised to create composite

samples, reducing spatial variability. Four sampling sites were chosen based on workshop operational duration: EOPS 1 from a mechanic workshop in Choba operating for 1 year, EOPS 2 from a workshop in operation for 5 years, EOPS 3 from a workshop in operation for 10 years, and EOPS 4 from a workshop that had been operating for 20 years. Control samples were collected from uncontaminated soils approximately 10 meters (relative to a terminal pollution point) from each workshop, in areas free of visible oil contamination or mechanical activity, ensuring minimal anthropogenic impact. All samples were placed in sterile, labelled polyethene bags, transported in ice-cooled containers, and stored at 4°C until analysis. To maintain sample integrity, processing was carried out within 48 hours of collection to minimise changes in physicochemical and microbiological properties.

2.2. Physicochemical Analysis

Soil samples were characterised for physicochemical attributes, including total petroleum hydrocarbons (TPH), polycyclic aromatic hydrocarbons (PAHs), total organic nitrogen (TON), total organic carbon (TOC), electrical conductivity, pH, and trace metal concentrations of copper, chromium, and iron.

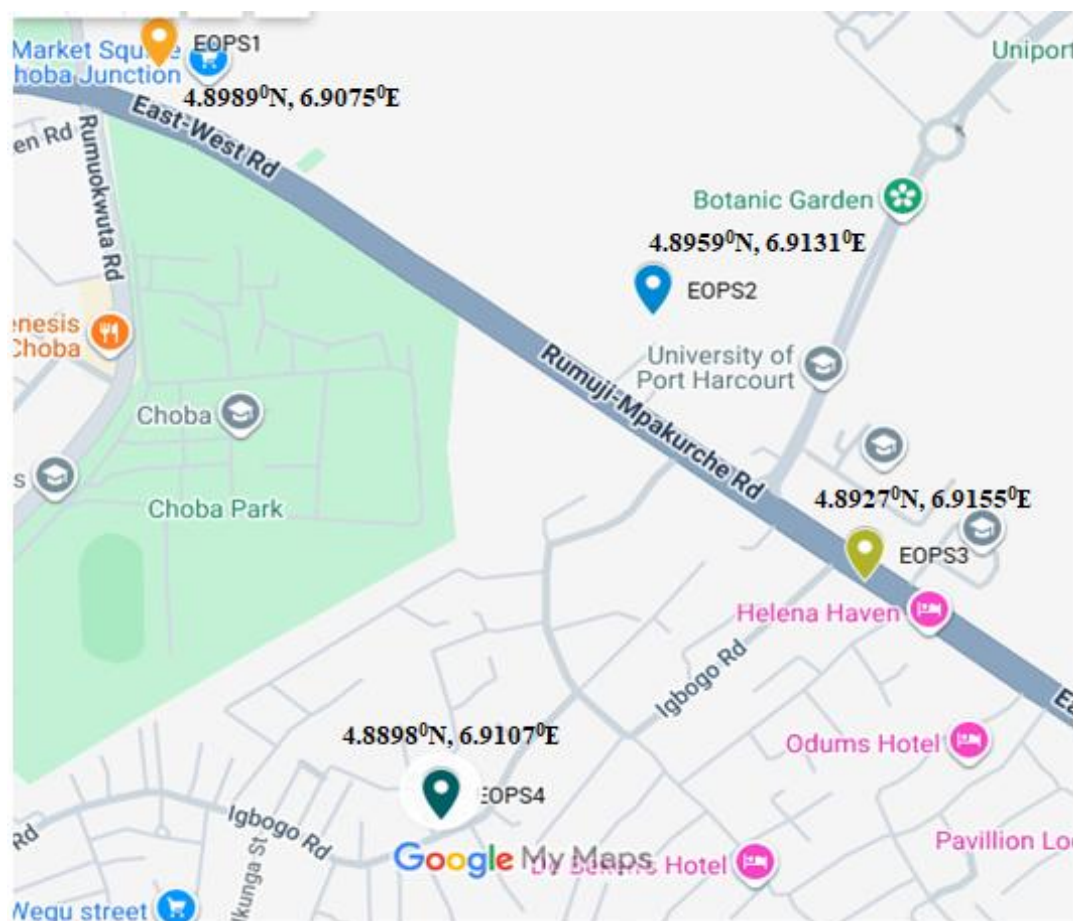


Figure 1. Map of the study site (Adapted from Google Map).

2.2.1. pH and electrical Conductivity Determination

Soil pH was determined using a calibrated digital pH meter. For each measurement, soil samples were suspended in distilled water, thoroughly mixed, and allowed to equilibrate. The meter was calibrated prior to use with standard buffer solutions (pH 4.0 and 10.0) according to the method of Dudala et al. (2020). Calibration accuracy was verified by measuring the pH of the standard solutions in “measure” rather than “calibrate” mode. The electrode was rinsed with deionised water between buffers and sample measurements to prevent cross-contamination. The instrument automatically compensated for temperature during analysis. The electrode was then immersed in the soil

suspension, and the pH was recorded. Electrical conductivity (EC) was measured using the same protocol, in conductivity mode.

2.2.2. Total Organic Carbon Determination

Total organic carbon (TOC) was quantified following the dichromate oxidation method described by Bassam and Battikhi (2005). Approximately 1 g of soil sample (in duplicate) was placed in a 250 ml conical flask, and 10 ml of 1 N potassium dichromate ($K_2Cr_2O_7$) solution was added; then the flask was gently swirled to disperse the soil. Subsequently, 20 ml of concentrated sulphuric acid (H_2SO_4) was added rapidly, directing the stream into the suspension as previously described by Azlan et al. (2012). The flask was gently swirled to ensure mixing, then vigorously for 1 min, and allowed to stand on filter paper for 30 min. After equilibration, 100 ml of distilled water and three drops of methyl red indicator were added. The mixture was titrated with 0.5 N ferrous ammonium sulphate solution until the colour shifted from a greenish cast to dark green, then to maroon red at the endpoint. TOC content was calculated from titration values, with blank determinations performed and subtracted.

The percentage total organic carbon was determined as:

$$\%TOC = \frac{(Vb - Vs) \times N \times 0.003 \times 100}{W}$$

W

where:

- Vb = volume (ml) of ferrous ammonium sulphate used for the blank
- Vs = volume (ml) of ferrous ammonium sulphate used for the sample
- N = normality of ferrous ammonium sulphate solution
- W = weight of soil sample (g)
- 0.003 = milliequivalent weight of carbon in grams

2.2.3. Total Organic Nitrogen Determination

Total nitrogen was determined using the Kjeldahl digestion–distillation method as described by APHA (1998). Approximately 10 g of air-dried soil, ground and sieved to 0.5 mm, was transferred into a 500 ml micro-Kjeldahl flask. Twenty (20) ml of distilled water was added, the mixture was swirled, and left to stand for 30 min. A mercury catalyst tablet and 10 g of potassium sulphate (K_2SO_4) were then introduced, followed by 30 ml of concentrated sulphuric acid (H_2SO_4). The flask was heated cautiously at low temperature until frothing ceased, after which the heat was increased, and digestion continued for 5 h until the solution became clear. The digest was allowed to cool before adding 100 mL of distilled water, then quantitatively transferred to a clean 750 mL Kjeldahl flask. For distillation, 50 mL of boric acid indicator solution was placed in a 500 mL Erlenmeyer flask, which was positioned under the condenser. To the digest, 150 ml of 10 M sodium hydroxide (NaOH) solution was added via the funnel stopcock, and distillation was continued until 150 ml of distillate was collected. The distillate was titrated with 0.01 M standard hydrochloric acid (HCl) until the colour changed from green to pink, indicating the endpoint.

The percentage total nitrogen was calculated as:

$$\%N = \frac{Vs - Vb \times 14.01 \times 100}{W \times 1000}$$

1000

where:

- Vs = volume (mL) of standard HCl used for the sample
- Vb = volume (mL) of standard HCl used for the blank
- N = normality of HCl solution
- 14.01 = atomic weight of nitrogen (mg/meq)
- W = weight of soil sample (g)

2.3. Chemical Analysis

2.3.1. Total Petroleum Hydrocarbon Determination

Total petroleum hydrocarbons (TPHs) in the soil samples were analysed using a modified EPA 8015 method, following the procedure of USEPA (2003). Soil samples were air-dried, homogenised, and ground. A 10 g portion of each ground sample was weighed into an amber glass bottle, mixed with 50 g of anhydrous sodium sulphate to remove residual moisture, and stirred until friable. Hydrocarbons were extracted with 50 mL of dichloromethane, mixed vigorously, and allowed to stand for 1 h before filtration. Sample clean-up was performed using a funnel packed with cotton wool, anhydrous sodium sulphate, and silica gel to eliminate particulates and pigments. The filtrate was passed through a clean-up column similarly packed with sodium sulphate and silica gel, separated by cotton wool. The eluate was concentrated under a gentle stream of nitrogen, reconstituted in 1 mL of dichloromethane, and 1 μ L of the extract was injected into an Agilent 6890N Gas Chromatograph (HP 5890, Hewlett Packard, Avondale, PA, USA) equipped with a Flame Ionisation Detector (FID; Agilent 7890A) for quantification.

2.3.2. Polyaromatic Hydrocarbon Determination

Quantification of PAHs was carried out using Gas Chromatography coupled with a Flame Ionisation Detector (GC-FID), following the American Society for Testing and Materials (ASTM, 1995) protocol. Ultrasonic extracts of the soil samples were analysed with an Agilent 6890N GC-FID system (Hewlett Packard, Wilmington, DE, USA) equipped with a 30 m \times 0.25 mm i.d. \times 0.25 μ m film thickness capillary column. Calibration was performed using a certified reference mixture of 16 priority PAHs (ChemServices, West Chester, USA), supplemented with the USEPA 8270 LCS mix (Supelco Inc., St. Louis, MO; Lot LB21442), which included acenaphthene, acenaphthylene, anthracene, benzo[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[g,h,i]perylene, benzo[k]fluoranthene, chrysene, dibenz[a,h]anthracene, dibenzofuran, fluoranthene, fluorene, indeno[1,2,3-cd]pyrene, naphthalene, phenanthrene, pyrene, and 1-methylnaphthalene. The stock calibration solution was prepared at 100 μ g/mL in a 90:10 (v/v) mixture of methylene chloride and acetone. Surrogate standards (2-fluorobiphenyl and 1-fluoronaphthalene) were included to verify method performance.

GC-FID Operating conditions and method performance

GC-FID analyses were conducted using an Agilent 6890N Gas Chromatograph (HP 5890, Hewlett Packard, Avondale, PA, USA) fitted with a Flame Ionisation Detector. PAH determination followed ASTM (1995), while TPH determination was based on a modified EPA 8015 method. The injector temperature was maintained at 280 $^{\circ}$ C, with detector temperatures set at 300 $^{\circ}$ C for PAHs and 320 $^{\circ}$ C for TPHs. Oven programming was as follows: initial temperature 60 $^{\circ}$ C (held 2 min), ramped to 180 $^{\circ}$ C at 15 $^{\circ}$ C/min for PAHs and 12 $^{\circ}$ C/min for TPHs, then further increased to 300 $^{\circ}$ C at 5 $^{\circ}$ C/min (held 10 min) for PAHs and 6 $^{\circ}$ C/min (held 15 min) for TPHs (X. Tan, 2023). For both PAH and TPH analyses, helium was used as the carrier gas at a constant flow of 1.0 mL/min, with injections performed in splitless mode using a 1.0 μ L injection volume. Retention times for all analytes and surrogates were verified by replicate injections, with consistency maintained within \pm 0.1 min. Quality control and method performance: Method detection limits (MDLs) ranged from 0.01 to 0.05 mg/kg (dry weight) for PAHs and from 0.05 to 0.10 mg/kg (dry weight) for TPH. Recovery efficiencies were within 78-112% for PAHs and 80-110% for TPH, while replicate analyses produced relative standard deviations (RSDs) below 10% for PAHs and 12% for TPH, confirming both accuracy and precision of the methods.

2.3.3. Heavy Metals Determination

The concentrations of Cu, Pb, Cd, Fe, Cr, and Ni in the samples were determined using Atomic Absorption Spectrophotometry (AAS) with a flame atomisation technique (Gandhi et al., 2022). Calibration standards at three concentrations were prepared, and their absorbance was measured to generate calibration curves. The absorbance of each test solution was then measured and adjusted to fall within the quantifiable range. The metal concentrations were determined from the calibration curves. Prior to analysis, the nebuliser was flushed with ASTM Type-1 water containing 1.5 mL concentrated HNO₃ per litre to minimise contamination and maintain instrument stability.

Concentrations were read directly from the instrument and corrected by the appropriate dilution factor, with results expressed in mg/L as:

$$\text{Metal concentration (mg/L)} = \text{Instrument reading (mg/L)} \times \text{Dilution factor}$$

Quality control and performance for AAS

Method performance was verified using procedural blanks, duplicate analyses, and certified reference materials (CRMs). Recovery rates for spiked samples ranged between acceptable limits (typically 85–110%), ensuring method accuracy (D. Thakur et al., 2024). Precision was assessed by calculating the relative standard deviation (RSD) of replicate measurements, which remained below 10%. Instrument calibration was routinely checked with mid-range standards. The method detection limits (MDLs) were determined as three times the standard deviation of replicate measurements of reagent blanks and were typically in the range of: Cu (0.01 mg/L), Pb (0.005 mg/L), Cd (0.002 mg/L), Fe (0.02 mg/L), Cr (0.01 mg/L), and Ni (0.01 mg/L). These values ensured reliable quantification of trace concentrations of the target metals.

2.4. Microbiological Analysis

2.4.1. Enumeration Of Total Heterotrophic Bacteria

Total heterotrophic bacterial counts were determined using the spread plate technique on nutrient agar (NA). A soil slurry was prepared by suspending 1 g of soil in 9 mL of sterile physiological saline solution (0.85% w/v). From this suspension, appropriate serial dilutions were prepared, and 0.1 mL aliquots were aseptically spread in triplicate onto pre-poured nutrient agar plates using a sterile bent glass rod, as adapted from Cao et al. (2024). The inoculated plates were incubated at 28 ± 2 °C for 24–48 hours, after which colonies were enumerated and expressed as colony-forming units per gram of soil (CFU g⁻¹).

2.4.2. Enumeration Of Hydrocarbon-Utilising Bacteria

Hydrocarbon-utilising bacterial (HUB) count was determined by serial dilution of 1 mL soil slurry (10^{-1} – 10^{-10}) using sterile physiological saline according to (Sokolo et al., 2018). Aliquots of 0.1 mL from the dilutions were spread in triplicate onto sterile mineral salt agar (MSA) prepared with Bushnell-Haas medium, using a sterile glass rod. To provide a hydrocarbon vapour phase, a 9 cm Whatman filter paper saturated with 10 mL of sterilised Bonny light crude oil was placed on the inner surface of the Petri dish lids. Plates were incubated in an inverted position at 37 °C for 48 hours, after which visible colonies were enumerated and recorded as viable hydrocarbon-utilising bacterial counts.

2.4.3. Purification Of Bacteria and Quality Control

Discrete bacterial colonies were purified by sub-culturing onto nutrient agar (NA) plates using the streak plate technique. Pure isolates were maintained as stock cultures on sterile NA slants in Bijou bottles, appropriately coded for identification, and stored at 4 °C until required for further analyses. All media and diluents were sterilised before use, and uninoculated control plates were included to confirm sterility. Triplicate plating and replicate counts were performed to ensure precision, with counts accepted only when variation between replicates was within $\pm 10\%$. Method performance was validated by repeatability checks across independent runs. The method detection limit (MDL), calculated as three times the standard deviation (3σ) of replicate blank and low-count plates, was approximately 3.0×10^2 CFU g⁻¹ of soil (Da Silva et al., 2018). Counts below this threshold were regarded as unreliable for quantification.

2.5. Statistical Analysis

Statistical analyses were conducted in Python 3.8.8, focusing on descriptive statistics, statistical significance testing, correlation analysis, and multiple regression. Descriptive statistics were computed to summarise key characteristics of the data, including measures of central tendency, spread, skewness, and kurtosis using the Pandas library. A two-sample t-test was performed to assess differences between groups (e.g., control vs polluted), with p-values < 0.05 (Hicks, 2024). considered significant. Pearson correlation coefficients were calculated to assess linear relationships, and line

graphs and box plots were generated for visualisation. Multiple regression analysis using Ordinary Least Squares (OLS) from statsmodels was used to model the relationship between a dependent variable and multiple independent variables, with coefficients, p-values, and R-squared values reported in the regression summary. These methods allowed for an in-depth examination of the data, identification of key predictors, and evaluation of statistical significance across groups.

3. Result and Discussions

3.1. Distribution Patterns of Physicochemical

The distribution of total petroleum hydrocarbons (TPH), polycyclic aromatic hydrocarbons (PAH), total heterotrophic bacteria (THB), and hydrocarbon-utilising bacteria (HUB) across 16 soil samples is shown in Table 1 and Figure 2. The contaminated soils showed significantly higher concentrations of both TPH and PAH, with statistical differences observed between control and polluted samples and across locations. However, no statistical difference was found based on depth. TPH levels often exceeded 1,600 ppm, with a peak of 3,272 ppm observed in the 20-year-old workshop. PAH concentrations consistently surpassed 17 ppm, reaching a maximum of 46 ppm in the same workshop. In contrast, the control samples showed much lower contamination levels, with TPH concentrations remaining below 110 ppm in the surface soil of the 20-year-old workshop and PAH concentrations under 1.6 ppm. In the control samples, surface samples have higher THM concentrations than their corresponding subsurface samples, although this difference is not statistically significant.

In contrast, the polluted subsurface samples display higher concentrations than the surface samples, showing a clear trend across the age-marked locations, with a statistically significant difference. A statistical difference was also observed based on sample status. The lowest concentration is found in the year-10 control sample (EOSP 3), at 1545 ppm, while the highest concentration is in the year-20 polluted surface sample, at 71,583.78 ppm. The control year-1 samples exhibit higher nitrogen content compared to their polluted counterparts, and the total organic nitrogen (TON) concentration in the polluted samples decreases with age. The TON in the polluted surface samples exceeds that of the subsurface samples. Regarding total organic carbon (TOC), the control surface samples show higher TOC levels than their corresponding subsurface samples, whereas the polluted samples exhibit higher TOC concentrations than their control counterparts. In terms of electrical conductivity (EC), the control surface samples demonstrate higher values than the subsurface samples, while the opposite is true for the polluted samples. Specifically, polluted surface samples have lower EC than their subsurface counterparts, and overall, polluted samples show lower EC than control samples.

Table 1. Physicochemical and biological data from the study site.

Sample	TON (%)	TOC (%)	EC ($\mu\text{S}/\text{cm}$)	pH	THM (ppm)	TPH (ppm)	PAH (ppm)	THB (log)	HUB (log)
Cont T S1	0.58±0.75	2.77±0.04	173.48±5.24	7.07±0.04	1923.301±3.21	137.75±2.91	1.69±0.05	6.78±0.56	3.32±0.18
Cont B S1	0.18±0.003	2.18±0.56	171.67±1.90	6.85±0.02	2452.008±20.43	134.78±1.94	1.68±0.03	4.69±0.03	2.90±0.15
Polt T S1	0.13±0.001	3.96±0.01	138.93±1.76	4.62±0.03	3389.011±12.09	1603.20±1.036	18.78±0.22	5.05±0.06	3.22±0.26
Polt B S1	0.11±0.006	3.05±0.03	142.77±2.83	4.14±0.01	3166.751±61.36	1632.23±4.006	17.81±0.21	4.05±0.06	2.21±0.03
Cont T S5	0.25±0.002	2.86±1.48	152.67±2.17	7.18±0.03	2201.931±2.35	131.50±6.22	1.66±0.17	6.94±0.05	3.96±0.05
Cont B S5	0.20±0.002	2.40±0.01	152.00±0.02	6.47±0.04	2271.73±7.02	124.13±4.97	1.65±0.06	4.86±0.06	4.09±0.35
Polt T S5	0.12±0.003	3.71±0.95	114.43±0.15	5.04±0.02	3747.432±11.69	1944.47±6.227	24.52±0.66	5.03±0.06	3.48±0.04
Polt B S5	0.06±0.001	2.62±0.03	118.03±1.11	5.13±0.02	3565.18±332.07	1818.00±34.19	23.04±1.22	3.97±0.04	2.66±0.01
Cont T S10	0.13±0.002	2.42±0.03	285.67±4.45	6.85±0.03	1545.30±8.92	131.56±5.86	1.82±0.075	6.44±0.11	4.05±0.13
Cont B S10	0.10±0.001	2.24±0.15	284.37±5.31	6.66±0.05	22832.77±4.67	133.02±4.64	1.87±0.04	4.66±0.07	4.04±0.19
Polt T S10	0.09±0.003	3.72±0.05	62.87±1.98	5.40±0.05	5098.358±11.21	2420.07±1.13.53	30.38±1.20	5.96±0.06	3.69±0.07
Polt B S10	0.08±0.003	3.89±0.03	62.43±3.3	5.55±0.02	4078.102±70.36	2442.07±6.8.00	29.31±0.61	3.90±0.01	3.27±0.15
Cont T S20	0.25±0.002	2.62±0.05	236.50±5.62	7.09±0.07	10138.78±25.72	110.55±10.08	1.87±0.13	6.44±0.09	4.09±0.02
Cont B S20	0.15±0.004	1.68±0.06	216.63±0.04	6.90±0.02	2054.763±6.67	135.48±2.97	1.85±0.06	4.33±0.07	3.26±0.12
Polt T S20	0.08±0.003	3.89±0.04	163.53±0.01	5.86±0.01	71583.78±10.11	3243.27±1.36.04	42.69±2.36	5.37±0.09	4.71±0.06
Polt B S20	0.06±0.002	3.69±0.01	163.47±0.01	5.43±0.02	6927.765±169.53	3272.33±1.33.30	46.02±0.91	4.77±0.04	3.48±0.17

TON: Total organic nitrogen TOC: Total organic carbon EC: Electrical conductivity THM: Total of heavy metals computed from Cu, Pb, Cd, Fe, Cr, and Ni TPH: Total petroleum hydrocarbon PAH: Polyaromatic hydrocarbon THB: Total heterotrophic bacteria HUB: Hydrocarbon utilising bacteria.



Figure 1. Distribution of the physicochemical and microbiological parameters across the 16 samples. All polluted samples exhibit lower pH than the unpolluted samples, while the control samples have higher pH. The pH of the year 1 polluted samples is lower initially but steadily increases with age. The THM (the sum of the six analysed heavy metals: Cu, Pb, Cd, Fe, Cr, and Ni) concentrations in the control samples do not follow a distinct trend, although surface values are generally higher than those of the subsurface. In contrast, the heavy metal concentrations in the polluted samples increase over time, following a trend similar to that observed for TPH. In summary, spent engine oil reduces soil TON, EC, and pH, but increases TOC, TPH, PAHs, and THMs.

A comparison between control and polluted soil samples shows that spent engine oil is a source of hydrocarbons and heavy metals. Dorsey et al. (1997), citing the Agency for Toxic Substances and Disease Registry (ATSDR, 1994), stated that spent engine oil (SEO) mainly consists of hydrocarbons (70-85%), followed by heavy metals (1-5%) from engine wear, fuel residues (2-10%) from incomplete combustion, and carbon particles (2-8%) from soot. It also contains acids (1-5%) resulting from oxidation, water (0.5-3%) from condensation, and breakdown products (2-7%) from the degradation of oil additives. The hydrocarbon fraction comprises a high proportion of aliphatic hydrocarbons (73-80%), along with monoaromatic hydrocarbons (11-15%) and diaromatic hydrocarbons (2-5%).

Of particular concern are polycyclic aromatic hydrocarbons (PAHs), which account for 4-8% and are known for their toxicity. Examples of diaromatic hydrocarbons include dibenzothiophene, biphenyl, and naphthalene, among others (Rhead & Hardy, 2003). These proportions can vary based on engine conditions, oil type, and usage. The introduction of these foreign chemical substances constitutes pollution, as they harm the environment and its receptors. However, this study primarily focuses on the PAHs, TPH, and THM due to their impact on other physicochemical and biological factors. Consistent with the current study, an increase in PAHs, TPH, and cumulative heavy metals was observed, as supported by (Okebalama et al., 2024), who demonstrated a rise in heavy metals in an auto spare part market in Nigeria, and by (Ibe et al., 2021), who confirmed an increase in PAH concentrations in a mechanic village in Nigeria. The SEO-polluted soil also shows lower total organic nitrogen and higher total organic carbon than the control sample. This can be explained by toxic substances in the oil, such as heavy metals and hydrocarbons, which inhibit soil microorganisms responsible for nitrogen cycling, thereby decreasing nitrogen levels. Additionally, the oil alters soil permeability, promoting nitrogen leaching. Conversely, the organic carbon in spent engine oil, primarily derived from hydrocarbons, increases TOC as the oil breaks down and decomposes, adding organic matter to the soil. Electrical conductivity (EC) measurements showed lower values in surface soils contaminated with spent engine oil compared to subsurface soils. This is because hydrocarbons do not dissolve to provide ions for conductivity, interfere with ion exchange, reduce microbial

activity, and can impair soil structure. Similarly, soil pH increased due to hydrocarbon degradation and the release of metallic ions into the soil. However, there are some studies, though limited, that show pH increases due to active microbial activity, the chemical nature of hydrocarbon degradation, and soil characteristics (Li et al., 2023; Mekonnen et al., 2024).

The impact of pollutants, including PAHs, TPH, and heavy metals, along with reduced nitrogen levels, led to a decrease in total heterotrophic bacteria. The selective pressure from SEO pollutants promoted the growth of hydrocarbon-degrading bacteria (HUBs), with the highest concentration observed in the 20-year polluted soil. The presence of these pollutants causes a shift in the bacterial community, resulting in a decrease in biomass but the development of functional taxa capable of resisting and metabolising hydrocarbons. Several mechanisms contribute to this functionally altered environment, including stress adaptation, metal resistance, biofilm formation, catabolic gene expression, metabolic adaptation, co-metabolism, and horizontal gene transfer (Das et al., 2025; Ren et al., 2025; Shukla et al., 2019). Hydrocarbon-utilising bacteria adapt to stress by altering their cell membranes, which act as barriers against external toxins, such as hydrocarbons and heavy metals. Under stress, bacteria increase the saturation of membrane fatty acids, making the membrane more rigid and less permeable to toxic compounds. The incorporation of lipids, such as hopanoids, further enhances membrane stability, enabling bacteria to withstand harsh conditions, including high hydrocarbon concentrations (Dutta et al., 2025). Also, bacteria produce heat shock proteins (HSPs) that stabilise proteins and maintain cellular integrity by ensuring proper protein folding. These proteins are essential for bacteria to recover from protein denaturation caused by toxic environmental conditions (Fauvet et al., 2021).

Additionally, bacteria activate efflux pumps that actively expel toxic substances, including hydrocarbons and heavy metals, reducing intracellular concentrations of these compounds and preventing cellular damage, thereby enabling the bacteria to metabolise hydrocarbons effectively. In some cases, stress-induced biofilm formation provides bacteria with enhanced protection by encasing them in a protective matrix. This shields them from stressors such as hydrocarbons and facilitates nutrient and horizontal gene transfer, thereby promoting survival under harsh conditions. Hydrocarbon-degrading bacteria possess specialised enzymes, such as alkane hydroxylases, dioxygenases, and cytochrome P450S, that allow them to break down hydrocarbons into less toxic, metabolisable compounds. These enzymes facilitate the oxidation of hydrocarbons, enabling bacteria to use them as an energy source despite their toxicity (Fenibo et al., 2024a). In addition, bacteria may produce enzymes such as glutathione S-transferase, which detoxify heavy metals and other harmful compounds by binding them to glutathione, making them less reactive and easier to excrete. This metabolic flexibility is essential for bacteria to thrive in polluted environments and contributes to detoxification efforts. As a necessity, HUBs shift their metabolic pathways to adapt to available nutrients in a contaminated environment.

3.2. Response of Microbiological Parameters to Pollutants

Fig. 3 clearly differentiates between polluted and control sites. Microbial analysis showed significant differences in THB community structure by depth within locations, whereas HUB differences were observed both within and between locations. The highest total heterotrophic bacteria (THB) count was recorded in the surface soil of the year-5 control site, with a log-transformed value of 6.94. The lowest THB count was found in the year-20 subsurface polluted soil. Similarly, the highest hydrocarbon-utilising bacteria (HUB) count was observed in the surface-polluted soil of the year-20 site, with a log-transformed value of 4.71, while the lowest HUB count was observed in the year-1 subsurface-polluted soil.

The observed increases in pollutants such as total petroleum hydrocarbons (TPH), polycyclic aromatic hydrocarbons (PAH), and total heavy metals (THM) have a notable impact on bacterial biomass in polluted soils. This impact is most evident in variations in bacterial populations, particularly in total heterotrophic bacteria (THB) and hydrocarbon-utilising bacteria (HUB) counts across depth, location, and age. These changes provide insight into the microbial dynamics of polluted soils, highlighting the selective pressures imposed by hydrocarbon contamination. The data indicate a significant reduction in the number of THBs in polluted soils, particularly in the deeper

layers of contaminated sites, due to a combination of nutrient scarcity and low oxygen levels. This reduction can be reinforced by the inhibitory effects of hydrocarbons, such as TPH, PAH, and THM, which are toxic to many aerobic and facultative bacteria. THBs are generalist microbes responsible for the breakdown of organic matter and are typically more abundant in non-polluted soils where organic material is more readily available and less toxic. However, in polluted soils, high concentrations of hydrocarbons and heavy metals create a hostile environment for these bacteria.

Hydrocarbons, particularly PAHs, are known to be toxic to a wide range of soil microorganisms, as they can interfere with cellular processes, disrupt membrane integrity, and inhibit enzyme activity (Innocent et al., 2024). In addition to their toxic effects, hydrocarbons can also reduce the availability of other organic carbon sources by dominating the nutrient pool, further stressing the microbial community. As a result, THBs, which rely on a diverse array of organic carbon sources, are less able to thrive in polluted conditions. This trend is particularly noticeable in the surface layers of polluted soils, where pollutant accumulation is often highest. In contrast to the reduction in THB counts, HUBs were observed to increase in polluted soils, particularly in the surface layers beyond year 1. HUBs are specialised bacteria that are capable of degrading hydrocarbons, including TPH and PAHs, making them better suited to survive and thrive in hydrocarbon-polluted environments. These bacteria play a crucial role in detoxification by breaking down complex hydrocarbons into simpler, less toxic compounds. The increase in HUBs in polluted soils suggests that hydrocarbon contamination acts as a selective force, favouring the growth of microorganisms capable of metabolising these pollutants.

The higher counts of HUBs in the surface layers of polluted soils reflect their ability to adapt to the presence of hydrocarbons in the environment. Surface soils are typically richer in oxygen, which is necessary for the aerobic degradation of hydrocarbons. Therefore, surface layers provide a more favourable environment for HUBs, which are often aerobic or microaerophilic bacteria. The increase in HUBs in response to hydrocarbon pollution indicates that these bacteria are actively participating in the detoxification of the contaminated site, breaking down the hydrocarbons and helping to reduce the overall pollutant load in the soil. However, it is important to note that while HUBs are stimulated by hydrocarbons, their presence alone may not be sufficient to fully mitigate the negative effects of pollution, particularly in areas where pollutant concentrations exceed the bacteria's degradation capacity.

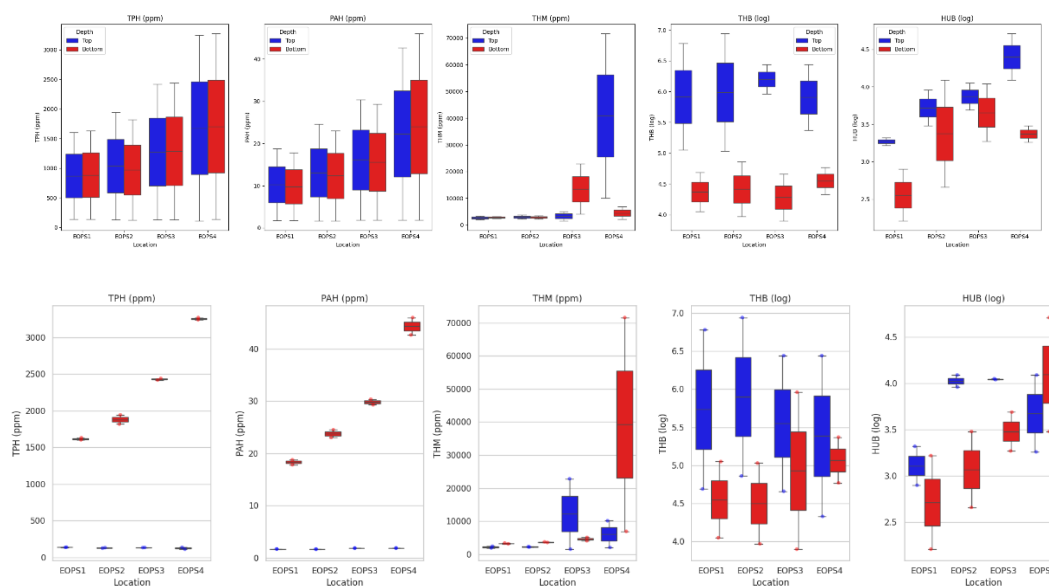


Figure 3. Discriminatory distribution of pollutants and bacterial response.

The reduction in bacterial biomass in surface samples supports the notion that aerobic bacteria, such as THBs, are more abundant and diverse in pollutant-free soils. Aerobic bacteria are more efficient at breaking down a wide range of organic materials and thrive in environments with abundant oxygen. The selective pressures exerted by hydrocarbon contamination on the microbial

community have significant implications for soil remediation and ecosystem health. While the increase in HUBs suggests some natural attenuation and potential for bioremediation, the reduction in THB counts and the overall loss of microbial diversity indicate adverse effects on soil health. The decreased microbial activity in polluted soils, especially in the surface layers, suggests that the soil's ability to perform essential ecosystem services is impaired. In the absence of chemical stress, they convert nitrogen into usable forms, recycle nutrients like carbon, nitrogen, and sulfur, and enhance soil fertility. Heterotrophic bacteria also assist in symbiotic relationships and help control pathogens, reducing reliance on pesticides. Additionally, they serve as environmental indicators, helping maintain ecosystem stability and support sustainability.

3.3. Influence of TPH on Other Soil Factors

The correlation matrix illustrates strong interrelationships among key physicochemical and microbiological parameters in the soil samples. Fig. 4 shows a very strong positive correlation between total petroleum hydrocarbons (TPH) and polycyclic aromatic hydrocarbons (PAH) ($r = 0.99$), and a moderate correlation with total heavy metals (THM) ($r > 0.41$). This suggests that petroleum contamination is closely linked to elevated PAH levels and moderately associated with heavy metals. PAH also correlated strongly with THM ($r \approx 0.98$), indicating potential common sources or similar environmental behaviour. Microbial indicators such as Total Heterotrophic Bacteria (THB) and Hydrocarbon-Utilising Bacteria (HUB) were moderately correlated ($r > 0.54$), reflecting microbial adaptation to hydrocarbon-rich conditions. In contrast, Total Organic Nitrogen (TON) showed a moderate negative correlation with TPH ($r = -0.56$), implying reduced nitrogen availability in contaminated soils. TON showed moderate positive correlations with pH and THB, but negative correlations with TPH and PAH, suggesting that petroleum pollution may suppress organic nitrogen and alter microbial activity. Soil pH exhibited moderate negative correlations with TPH and weak negative correlations with THM, suggesting contamination-induced acidification. Total Organic Carbon (TOC) showed negligible correlations with THB ($r = 0.02$) and HUB ($r = 0.066$), indicating that organic matter alone does not significantly influence microbial abundance in these soils. TOC was moderately negatively correlated with Electrical Conductivity (EC) and pH, possibly due to the organic matter's buffering effect. A moderate positive correlation was observed between EC and pH ($r = 0.6$). Other variables showed weak or negligible correlations, indicating independent variation. These relationships, visualised in a heatmap (Fig. 4), underscore the complex interactions between chemical pollutants and microbial dynamics in contaminated soils.

The correlations observed between physicochemical and microbiological parameters underscore the complex dynamics of soil contamination and its ecological consequences. The strong positive correlations between total petroleum hydrocarbons (TPH), polycyclic aromatic hydrocarbons (PAH), and total heavy metals (THM) indicate a shared origin or co-migration pattern, likely linked to petroleum-related activities such as oil spills, industrial discharge, and improper waste disposal, as observed in the work of Lu et al. (2025). These pollutants often enter the soil simultaneously, where they interact both chemically and physically, intensifying contamination. Hydrocarbons, especially PAHs, are known for their persistence and hydrophobic properties, which enable them to strongly bind to soil particles and organic matter. This binding reduces their bioavailability and creates microenvironments that trap heavy metals, promoting their accumulation (Rahman & Singh, 2020). The observed positive correlations between TPH, PAH, and THM in this study align with the findings of Zhang et al. (2023), which demonstrated that petroleum hydrocarbon contamination in soils frequently coincides with heavy metal accumulation due to shared sources and environmental behaviour. Truskewycz et al.'s (2019) research highlighted that petroleum hydrocarbon contamination alters soil permeability, moisture content, and nutrient availability, thereby impacting microbial diversity and activity. Contamination with TPH, PAHs, and heavy metals significantly affects the soil's physical properties. These pollutants bind tightly to soil particles, particularly in organic-rich layers, leading to compaction and reduced porosity. This compromises water infiltration, aeration, and root penetration, negatively affecting plant growth and microbial activity. Hydrocarbons also create hydrophobic conditions, decreasing water retention and increasing surface runoff, while heavy metals can alter soil pH, often leading to acidification (Mohanta et al., 2024b).

These alterations degrade soil structure, reduce fertility, and disrupt vital ecological processes such as nutrient cycling and microbial respiration.

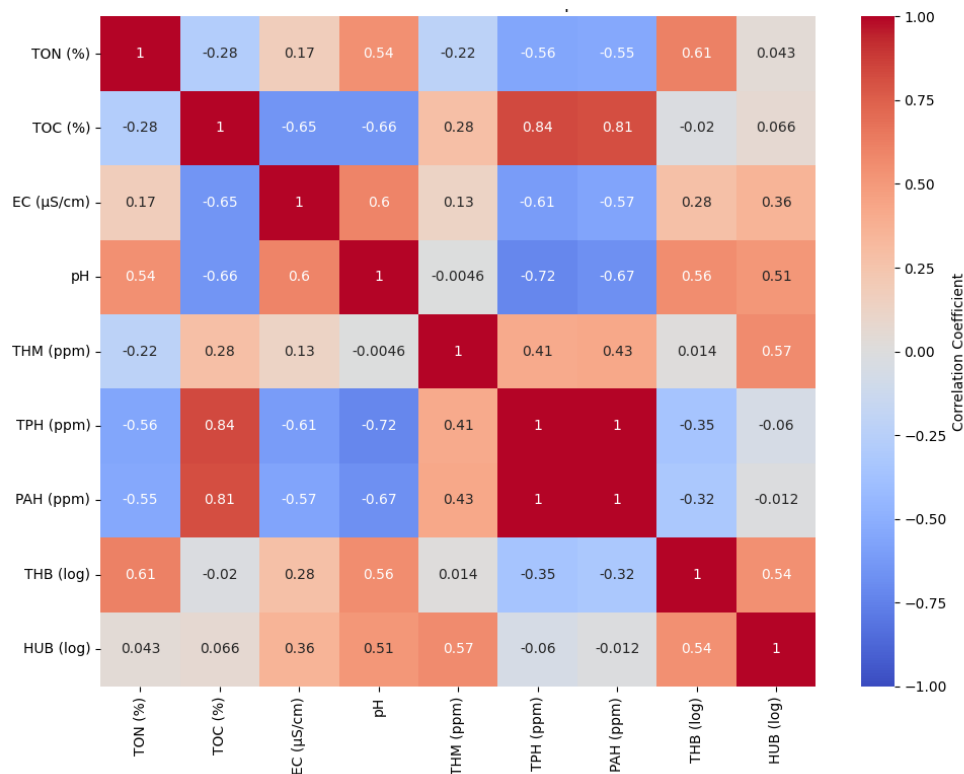


Figure 4. Correlation matrix of soil quality parameters. Pearson correlation coefficients between soil quality parameters are shown. Red indicates a positive correlation, blue indicates a negative correlation, with colour intensity reflecting strength. The persistence of these contaminants further exacerbates the damage and complicates long-term remediation efforts.

The moderate correlation observed between total heterotrophic bacteria (THB) and hydrocarbon-utilising bacteria (HUB) indicates microbial adaptation to hydrocarbon-rich environments, where selective pressure favours taxa capable of metabolising petroleum-derived compounds. Microorganisms, particularly bacteria, are integral to soil ecosystem functioning, contributing to nutrient cycling, decomposition of organic matter, and overall soil health. However, the introduction of pollutants such as total petroleum hydrocarbons (TPH), polycyclic aromatic hydrocarbons (PAH), and heavy metals can significantly disrupt microbial communities and impair their ecological roles. Heavy metals such as cadmium, lead, and mercury further exacerbate microbial stress. At elevated concentrations, these metals disrupt enzymatic functions, impair cellular respiration, and inhibit key metabolic processes (Fu & Xi, 2020). While some bacteria develop resistance mechanisms, such as metal efflux systems or chelation, these adaptations often come at the cost of reduced growth and metabolic efficiency. Consequently, heavy metal contamination can lead to a narrowed microbial community dominated by resistant strains, while suppressing beneficial organisms involved in nitrogen fixation, organic matter turnover, and other essential soil functions. The co-occurrence of hydrocarbons and heavy metals may produce synergistic toxic effects, where hydrocarbons enhance the mobility or bioavailability of metals, and metals inhibit microbial degradation of hydrocarbons. This interaction intensifies ecological stress, further diminishing microbial diversity and functionality. Despite these adverse effects, some soils may exhibit resilience through natural attenuation, microbial adaptation, or bioremediation. Recovery potential depends on the extent of contamination, the presence of competent microbial strains, and environmental factors such as moisture, temperature, and pH. In severely polluted soils, however, microbial recovery may be limited, leading to long-term degradation of soil health and ecosystem services.

The strong negative correlation between total nitrogen (TON) and total petroleum hydrocarbons (TPH) ($r = -0.85$) highlights the adverse impact of petroleum contamination on soil fertility. Nitrogen

is a fundamental nutrient for plant growth and microbial metabolism, and its depletion in hydrocarbon-polluted soils suggests a disruption of key biogeochemical processes. One plausible explanation is the toxic effect of hydrocarbons on nitrogen-fixing bacteria, which are crucial for converting atmospheric nitrogen into bioavailable forms (Ling et al., 2021). Hydrocarbons, particularly complex PAHs, can interfere with microbial enzymatic systems, inhibit nitrogenase activity, and reduce microbial biomass, thereby impairing nitrogen fixation and mineralisation. The hydrophobic nature of hydrocarbons can reduce water retention and oxygen diffusion, creating anaerobic conditions that further suppress microbial function (Kebede et al., 2021). In such environments, denitrification may be enhanced, leading to nitrogen loss as gaseous emissions, while ammonification and nitrification processes are inhibited. The depletion of nitrogen has cascading ecological consequences. Reduced nitrogen availability limits plant growth, weakens root development, and diminishes vegetative cover, which in turn affects soil structure and erosion resistance. Furthermore, nitrogen scarcity can shift microbial community composition, favouring stress-tolerant or hydrocarbon-degrading species at the expense of functionally diverse microbial populations. This loss of microbial diversity compromises soil resilience and its ability to recover from contamination. The negative correlation between TON and TPH underscores the broader ecological degradation associated with petroleum pollution. It reflects not only a chemical imbalance but also a biological disruption that threatens the integrity of soil ecosystems and their capacity to support vegetation, microbial life, and essential nutrient cycles. The moderate negative correlation between soil pH and both TPH and heavy metals suggests that petroleum contamination and metal accumulation may lead to soil acidification (Kebede et al., 2021).

This pH shift can significantly affect microbial activity and nutrient availability, as most soil microorganisms thrive within a specific pH range of 6.5-7.5. Acidification can inhibit microbial growth and disrupt essential processes such as nutrient cycling, organic matter decomposition, and pollutant degradation (C. Wang & Kuzyakov, 2024). These changes in soil pH can further hinder the natural recovery of contaminated environments, making it more difficult to restore soil health through conventional means. The correlation between pH and pollutants emphasises the interconnectedness of chemical and biological factors in polluted soils and the need for comprehensive strategies that address both physical and chemical soil properties. This correlation study serves as a crucial tool for understanding the interactions between pollutants and microbial communities in hydrocarbon-contaminated soils and also lays the groundwork for predictive modelling and risk assessment in polluted environments.

3.4. Prediction of Microbial Indicators

The multiple regression analysis conducted on the dataset provides valuable insights into the relationship between environmental parameters, specifically total organic carbon (TOC) and total petroleum hydrocarbon (TPH), and microbial populations, namely total heterotrophic bacteria (THB) and hydrocarbon-utilising bacteria (HUB). For THB, the regression model demonstrated moderate explanatory power ($R^2 = 0.373$), indicating that TOC and TPH together account for approximately 37% of the variation in THB levels. TOC emerged as a statistically significant positive predictor, highlighting its importance as a vital nutrient source that fosters microbial growth (Ficetola et al., 2024). This aligns with the role of organic carbon in sustaining general microbial activity in various environments.

In contrast, TPH showed a significant negative association with THB, suggesting that elevated hydrocarbon concentrations may inhibit the growth and activity of general heterotrophic bacteria. The multiple linear regression output, shown in Figs. 5 and 6, illustrates the relationship between THB, TPH, and other environmental parameters. This negative relationship could be due to the toxicity of hydrocarbons or the environmental stress they impose on microbial communities. On the other hand, the regression model for HUB exhibited weak explanatory power, with an R^2 of only 0.047, indicating that TOC and TPH accounted for only 4.7% of the variation in HUB populations. Neither TOC nor TPH was a statistically significant predictor in this case, suggesting that other factors not captured in the model may influence HUB populations. These factors could include

specific types of hydrocarbons that are more readily metabolised by HUB, microbial adaptation mechanisms to hydrocarbon stress, or additional nutrient sources that support their growth.

The results of the multiple regression analysis, along with the scatter plots, provide valuable insights into how various environmental factors influence microbial populations, particularly total heterotrophic bacteria (THB) and hydrocarbon-utilising bacteria (HUB). The regression analysis indicates that total organic carbon (TOC) is a significant positive predictor of THB, highlighting its role as a key nutrient supporting general microbial growth. In contrast, total petroleum hydrocarbons (TPH) negatively affected THB, suggesting that high hydrocarbon concentrations may inhibit microbial activity, likely due to the toxicity or environmental stress that hydrocarbons impose on these bacteria. The model explains 37% of the variation in THB levels, underscoring the importance of TOC and TPH in shaping microbial populations in polluted environments (Chicca et al., 2022). Interestingly, TOC and TPH were not significant predictors for HUB populations, indicating that other factors may be influencing their abundance. This suggests that HUBs, which specialise in hydrocarbon degradation, may be more responsive to specific types of hydrocarbons or other environmental conditions not captured in the model, such as the presence of particular hydrocarbon compounds or microbial adaptation mechanisms. The regression analysis, therefore, suggests that while TOC plays a central role in supporting overall microbial activity, the presence of TPH alone is insufficient to predict HUB abundance. The scatter plots further reinforce these findings, illustrating significant correlations between THB and factors such as TOC, pH, and TPH. The positive correlation with TOC aligns with the regression results, affirming that organic carbon supports heterotrophic microbial growth. Additionally, the negative correlation with TPH supports the idea that higher hydrocarbon levels suppress general microbial activity.

In contrast, weak or no associations were observed between HUB and TOC, THM, EC, and PAH, further suggesting that HUB populations are influenced by more specific environmental cues beyond the general availability of organic carbon and hydrocarbons. These results highlight the complexity of microbial responses in contaminated environments, where multiple factors interact to shape microbial community structure (Birrer et al., 2017; Chikere et al., 2018). While TOC is a critical driver of general microbial growth, TPH appears to suppress THB, and HUB populations may respond to more specific environmental conditions, such as the presence of certain hydrocarbons. Understanding these interactions is crucial for designing effective bioremediation strategies, underscoring the need to consider a broader range of factors beyond organic carbon and hydrocarbons when assessing microbial dynamics in polluted ecosystems. Based on the findings, several studies are needed to deepen our understanding of the factors influencing hydrocarbon-utilising bacteria (HUB) populations and microbial responses in contaminated environments. First, specific hydrocarbon characterisation studies should identify and assess the types of hydrocarbons that HUBs readily utilise, with a focus on their biodegradability and toxicity. Additionally, research into microbial adaptation mechanisms is needed to explore the genetic and metabolic pathways HUBs use to metabolise specific hydrocarbons, including stress-response proteins and enzymes (Y.-S. Tan et al., 2022). Comprehensive environmental factor studies should examine how variables such as soil texture, temperature, moisture, pH, and heavy metal concentrations interact with hydrocarbons to shape microbial community structure.

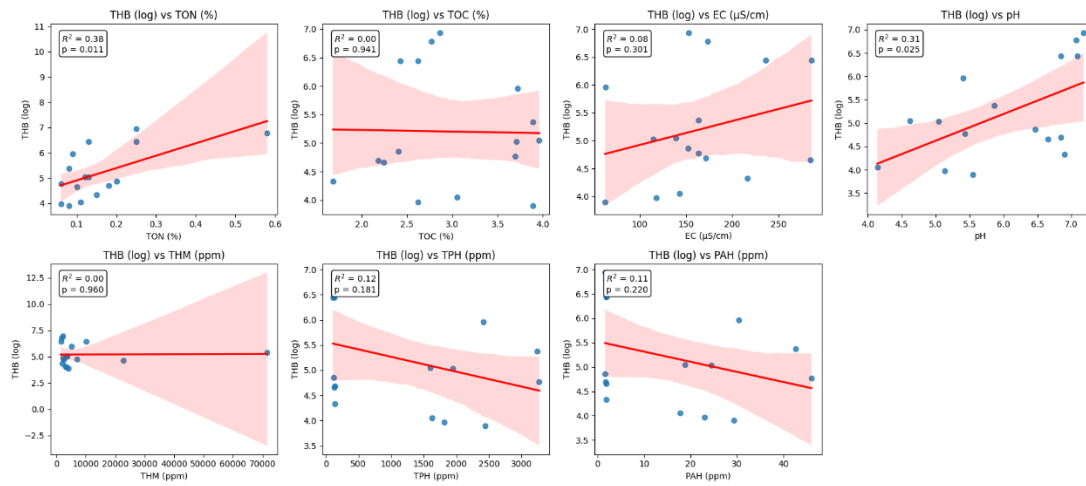


Figure 5. Regression analysis of THB impact on various parameters. Each dot represents a soil sample showing the relationship between TPH and a parameter (TON, TOC, EC, THM, TPH or PAH). The red line is the regression line, with the shaded area indicating the confidence interval.

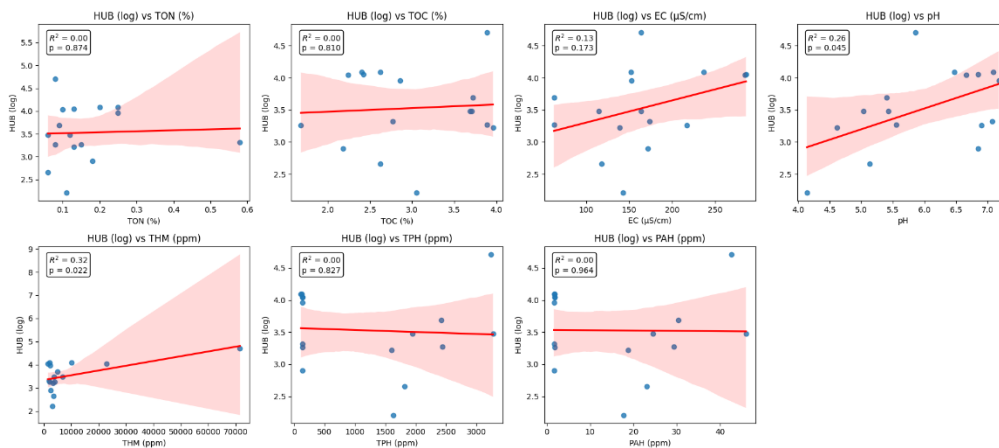


Figure 6. Regression analysis of HUB impact on various parameters. Each dot represents a soil sample showing the relationship between TPH and a parameter (TON, TOC, EC, THM, TPH or PAH). The red line is the regression line, with the shaded area indicating the confidence interval. Longitudinal bioremediation studies should track the performance of HUB populations over time to assess their role in pollutant degradation and the effectiveness of bioremediation efforts (Lin et al., 2023). Research on the environmental impact of other pollutants, such as heavy metals and PAHs, is also needed to examine how mixtures of contaminants affect microbial diversity and function. Functional genomic and metagenomic studies could help identify key genes involved in hydrocarbon degradation and stress resistance in HUBs (Baev, 2025). Finally, bioaugmentation and biostimulation studies should explore strategies to enhance HUB populations or create conditions that optimise bioremediation in hydrocarbon-polluted ecosystems. Collectively, these studies will offer essential insights for designing more effective and targeted bioremediation strategies, which are crucial for mitigating the health risks associated with spent engine oil pollution.

3.5. Spent Engine Oil Pollution and Public Health Implications

The increasing levels of total petroleum hydrocarbons (TPH), polycyclic aromatic hydrocarbons (PAH), and heavy metals in spent engine oil over time (as indicated in location 4 with 20 years of SEO exposure) present a critical environmental and public health concern. These pollutants are primarily the result of improper disposal practices, particularly in mechanical workshops, where spent engine oil is often carelessly discarded into the environment. As these pollutants accumulate in soil, water bodies, and the atmosphere, they pose significant risks to animals, fish, and humans, as well as to ecological health. The implications of TPH and PAHs for public health are significant, particularly for respiratory and skin diseases and cancer (Alao et al., 2025). Workers exposed to these chemicals, especially in environments where engine oil is handled or disposed of improperly, face

heightened risks for respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD) (Ferguson et al., 2020; Hart et al., 2006). One of the key contributors to COPD development is the generation of reactive oxygen species (ROS) as a byproduct of the metabolism of PAHs and TPH. These ROS are highly reactive molecules that cause oxidative stress, damaging cellular structures such as lipids, proteins, and DNA. The oxidative damage is especially harmful to the lining of the airways and the alveoli, the tiny air sacs in the lungs responsible for oxygen exchange. This damage impairs lung function by reducing the lungs' ability to expel air efficiently, which is a characteristic symptom of COPD. The continuous production of ROS from sustained exposure to these pollutants exacerbates the inflammation, resulting in further degradation of lung tissue.

Additionally, PAHs and TPH can impair the function of the mucociliary escalator, the mechanism responsible for clearing mucus and foreign particles from the lungs (Black, 2013). These pollutants damage the cilia, small hair-like structures in the airways, reducing their ability to effectively remove mucus and debris. As a result, mucus accumulates in the airways, increasing the risk of infections. Chronic bronchitis and respiratory infections caused by this mucus buildup contribute to the progression of COPD, further irritating and inflaming the airways. Prolonged exposure to PAHs and TPH also contributes to the development of emphysema, a condition where the alveolar walls of the lungs are destroyed (Nwaozuzu et al., 2021). The inflammation and oxidative damage caused by these pollutants activate enzymes that degrade structural proteins such as elastin and collagen in lung tissue (Albano et al., 2022). As the alveolar walls deteriorate, the lungs lose elasticity, making it more difficult to fully exhale. This results in air trapping, where air becomes stuck in the lungs during exhalation, leading to difficulty breathing and reduced oxygen intake, which are key symptoms of COPD. Finally, chronic exposure to TPH and PAHs can impair the immune system's normal function, particularly in the lungs. These pollutants can alter the balance of immune cells, such as macrophages and neutrophils, leading to a hyperactive immune response. This dysfunction exacerbates inflammation and lung damage, contributing to the progression of COPD. Over time, the immune system's reduced ability to repair lung tissue and its increased susceptibility to infections worsen respiratory symptoms.

Chronic exposure to these pollutants can also cause a range of severe dermatological issues due to their toxic and carcinogenic properties. When these SEOs and associated pollutants come into direct contact with the skin, they can cause a range of harmful effects, particularly with prolonged exposure. One key dermatological condition associated with PAH exposure is photoallergic dermatitis. PAHs are photosensitive compounds, meaning that they can interact with ultraviolet (UV) light from the sun. When the skin is exposed to sunlight, PAHs can absorb UV radiation and undergo chemical transformations that trigger an immune response. This response leads to skin inflammation, causing the skin to become red, itchy, and swollen. Over time, this can develop into a more chronic condition in which the skin becomes increasingly sensitive to sunlight, leading to photosensitivity. People with photoallergic dermatitis often experience flare-ups upon exposure to light, resulting in painful skin reactions that worsen with continued UV exposure (Borska et al., 2006; Yanagisawa et al., 2021). In addition to causing dermatological irritation, PAHs pose a significant risk for skin cancer. PAHs, such as benzo[a]pyrene, are potent carcinogens (Yanagisawa et al., 2021). When absorbed by the skin, they can penetrate the skin cells and undergo metabolic activation, leading to the formation of reactive intermediates. These intermediates can bind to DNA in skin cells, leading to mutations (Pashin & Bakhitova, 1979). These mutations disrupt normal cellular processes, especially those that regulate cell growth and repair. As a result, skin cells can begin to grow uncontrollably, forming abnormal growths that can progress to malignant tumours, such as skin cancer. The toxic nature of PAHs further increases the risk of skin cancer through their ability to cause DNA damage (Chan et al., 2021). The binding of PAHs to DNA creates DNA adducts that distort the DNA helix, making it difficult for the cell to replicate and repair DNA properly. These DNA adducts also distort the DNA helix, impairing its ability to replicate and transcribe properly. This binding triggers the activation of cytochrome P450 enzymes, further enhancing the bioactivation of PAHs into more potent carcinogenic forms. The accumulation of these mutations can overwhelm the skin's natural repair mechanisms, leading to the development of skin cancer. The risk is further elevated with repeated or prolonged exposure to PAHs, as they continually induce cellular damage and

inflammation, promoting the formation of malignant tumour Spent engine oil also possesses hazardous heavy metals.

One of the most concerning aspects of heavy metal contamination, particularly with lead, cadmium, and arsenic, is their bioaccumulation in the environment. These metals do not readily degrade or break down, so they persist in soil, water, and air for extended periods. They accumulate in the tissues of plants, animals, and humans. Unlike organic pollutants, which may degrade over time, heavy metals tend to remain in the ecosystem, increasing the potential for long-term exposure. As these metals are absorbed by plants and animals, they enter the food chain, where their concentrations increase at each step, magnifying the potential health risks for humans and animals alike. When humans or animals ingest food or water contaminated with heavy metals, these toxic substances enter the body and are stored in various tissues, such as the liver, kidneys, and bones. In some cases, the metals accumulate in the bloodstream and soft tissues, where they interfere with normal biological functions. Bioaccumulation means that even low-level, chronic exposure to these metals over time can lead to a gradual buildup of harmful concentrations in the body. This persistent accumulation significantly increases the likelihood of adverse health effects, including organ damage, neurological impairment, immune system dysfunction, and, in the long term, an elevated risk of cancer (Parida & Patel, 2023). In agricultural communities where farming is a primary livelihood, contamination of soil and water with heavy metals poses a serious, direct threat to food safety and public health. These metals can easily contaminate crops and groundwater through the deposition of industrial waste, runoff of polluted water, or improper disposal of materials such as spent engine oil. As plants absorb heavy metals from the soil, these metals accumulate in the edible portions of the crops, such as fruits, vegetables, and grains. When livestock graze on contaminated pasture or drink polluted water, the metals accumulate in their tissues, including their meat, milk, and eggs, which can then be consumed by humans. The transfer of these metals through the food chain is known as biomagnification, a process where the concentration of toxic substances increases as they move up the food chain. For example, a small amount of lead or cadmium in soil may be absorbed by plants, and then by herbivores that eat those plants. Carnivores, in turn, may consume these herbivores, further concentrating the metals in their own tissues. Humans, at the top of the food chain, are particularly at risk because they consume both plants and animals contaminated with heavy metals, often resulting in higher exposure levels than those of other organisms in the ecosystem. The health implications of bioaccumulation in agricultural communities are significant.

Spent engine oil contains several heavy metals, including lead, cadmium, and arsenic, each of which poses significant health risks due to its toxic properties. These heavy metals are hazardous not only when directly exposed to humans but also through bioaccumulation in the environment, leading to long-term health impacts. Lead exposure is particularly harmful, especially in children, where it can severely impair cognitive function (Santa Maria et al., 2019). In children, lead exposure can cause learning disabilities, developmental delays, and behavioural issues. The effects on the developing brain can be permanent, leading to long-term academic challenges and neurological deficits. In adults, lead exposure causes cardiovascular problems, such as hypertension, and damages the kidneys, contributing to kidney disease (Alissa & Ferns, 2011). Additionally, lead is toxic to the reproductive system, potentially causing infertility and complications in pregnancy. Chronic exposure to lead in any form increases the risk of developing severe, irreversible health problems, including organ failure. Cadmium, another heavy metal found in spent engine oil, is highly toxic and accumulates in the kidneys and liver, where it interferes with their normal functions. Prolonged exposure to cadmium leads to renal dysfunction, as the metal accumulates in kidney tissues, causing damage to the renal tubules and potentially leading to kidney failure (Charkiewicz et al., 2023). In addition to kidney damage, cadmium weakens bone structure, increasing the risk of fractures and bone diseases such as osteomalacia and osteoporosis (Tang et al., 2025). Cadmium is a potent carcinogen that promotes cellular mutations, contributing to the development of malignant tumors over time. Arsenic, a well-known carcinogen, is associated with an increased risk of several cancers, including skin, lung, and bladder cancers (Oberoi et al., 2014; Palma-Lara et al., 2020). Chronic exposure to arsenic, either through ingestion or inhalation, leads to the formation of DNA adducts, which disrupt cellular processes and increase the likelihood of cancerous transformations. The

carcinogenic nature of arsenic is particularly concerning in areas where drinking water and food sources are contaminated with this metal. In addition to cancer, arsenic exposure has been linked to cardiovascular disease, neurotoxicity, and skin lesions (M. Thakur et al., 2021), further compounding the public health risks. Table 2 summarizes the health risks associated with pollution from spent engine oil.

In addition to the direct health risks posed by heavy metal contamination, the economic consequences for farming communities are substantial and far-reaching. The contamination of soil and water with metals such as lead, cadmium, and arsenic undermines agricultural productivity by harming crops and livestock. Heavy metals can directly interfere with plant growth by affecting nutrient absorption and disrupting photosynthesis, leading to lower yields and reduced crop quality (Rai et al., 2016). Crops may also become contaminated with toxic substances, rendering them unsafe for consumption and further impacting harvests. For livestock, the ingestion of contaminated water or feed leads to increased mortality rates and health issues, such as organ damage and reproductive failures. As a result, farmers face higher veterinary care costs, animal losses, and the need to replace livestock, further depleting their income. This reduction in agricultural productivity significantly reduces farmers' incomes, creating a cycle of poverty, especially in rural communities where farming is the primary source of livelihood. With reduced yields and higher livestock maintenance costs, farmers are unable to generate the revenue needed to sustain their operations. Food insecurity becomes a pressing issue as the local population struggles to access affordable, healthy, and safe food. This is particularly critical in areas with few alternative income sources, leaving the community heavily reliant on agriculture.

Moreover, the stigma associated with contaminated agricultural products presents an additional challenge. Consumers are increasingly aware of the potential health risks posed by food contaminated with heavy metals, and demand for such products tends to decrease. Contamination of crops and livestock reduces their marketability, as both local and international buyers may avoid purchasing goods from areas with high contamination levels. The fear of potential health risks can lead to reduced market access, forcing farmers to sell their produce at lower prices or, in some cases, face a complete loss of income from unsellable goods. This creates an economic barrier that can drive farmers into further financial hardship. The economic fallout from heavy metal contamination is not confined to individual farms; it extends to the broader local economy. Agriculture is often the backbone of rural economies, and when this sector suffers, the effects ripple throughout the entire community (Gelaye, 2024). Lower incomes in farming communities reduce purchasing power, leading to a decline in demand for local goods and services, which in turn affects local businesses. As farmers struggle to survive financially, they may be forced to abandon farming altogether, leading to increased unemployment and migration to urban areas, further straining city infrastructure and social systems. The long-term economic decline caused by environmental contamination can create a vicious cycle of poverty, leaving families unable to access basic necessities, including adequate healthcare, education, and nutrition.

The contamination of local water bodies with heavy metals significantly exacerbates the economic challenges faced by communities, particularly those that rely on water resources for agriculture, fishing, and daily life. Water is an essential resource for farming, as it is needed for irrigation, livestock hydration, and crop growth. When local rivers, lakes, or groundwater sources are polluted with heavy metals such as lead, cadmium, and arsenic, the negative impacts ripple through various sectors, affecting both human health and economic stability (Gelaye, 2024). Agricultural productivity is directly compromised when water sources become contaminated. Irrigation, which is vital for crops in many farming regions, relies on clean, uncontaminated water. Polluted water can introduce toxic heavy metals into the soil, which harm plants by disrupting their ability to absorb nutrients and water. Contamination of agricultural systems can significantly impair crop productivity, thereby reducing overall yield and farmer income. These disruptions have direct implications for Sustainable Development Goals (SDGs) 6.4 and 2.4, which emphasise the efficient use of water resources and resilient, sustainable agricultural practices (Sabir et al., 2024). Furthermore, heavy metals in irrigation water may accumulate in the soil over time, leading to long-

term degradation of soil quality. This makes it even more difficult for farmers to sustain healthy crops, thereby exacerbating food insecurity in the region.

Table 2. Health risks affiliated to spent engine oil pollution in soil with respect to limit set by regulatory bodies.

Pollutant	Regulatory limit	Negative effect	Exposure pathways	Reference
BTEX	Benzene ≤ 0.01 mg/L (WHO drinking water)	Neurotoxicity, carcinogenicity, reproductive toxicity	Inhalation, ingestion of contaminated water	WHO (2004)
Polycyclic aromatic hydrocarbons	≤ 1 mg/kg (Benzo[a]pyrene, EU soil guideline)	Carcinogenic, mutagenic, immunotoxic	Inhalation of fumes, dermal contact, ingestion	Besse-Lototskaya et al. (2024)
Total Petroleum Hydrocarbons	≤ 2000 mg/kg (soil, Nigeria DPR guideline)	Soil infertility, groundwater contamination	Soil leaching, water runoff, and dermal contact	Akporido et al. (2018)
Polychlorinated Biphenyls (PCBs)	≤ 0.1 mg/L (Dutch standard)	Endocrine disruption, cancer, bioaccumulation	Ingestion via the food chain, dermal contact	Annema et al. (1995)
Lead (Pb)	≤ 400 mg/kg (soil, US EPA guideline)	Neurotoxicity, developmental delays, and kidney damage	Inhalation of dust, ingestion, and dermal contact	Darma et al. (2022)
Cadmium (Cd)	≤ 3 mg/kg (soil, WHO guideline)	Kidney damage, bone demineralization, carcinogenicity	Ingestion via crops, dermal contact	Kubier et al. (2019)
Copper (Cu)	≤ 36 mg/kg (soil, WHO guideline)	Liver and kidney damage, ecological toxicity	Inhalation, ingestion, dermal contact	Etana et al. (2025)
Zinc (Zn)	300 mg/kg (soil, WHO guideline)	Disruption of enzyme function, phytotoxicity	Inhalation, ingestion, dermal contact	Ben Chabchoubi et al. (2020)
Zinc Dithiophosphate		Skin and eye irritation, potential reproductive effects	Dermal contact, inhalation of fumes	Huynh et al. (2021)
Arsenic	10–20 mg/kg	Cancers of the skin, Neuropathy, multi-organ failure	Inhalation, ingestion, dermal contact	Huang et al. (2024)
Halogens	$>1,000$ ppm halogens	Toxicity to aquatic life, potential human health effects	Toxicity to aquatic life, potential human health effects	Rauckyte et al. (2006)

The fishing industry, too, suffers severe setbacks due to water contamination. Rivers, lakes, and coastal ecosystems are home to diverse aquatic life, including fish, shellfish, and other organisms, which are critical not only to local diets but also to the livelihoods of communities dependent on

fishing. When these water bodies are contaminated with heavy metals, these pollutants bioaccumulate. As fish and other aquatic species absorb metals from water and sediment, they can experience physiological harm, including weakened immune systems, reproductive failure, and abnormal growth. This reduces fish populations, significantly impacting local fisheries. As fish stocks decline, fishing communities face reductions in both food availability and income. For communities that rely heavily on fishing as their primary source of income, the depletion of aquatic life can be devastating. Fishermen may struggle to catch enough fish to support their families or sell at market prices. The reduced availability of fish and other aquatic species also increases food prices, making it harder for local populations to afford nutritious food. As fish populations dwindle, fishing communities are forced to either move to different areas in search of cleaner water sources or find alternative sources of income, which may not be readily available. The depletion of aquatic life due to heavy metal contamination disrupts local food chains, leading to broader ecological imbalances. Aquatic ecosystems function as interconnected webs of life, with each species playing a role in maintaining the balance. Fish, for example, are a critical food source for larger animals, including birds and humans. The reduction in fish populations caused by contamination can have a cascading effect on the entire ecosystem. When one species declines, it impacts the species that depend on it for food, causing a ripple effect throughout the ecosystem. Additionally, the decline in biodiversity within local water bodies reduces the resilience of ecosystems to external stressors, such as climate change. Healthy ecosystems with diverse species can better withstand disturbances, whereas ecosystems contaminated with pollutants are more vulnerable to further degradation. This not only affects food security and local economies but also threatens the sustainability of natural resources essential for human well-being.

3.6. Exposure Pathway, Prevention, and Mitigation of Spent Engine Oil Pollution

Spent engine oil is a hazardous waste byproduct that poses significant risks to human health and the environment, particularly when not properly disposed of. The exposure pathways for spent engine oil pollution are diverse, affecting soil, water, and air quality. Understanding these pathways, along with effective prevention and mitigation strategies, is essential for minimising the harmful effects of this pollution.

3.6.1. Exposure Pathways

Spent engine oil can contaminate the soil when it is spilled or improperly disposed of. This contamination occurs either through direct dumping, leakage, or runoff from disposal sites that aren't adequately managed. Toxic substances in engine oil, including polycyclic aromatic hydrocarbons (PAHs) and heavy metals such as lead, cadmium, and arsenic, can persist in soil for extended periods. These contaminants can be absorbed by plants, affecting their growth and quality. As crops grow, they may take up these toxins, impacting food safety and agricultural productivity. When spent engine oil enters water bodies, such as rivers, lakes, or groundwater, it forms a thin, persistent surface layer that reduces oxygen exchange, suffocating aquatic life. The contaminants from the oil seep into the water, disrupting ecosystems and bioaccumulating in aquatic organisms. Fish and shellfish absorb these metals and hydrocarbons, which can then enter the food chain, posing long-term risks to both wildlife and humans who consume these animals (Sonone et al., 2020). Airborne contaminants are another significant exposure pathway. In areas where spent engine oil is exposed to heat or sunlight, volatile organic compounds (VOCs) can evaporate, contributing to air pollution. These compounds can degrade air quality, leading to respiratory issues, particularly in workers who handle the oil without proper protection. Inhaling these fumes over time can lead to chronic respiratory diseases. Lastly, workers who handle spent engine oil are at direct risk of exposure through skin contact and inhalation of fumes. Prolonged exposure can cause dermatitis, rashes, and other skin conditions, as well as long-term health effects, such as liver and kidney damage, neurological disorders, and cancer, especially if the oil contains carcinogenic PAHs and heavy metals. Understanding these exposure pathways will inform prophylactic measures.

One of the most effective ways to prevent spent engine oil pollution is through proper disposal and recycling. Used engine oil should never be disposed of in the environment, such as by pouring

it down drains, onto the ground, or into water bodies. This is because it contains harmful substances, such as heavy metals and toxic compounds, which can contaminate soil and water, causing long-term environmental damage. Proper disposal involves collecting the used oil in secure, leak-proof containers immediately after draining it from vehicles or machinery (Rajalakshmi et al., 2023). These containers should be sealed to avoid spills during transportation, ensuring that the oil does not leak into the environment. Once collected, the used engine oil should be taken to certified recycling facilities. These facilities specialise in safely processing and re-refining the oil, preventing it from being released into the environment and transforming it into a valuable resource once again. Recycling used engine oil offers several benefits. One of the primary advantages is re-refining, which filters and cleans the oil to remove impurities. This re-refined oil can be reused as base stock for lubricants or as fuel in industrial applications. This reduces the demand for new raw oil and helps conserve energy and natural resources. Furthermore, it significantly reduces the environmental impact that improper disposal can have on ecosystems. In addition to the technical benefits of oil recycling, it plays a crucial role in reducing environmental pollution. Proper recycling minimizes the risk of contamination to groundwater, soil, and aquatic ecosystems, which can be severely impacted by oil spills. By ensuring that spent oil is processed correctly, we can mitigate its harmful effects and protect natural habitats from long-lasting damage.

Containment systems are critical for preventing the release of hazardous chemicals, waste, or industrial byproducts into the environment. Systems such as lined pits, sealed containers, and specially designed storage areas play a significant role in containing and isolating these substances from surrounding soil, water, and air (Kuwayama et al., 2015). These containment measures help prevent the spread of pollutants, thereby protecting both the environment and public health. Lined pits are one of the most commonly used containment systems for residuals. These pits are typically excavated and then lined with impermeable materials, such as plastic or geomembranes, to prevent leakage. By sealing the bottom and sides of the pit, these liners ensure that hazardous substances are contained and cannot seep into the surrounding soil or groundwater. The design of these pits can vary depending on the type of residuals being stored, but the primary objective is always to create a barrier that isolates the pollutants from the natural environment. Sealed containers offer another effective method of containing residuals, especially when the substances are liquid or semi-solid (L. K. Wang & Wang, 2025). These containers, made from durable materials such as steel, plastic, or concrete, are designed to hold hazardous substances securely without risk of leakage or spill. Containers are typically equipped with tight-fitting lids and often feature additional safety mechanisms, such as pressure relief valves or corrosion-resistant coatings, to ensure that the contents remain contained under various environmental conditions. Specially designed storage areas can include a variety of structures, such as warehouses or specialised tanks, designed to safely store residuals. These storage areas are usually equipped with secondary containment features, such as spill containment trays or bunds, to capture any potential leaks or spills. The design of these areas takes into account factors such as the type of residual being stored, its volume, and the potential risks of chemical reactions or leakage. While these containment systems are essential for the safe management of residuals, their effectiveness depends on regular monitoring and maintenance. Environmental factors, such as extreme weather, chemical reactions, or mechanical wear and tear, can gradually weaken containment materials or cause unexpected breaches. Monitoring these systems helps ensure that any signs of damage or degradation, such as cracks in pit linings or rust on containers, are detected early. This allows for timely repairs or replacements, which are critical for preventing the release of harmful substances into the environment.

In addition to traditional physical inspections, the integration of modern technologies has significantly enhanced the ability to monitor and manage containment systems. One of the most effective ways to improve monitoring is to use sensors. These devices can be installed within containment systems to continuously measure key environmental factors, such as pressure, temperature, and moisture levels. For instance, a change in temperature or moisture could signal an imminent leak or spill, while variations in pressure might indicate structural weaknesses in the containment system. By providing real-time data, sensors can alert operators to potential issues before they escalate into serious environmental hazards. The use of sensors offers a proactive

approach to contamination prevention (Palla & Iwunwa, 2025). For example, if a sensor detects an increase in pressure inside a sealed container, it may indicate that the contents are undergoing a chemical reaction that could lead to rupture. Similarly, if moisture levels rise in a lined pit, it could indicate that water is infiltrating the storage, potentially leading to contamination or flooding. Early detection of such problems allows operators to take corrective actions, such as reinforcing structural components or redirecting water flow, thereby preventing contamination. Automated systems further enhance the management of containment systems by streamlining monitoring and reporting. These systems can track critical variables, such as the volume of residuals stored, to ensure containment capacities are not exceeded. By keeping precise records of how much material is stored and its movement, automated systems help avoid overfilling, which could lead to spills or leaks.

Furthermore, these systems can provide real-time updates on the status of residuals, alerting operators when it's time to remove or treat the materials to prevent overflow or degradation. Routine maintenance is equally crucial to ensuring that containment systems remain functional and effective over time. While modern technology provides early warnings and continuous monitoring, regular maintenance is still needed to ensure the system's integrity. This includes tasks such as cleaning, which prevents the build-up of hazardous residues that might impair the system's performance. It also involves reinforcing structural elements, such as replacing worn-out seals or repairing cracks in containment liners, to preserve their protective properties. Another important aspect of maintenance is the replacement of damaged components (Abdi & Taghipour, 2019). Over time, parts of the containment system, whether mechanical, electrical, or structural, may wear out due to exposure to chemicals, extreme weather, or normal wear and tear. Identifying and promptly replacing these components ensures the system continues to function as designed without failure. For example, seals or liners made from materials that degrade over time should be replaced to prevent leaks. Scheduled maintenance checks offer an opportunity not only to perform routine repairs but also to assess the overall performance of the containment system. These checks can help identify areas for improvement or potential upgrades, whether it's adjusting the system for increased capacity or enhancing its ability to handle different types of residuals. During these reviews, operators can also evaluate the effectiveness of the monitoring technologies, ensuring that sensors are properly calibrated and that automated systems are functioning as intended. This ongoing evaluation ensures that the containment system remains capable of protecting the environment and minimizing risks to public health.

Residuals from various industrial or chemical processes that end up in soil can have harmful effects on the environment, particularly when exposed to elements such as rain, heat, and sunlight. These residuals can include chemicals, heavy metals, or other pollutants that, if left unprotected, can leach into the soil and water, further contaminating the environment and impacting plant, animal, and human health. To mitigate the risks, it is crucial to protect these residuals from rain, heat, and sunlight. Rainwater can wash away pollutants from residuals, spreading them over a larger area and allowing them to infiltrate the soil, contaminating groundwater sources and increasing the chances of widespread pollution. Covering residuals with protective layers, such as tarps or impermeable materials, can help prevent rain from directly contacting the harmful substances and dispersing them. Similarly, heat and sunlight can cause chemical reactions that may make the residuals even more toxic or volatile. Some chemicals, when exposed to high temperatures or UV radiation, can break down into more hazardous substances (Cormier et al., 2006). Heat can also cause the evaporation of harmful compounds into the air, leading to air pollution or potential health risks for surrounding communities. To prevent this, residuals should be stored in shaded, cool areas where direct sunlight is minimised. Using materials such as UV-resistant covers or creating physical barriers, such as sheds, can protect these residuals from excessive heat and sunlight.

Public education plays a crucial role in addressing the pollution caused by spent engine oil. One of the primary steps in reducing this environmental hazard is ensuring that communities understand the importance of proper oil disposal and other precautionary practices. Many individuals may not be aware of the dangers of improper disposal of used engine oil, including its potential to contaminate soil and water sources. Educating the public about these risks and the need for safe disposal practices can foster responsible behaviour. To promote proper disposal, communities should be encouraged

to utilise designated drop-off points and participate in recycling programs. Establishing easily accessible collection centers for used oil ensures that individuals have a straightforward option to dispose of it correctly. In addition, public awareness campaigns should highlight the environmental impacts of improper disposal, including contamination of local water supplies and potential harm to wildlife and plant life. These efforts, coupled with accessible disposal infrastructure, can significantly reduce the number of people who improperly dispose of engine oil, leading to a cleaner, safer environment (Barra & González, 2018). Furthermore, governments play a key role in enforcing policies that ensure proper oil management practices are adhered to. Establishing and enforcing regulatory frameworks for the collection, storage, transportation, and disposal of used engine oil is essential. These regulations should include clear guidelines for proper oil disposal and penalties for non-compliance to encourage accountability among individuals, businesses, and industries. Governments must also ensure regular inspections to monitor oil disposal practices and ensure that businesses and individuals follow the prescribed standards. In addition to regulations, establishing collection points in local communities is crucial to facilitate proper disposal. These collection points should be well-publicized and easily accessible to the public, thereby reducing the likelihood that individuals will resort to improper disposal methods. Communities should be actively involved in the setup and management of these collection points, making it a shared responsibility that encourages greater community participation.

Another promising strategy for preventing oil pollution is promoting biodegradable lubricants or alternative engine oils that break down more readily in the environment. These alternatives reduce the persistence of pollutants when oil is accidentally spilled or improperly disposed of. Encouraging industries and individuals to make the switch to these safer, more eco-friendly lubricants can mitigate the long-term environmental impact of engine oil contamination (Petruaru & Gavrilescu, 2010). Governments, in collaboration with industries, can offer incentives for using biodegradable alternatives, such as tax breaks or subsidies, to make them more accessible and attractive to consumers. In industrial settings, adopting best management practices (BMPs) is critical for preventing oil spills and minimizing contamination. This includes using spill containment systems to prevent oil from spreading in the event of an accident, and ensuring machinery is properly maintained to reduce the likelihood of leaks or spills. Safe storage facilities for spent oil must be available to ensure that it is securely contained until it can be recycled or disposed of properly. Regular maintenance checks of machinery and storage systems can help identify potential risks before they lead to contamination. Furthermore, worker training is essential to ensure they understand and follow proper handling procedures when working with engine oil. This includes knowledge of how to safely store, handle, and dispose of used oil. Emergency response protocols should be in place to address accidental releases quickly and effectively, limiting the environmental impact. Workers should be familiar with these protocols through regular drills and education, which helps ensure that any accidental spills are contained swiftly and safely.

3.6.2. Mitigation of Spent Engine Oil Pollution

When contamination occurs, various mitigation techniques can be employed to reduce the environmental impact and promote recovery. One of the most widely recognized and effective methods is bioremediation, which uses natural processes to clean up polluted sites. Bioremediation relies on microorganisms, such as bacteria and fungi, to break down toxic hydrocarbons found in spent engine oil. These microorganisms can metabolize the harmful components of oil, making bioremediation an eco-friendly and sustainable approach to environmental cleanup. Bioremediation can be applied through several processes, including land farming and bioventing. Land farming involves treating contaminated soil by spreading it out in thin layers and then adding nutrients to enhance microbial activity (Lukić et al., 2017). The added nutrients stimulate the growth and activity of naturally occurring oil-degrading microbes, allowing them to break down the hydrocarbons in the soil more efficiently. This method is effective in restoring soil quality over time and can be implemented at relatively low cost. On the other hand, bioventing involves introducing air or oxygen into the contaminated soil (Anekwe & Isa, 2024). This process increases the oxygen supply, encouraging the growth of aerobic microbes that specialize in degrading oil and other organic

pollutants. Bioventing is often used in areas with deeper contamination or where soil aeration is required for microbial degradation to occur effectively. Another innovative and natural remediation strategy is phytoremediation, which uses plants to absorb, degrade, or transform contaminants in the soil (Terry & Banuelos, 2020). Certain plants, known as hyperaccumulators, can absorb heavy metals and hydrocarbons from the environment and store them in their tissues. Plants such as sunflowers and mustard are particularly effective at absorbing oil residues and other pollutants. These plants can be strategically planted in contaminated areas, where they act as natural filters, removing harmful substances from the soil and improving its overall health. Once the plants have absorbed the contaminants, they can be harvested and safely disposed of, effectively removing pollutants from the environment. This method is both eco-friendly and cost-effective, as it relies on natural processes to restore soil quality without the need for complex machinery or chemical treatments.

However, in cases of severe contamination, biological methods such as bioremediation and phytoremediation may not be sufficient on their own, and additional physical methods may be required. Soil washing and excavation are two physical techniques that can be employed to treat heavily contaminated soil. Soil washing involves treating contaminated soil with water, chemical agents, or surfactants to extract pollutants from soil particles (J. Liu et al., 2022). The contaminants are then separated from the soil, allowing the cleaned material to be returned to the site or treated further. This method can effectively reduce contamination levels, but it is typically costlier and labour-intensive than biological approaches. When contamination is extensive or deeply ingrained, excavation may be the most effective solution. This process involves removing the contaminated soil from the site and transporting it to a treatment facility for proper treatment or disposal. Excavation is often necessary when pollutants have penetrated deep into the soil or when bioremediation methods cannot address the extent of the contamination. Although excavation can be effective at rapidly reducing contamination levels, it is much more expensive and more disruptive to the environment than other remediation methods. For water contamination, filtration systems such as activated carbon filters, membrane filtration, and chemical precipitation can be used to remove toxic metals and hydrocarbons from polluted water (Sbardella et al., 2018). These systems can help restore water quality, making it safe for human consumption and agricultural use. Monitoring local water sources is crucial to detecting contamination early and preventing the spread of pollutants. Environmental restoration projects are also essential to mitigate the long-term effects of spent engine oil pollution. These projects focus on rebuilding ecosystems damaged by oil spills, including replanting vegetation to restore soil health, introducing new fish populations to affected water bodies, and cleaning up contaminated habitats. Environmental restoration aims to restore natural functions, such as water filtration, nutrient cycling, and biodiversity, which are critical for the sustainability of local ecosystems and food security.

4. Conclusions

This study aimed to assess the trend of spent engine oil (SEO) pollution across mechanic shops with varying pollution ages, ranging from one to 20 years. The findings reveal that as the pollution age increases, levels of pollutants such as total petroleum hydrocarbons (TPH), polycyclic aromatic hydrocarbons (PAH), and cumulative heavy metals also rise. Concurrently, the contaminated environment showed a decrease in heterotrophic bacteria and an increase in hydrocarbon-utilising bacteria (HUB). While HUBs play a beneficial role in the biodegradation of hydrocarbons, their capacity to mitigate the accumulation of hydrocarbons and heavy metals is insufficient, especially in older polluted soils. This suggests that the microbial degradation of pollutants is not occurring at a fast rate to prevent the ongoing accumulation of contaminants. The build-up of these pollutants poses significant health risks, particularly in densely populated areas where mechanic workshops are often located. Given these findings, it is crucial to implement effective remediation strategies in mechanic workshops to reduce the health risks associated with long-term exposure to such pollutants. This highlights the need for intentional actions, such as regular pollution monitoring and soil remediation, to protect public health.

Author Contributions: CN, EOF and HOS contributed to the conceptualisation of the study. EOF, CN, CCU and JOP drafted the original manuscript under the supervision of HOS. All authors approved the final manuscript and agreed to its submission.

Conflicts of Interest: The authors declare that there are no conflicts of interest related to this work.

Funding statement: This research received no specific grant from any funding agency, commercial, or not-for-profit sectors.

Generative AI Statement: The authors declare that no generative artificial intelligence was used except for language editing.

References

1. Abdi, A., & Taghipour, S. (2019). Sustainable asset management: A repair-replacement decision model considering environmental impacts, maintenance quality, and risk. *Computers & Industrial Engineering*, *136*, 117–134.
2. Agency (USEPA), U. S. E. P. (2003). US EPA Method 8015D (SW-846): Nonhalogenated Organics Using GC/FID. USEPA Washington, DC, USA.
3. Akporido, S. O., Emoyan, O. O., & Ipeaiyeda, A. R. (2018). Soil quality and extent of soil-plant transfer of trace metals in areas adjoining the Benin-Ethiopia fluvial system in the vicinity of Sapele, Nigeria. *Environment Conservation Journal*, *19*(1 & 2), 17–37.
4. Alao, J. O., Saqr, A. M., Ayejoto, D. A., Otokpa, O. J., Abubakar, F., Mohammed, M. A., & Ibe, A. A. (2025). Environmental impacts of hydrocarbon contaminants and associated potential public health risks. *Journal of Hazardous Materials Advances*, 100853.
5. Albano, G. D., Gagliardo, R. P., Montalbano, A. M., & Profita, M. (2022). Overview of the mechanisms of oxidative stress: Impact in inflammation of the airway diseases. *Antioxidants*, *11*(11), 2237.
6. Alissa, E. M., & Ferns, G. A. (2011). Heavy Metal Poisoning and Cardiovascular Disease. *Journal of Toxicology*, *2011*, 1–21. <https://doi.org/10.1155/2011/870125>
7. Anekwe, I. M. S., & Isa, Y. (2024). Application of biostimulation and bioventing system as bioremediation strategy for the treatment of crude oil contaminated soils. *Soil & Water Research*, *19*(2). <https://www.agriculturejournals.cz/pdfs/swr/2024/02/03.pdf>
8. Angon, P. B., Islam, M. S., Das, A., Anjum, N., Poudel, A., & Suchi, S. A. (2024). Sources, effects and present perspectives of heavy metals contamination: Soil, plants and human food chain. *Heliyon*, *10*(7). [https://www.cell.com/heliyon/fulltext/S2405-8440\(24\)04388-3](https://www.cell.com/heliyon/fulltext/S2405-8440(24)04388-3)
9. Annema, J. A., Beurskens, J. E. M., & Bodar, C. W. M. (1995). NATIONAL INSTITUTE OF PUBLIC HEALTH AND ENVIRONMENTAL PROTECTION BILTHOVEN, THE NETHERLANDS. <https://www.rivm.nl/bibliotheek/rapporten/601014011.pdf>
10. Association, A. P. H. (1926). *Standard methods for the examination of water and wastewater* (Vol. 6). American public health association.
11. Baev, V. (2025). Bioinformatics Research in Bacterial Genomics and Metagenomics. In *Current Issues in Molecular Biology* (Vol. 47, Issue 4, p. 258). MDPI. <https://www.mdpi.com/1467-3045/47/4/258>
12. Barra, R., & González, P. (2018). Sustainable chemistry challenges from a developing country perspective: Education, plastic pollution, and beyond. *Current Opinion in Green and Sustainable Chemistry*, *9*, 40–44.
13. Ben Chabchoubi, I., Mtibaa, S., Ksibi, M., & Hentati, O. (2020). Health risk assessment of heavy metals (Cu, Zn, and Mn) in wild oat grown in soils amended with sediment dredged from the Joumine Dam in Bizerte, Tunisia. *Euro-Mediterranean Journal for Environmental Integration*, *5*(3), 60. <https://doi.org/10.1007/s41207-020-00193-9>
14. Besse-Lototskaya, A., Matson, A., Hendriks, C., & Hazeu, G. (2024). *Reflection on the Dutch points of interest regarding the proposed EU Directive on Soil Monitoring and Resilience*. Wageningen University & Research. <https://edepot.wur.nl/651722>
15. Birrer, S. C., Dafforn, K. A., & Johnston, E. L. (2017). Microbial Community Responses to Contaminants and the Use of Molecular Techniques. In C. Cravo-Laureau, C. Cagnon, B. Lauga, & R. Duran (Eds.), *Microbial Ecotoxicology* (pp. 165–183). Springer International Publishing. https://doi.org/10.1007/978-3-319-61795-4_8

16. Black, C. (2013). *Screening Assessment for the Challenge*. https://www.canada.ca/content/dam/eccc/migration/ese-ees/2cf34283-cd2b-4362-a5d6-ad439495d0d1/fsar_b12-20-201333-86-4-20-carbon-20black-_en.pdf
17. Borska, L., Fiala, Z., Krejsek, J., Hamáková, K., Andrýs, C., Šmejkalová, J., Vokurkova, D., & Kremláček, J. (2006). Cytogenetic and Immunological Changes after Dermal Exposure to Polycyclic Aromatic Hydrocarbons and UV Radiation. *Physiological Research*, 55(3). https://www.researchgate.net/profile/Jan-Kremlacek/publication/7677411_Cytogenetic_and_immunological_changes_after_dermal_exposure_to_polycyclic_aromatic_hydrocarbons_and_UV_radiation/links/09e4151222154e96a9000000/Cytogenetic-and-immunological-changes-after-dermal-exposure-to-polycyclic-aromatic-hydrocarbons-and-UV-radiation.pdf
18. Cao, X., Xiong, H., Fan, Y., & Xiong, L. (2024). Comparing the Effects of Two Culture Methods to Determine the Total Heterotrophic Bacterial Colony Count in Hospital Purified Water. *Journal of Epidemiology and Global Health*, 14(1), 184–192. <https://doi.org/10.1007/s44197-023-00186-1>
19. Chan, T. K., Bramono, D., Bourokba, N., Krishna, V., Wang, S. T., Neo, B. H., Lim, R. Y., Kim, H., Misra, N., & Lim, S. (2021). Polycyclic aromatic hydrocarbons regulate the pigmentation pathway and induce DNA damage responses in keratinocytes, a process driven by systemic immunity. *Journal of Dermatological Science*, 104(2), 83–94.
20. Charkiewicz, A. E., Omeljaniuk, W. J., Nowak, K., Garley, M., & Nikliński, J. (2023). Cadmium toxicity and health effects—A brief summary. *Molecules*, 28(18), 6620.
21. Chicca, I., Becarelli, S., & Di Gregorio, S. (2022). Microbial involvement in the bioremediation of total petroleum hydrocarbon polluted soils: Challenges and perspectives. *Environments*, 9(4), 52.
22. Chikere, C. B., Fenibo, E. O., & Akaranta, O. (2018). Comparative effectiveness of activated soil in bioremediation of a farmland polluted soil by polyaromatic hydrocarbon in the Niger Delta. *Journal of Bioremediation and Biodegradation*, 6(9), 456.
23. Cormier, S. A., Lomnicki, S., Backes, W., & Dellinger, B. (2006). Origin and health impacts of emissions of toxic by-products and fine particles from combustion and thermal treatment of hazardous wastes and materials. *Environmental Health Perspectives*, 114(6), 810–817.
24. Da Silva, N., Taniwaki, M. H., Junqueira, V. C., Silveira, N., Okazaki, M. M., & Gomes, R. A. R. (2018). *Microbiological examination methods of food and water: A laboratory manual*. CRC Press. <https://www.taylorfrancis.com/books/mono/10.1201/9781315165011/microbiological-examination-methods-food-water-neusely-da-silva-val%C3%A9ria-junqueira-neliane-silveira-marta-taniwaki-renato-abear-romeiro-gomes-margarete-midori-okazaki>
25. Darma, A., Ibrahim, S., Sani, A., Zandi, P., & Yang, J. (2022). Appraisal of lead (Pb) contamination and potential exposure risk associated with agricultural soils and some cultivated plants in gold mines. *Environmental Systems Research*, 11(1), 14. <https://doi.org/10.1186/s40068-022-00259-3>
26. Das, N., Kumar, V., Chaure, K., & Pandey, P. (2025). Environmental restoration of polyaromatic hydrocarbon-contaminated soil through sustainable rhizoremediation: Insights into bioeconomy and high-throughput systematic analysis. *Environmental Science: Advances*, 4(6), 842–883.
27. Dudala, S., Dubey, S. K., & Goel, S. (2020). Microfluidic soil nutrient detection system: Integrating nitrite, pH, and electrical conductivity detection. *IEEE Sensors Journal*, 20(8), 4504–4511.
28. Dutta, S., Islam, Z., Das, S., Barman, A., Chowdhury, M., Mondal, B. P., Ajnabi, J., & Manna, D. (2025). Harmonizing plant resilience: Unveiling the symphony of membrane lipid dynamics in response to abiotic stresses: a review. *Discover Plants*, 2(1), 61. <https://doi.org/10.1007/s44372-025-00152-0>
29. Etana, E., Hussein, R., & Huluka, A. (2025). Evaluation of some physicochemical parameters and health risks associated with potentially toxic elements (PTEs) in agricultural soils from the southwest region of Ethiopia. *Journal of Hazardous Materials Advances*, 17, 100561.
30. Fauvet, B., Rebeaud, M. E., Tiwari, S., De Los Rios, P., & Goloubinoff, P. (2021). Repair or degrade: The thermodynamic dilemma of cellular protein quality-control. *Frontiers in Molecular Biosciences*, 8, 768888.
31. Fenibo, E. O., Nkuna, R., & Matambo, T. (2024a). Impact of artisanal refining activities on bacterial diversity in a Niger Delta fallow land. *Scientific Reports*, 14(1), 3866.

32. Fenibo, E. O., Nkuna, R., & Matambo, T. (2024b). Impact of artisanal refining activities on bacterial diversity in a Niger Delta fallow land. *Scientific Reports*, *14*(1), 3866.
33. Ferguson, J. M., Costello, S., Elser, H., Neophytou, A. M., Picciotto, S., Silverman, D. T., & Eisen, E. A. (2020). Chronic obstructive pulmonary disease mortality: The Diesel Exhaust in Miners Study (DEMS). *Environmental Research*, *180*, 108876.
34. Ficetola, G. F., Marta, S., Guerrieri, A., Cantera, I., Bonin, A., Cauvy-Fraunié, S., Ambrosini, R., Caccianiga, M., Anthelme, F., & Azzoni, R. S. (2024). The development of terrestrial ecosystems emerging after glacier retreat. *Nature*, *632*(8024), 336–342.
35. Fu, Z., & Xi, S. (2020). The effects of heavy metals on human metabolism. *Toxicology Mechanisms and Methods*, *30*(3), 167–176. <https://doi.org/10.1080/15376516.2019.1701594>
36. Gandhi, K., Sharma, N., Gautam, P. B., Sharma, R., Mann, B., & Pandey, V. (2022). Atomic Absorption Spectroscopy and Flame Photometry. In K. Gandhi, N. Sharma, P. B. Gautam, R. Sharma, B. Mann, & V. Pandey, *Advanced Analytical Techniques in Dairy Chemistry* (pp. 219–247). Springer US. https://doi.org/10.1007/978-1-0716-1940-7_11
37. Gelaye, Y. (2024). Public health and economic burden of heavy metals in Ethiopia. *Heliyon*, *10*(19). [https://www.cell.com/heliyon/fulltext/S2405-8440\(24\)15053-0](https://www.cell.com/heliyon/fulltext/S2405-8440(24)15053-0)
38. Hart, J. E., Laden, F., Schenker, M. B., & Garshick, E. (2006). Chronic Obstructive Pulmonary Disease Mortality in Diesel-Exposed Railroad Workers. *Environmental Health Perspectives*, *114*(7), 1013–1017. <https://doi.org/10.1289/ehp.8743>
39. Hicks, J. D. (2024). The potential for micropollutants and microbial indicators as tracers of domestic sewage contamination along the Bow River. <https://era.library.ualberta.ca/items/b11c6361-9486-4744-898b-c0c612e28321>
40. Huang, Y., Zhang, N., Ge, Z., Lv, C., Zhu, L., Ding, C., Liu, C., Peng, P., Wu, T., & Wang, Y. (2024). Determining soil conservation strategies: Ecological risk thresholds of arsenic and the influence of soil properties. *Eco-Environment & Health*, *3*(2), 238–246.
41. Huynh, K. K., Tieu, K. A., & Pham, S. T. (2021). Synergistic and competitive effects between zinc dialkyldithiophosphates and modern generation of additives in engine oil. *Lubricants*, *9*(4), 35.
42. Ibe, F. C., Duru, C. E., Isiuku, B. O., & Akalazu, J. N. (2021). Ecological risk assessment of the levels of polycyclic aromatic hydrocarbons in soils of the abandoned sections of Orji Mechanic Village, Owerri, Imo State, Nigeria. *Bulletin of the National Research Centre*, *45*(1), 18. <https://doi.org/10.1186/s42269-021-00485-2>
43. Innocent, M. O., Mustapha, A., Abdulsalam, M., Livinus, M. U., Samuel, J. O., Elelu, S.-A., Lateefat, S. O., & Muhammad, A. S. (2024). Soil Microbes and Soil Contamination. In S. A. Aransiola, H. I. Atta, & N. R. Maddela (Eds.), *Soil Microbiome in Green Technology Sustainability* (pp. 3–35). Springer Nature Switzerland. https://doi.org/10.1007/978-3-031-71844-1_1
44. Kebede, G., Tafese, T., Abda, E. M., Kamaraj, M., & Assefa, F. (2021). Factors Influencing the Bacterial Bioremediation of Hydrocarbon Contaminants in the Soil: Mechanisms and Impacts. *Journal of Chemistry*, *2021*, 1–17. <https://doi.org/10.1155/2021/9823362>
45. Kubier, A., Wilkin, R. T., & Pichler, T. (2019). Cadmium in soils and groundwater: A review. *Applied Geochemistry*, *108*, 104388.
46. Kuwayama, Y., Roeshot, S. S., Krupnick, A., & Richardson, N. D. (2015). Pits versus tanks: Risks and mitigation options for on-site storage of wastewater from shale gas and tight oil development. *Resources for the Future Discussion Paper*, 15–53.
47. Li, C., Cui, C., Zhang, J., Shen, J., He, B., Long, Y., & Ye, J. (2023). Biodegradation of petroleum hydrocarbons based pollutants in contaminated soil by exogenous effective microorganisms and indigenous microbiome. *Ecotoxicology and Environmental Safety*, *253*(15), 114673.
48. Lin, X., Qiao, B., Chang, R., Li, Y., Zheng, W., He, Z., & Tian, Y. (2023). Characterization of two keystone taxa, sulfur-oxidizing, and nitrate-reducing bacteria, by tracking their role transitions in the benzo[a]pyrene degradative microbiome. *Microbiome*, *11*(1), 139. <https://doi.org/10.1186/s40168-023-01583-1>

49. Ling, J., Zhou, W., Yang, Q., Lin, X., Zhang, Y., Ahmad, M., Peng, Q., & Dong, J. (2021). Effect of PAHs on nitrogen-fixing and sulfate-reducing microbial communities in seagrass *Enhalus acoroides* sediment. *Archives of Microbiology*, 203(6), 3443–3456. <https://doi.org/10.1007/s00203-021-02321-7>
50. Liu, J., Zhao, L., Liu, Q., Li, J., Qiao, Z., Sun, P., & Yang, Y. (2022). A critical review on soil washing during soil remediation for heavy metals and organic pollutants. *International Journal of Environmental Science and Technology*, 19(1), 601–624.
51. Liu, P., Wen, S., Zhu, S., Hu, X., & Wang, Y. (2025). Microbial Degradation of Soil Organic Pollutants: Mechanisms, Challenges, and Advances in Forest Ecosystem Management. *Processes*, 13(3), 916.
52. Lu, F., Song, Q., & Liu, W. (2025). Mobilization of PAHs by Wave-Induced Resuspension and Liquefaction in Silty Sediment. *Journal of Marine Science and Engineering*, 13(9), 1661.
53. Lukić, B., Panico, A., Huguenot, D., Fabbicino, M., Van Hullebusch, E. D., & Esposito, G. (2017). A review on the efficiency of landfarming integrated with composting as a soil remediation treatment. *Environmental Technology Reviews*, 6(1), 94–116. <https://doi.org/10.1080/21622515.2017.1310310>
54. Mekonnen, B. A., Aragaw, T. A., & Genet, M. B. (2024). Bioremediation of petroleum hydrocarbon contaminated soil: A review on principles, degradation mechanisms, and advancements. *Frontiers in Environmental Science*, 12, 1354422.
55. Mohanta, S., Pradhan, B., & Behera, I. D. (2024a). Impact and Remediation of Petroleum Hydrocarbon Pollutants on Agricultural Land: A Review. *Geomicrobiology Journal*, 41(4), 345–359. <https://doi.org/10.1080/01490451.2023.2243925>
56. Mohanta, S., Pradhan, B., & Behera, I. D. (2024b). Impact and Remediation of Petroleum Hydrocarbon Pollutants on Agricultural Land: A Review. *Geomicrobiology Journal*, 41(4), 345–359. <https://doi.org/10.1080/01490451.2023.2243925>
57. Monteiro, J. P. S. (2025). Exploring the potential of native microorganisms from diesel-contaminated areas for enhanced petroleum hydrocarbon degradation. <https://repositorio.ufsc.br/handle/123456789/265388>
58. Nwaozuzu, C. C., Partick-Iwuanyanwu, K. C., & Abah, S. O. (2021). Systematic Review of Exposure to Polycyclic Aromatic Hydrocarbons and Obstructive Lung Disease. *Journal of Health and Pollution*, 11(31), 210903. <https://doi.org/10.5696/2156-9614-11.31.210903>
59. Oberoi, S., Barchowsky, A., & Wu, F. (2014). The global burden of disease for skin, lung, and bladder cancer caused by arsenic in food. *Cancer Epidemiology, Biomarkers & Prevention*, 23(7), 1187–1194.
60. Okebalama, C. B., Onwurah, C. L., Jidere, C. M., & Okolo, C. C. (2024). Disposal of spent oil into soils around auto parts markets impacts heavy metal concentrations and poses a potential ecological risk. *Environmental Systems Research*, 13(1), 15. <https://doi.org/10.1186/s40068-024-00343-w>
61. Organization, W. H. (2004). *Guidelines for drinking-water quality* (Vol. 1). World health organization. [https://books.google.com/books?hl=en&lr=&id=SJ76COTm-nQC&oi=fnd&pg=PR15&dq=WHO+\(2003\)+Benzene+in+drinking-water.+Background+document+for+preparation+of+WHO+Guidelines+for+drinking-water+quality.+Geneva,+World+Health+Organization+\(WHO/SDE/WSH/03.04/24\).&ots=Vat1ocQ7_c&sig=YwcJf5TrdOOvR0Tx5X3Ez08_8VY](https://books.google.com/books?hl=en&lr=&id=SJ76COTm-nQC&oi=fnd&pg=PR15&dq=WHO+(2003)+Benzene+in+drinking-water.+Background+document+for+preparation+of+WHO+Guidelines+for+drinking-water+quality.+Geneva,+World+Health+Organization+(WHO/SDE/WSH/03.04/24).&ots=Vat1ocQ7_c&sig=YwcJf5TrdOOvR0Tx5X3Ez08_8VY)
62. Palla, U., & Iwunwa, M.-G. C. (2025). Leveraging predictive models to enhance infection control and reduce cross-contamination in public health settings. *International Journal of Research Publication and Reviews*, 6(1), 407.
63. Palma-Lara, I., Martínez-Castillo, M., Quintana-Pérez, J. C., Arellano-Mendoza, M. G., Tamay-Cach, F., Valenzuela-Limón, O. L., García-Montalvo, E. A., & Hernández-Zavala, A. (2020). Arsenic exposure: A public health problem leading to several cancers. *Regulatory Toxicology and Pharmacology*, 110, 104539.
64. Parida, L., & Patel, T. N. (2023). Systemic impact of heavy metals and their role in cancer development: A review. *Environmental Monitoring and Assessment*, 195(6), 766. <https://doi.org/10.1007/s10661-023-11399-z>
65. Pashin, Y. V., & Bakhitova, L. M. (1979). Mutagenic and carcinogenic properties of polycyclic aromatic hydrocarbons. *Environmental Health Perspectives*, 30, 185–189. <https://doi.org/10.1289/ehp.7930185>
66. Petraru, M., & Gavrilescu, M. (2010). Pollution prevention, a key to economic and environmental sustainability. *Environmental Engineering and Management Journal*, 9(4), 597–614.
67. Properties, A. S. D. 10 on M. (1995). Standard test method for tensile properties of thin plastic sheeting.

68. Rahman, Z., & Singh, V. P. (2020). Bioremediation of toxic heavy metals (THMs) contaminated sites: Concepts, applications and challenges. *Environmental Science and Pollution Research*, 27(22), 27563–27581. <https://doi.org/10.1007/s11356-020-08903-0>
69. Rai, R., Agrawal, M., & Agrawal, S. B. (2016). Impact of Heavy Metals on Physiological Processes of Plants: With Special Reference to Photosynthetic System. In A. Singh, S. M. Prasad, & R. P. Singh (Eds.), *Plant Responses to Xenobiotics* (pp. 127–140). Springer Singapore. https://doi.org/10.1007/978-981-10-2860-1_6
70. Rajalakshmi, S., Amzad Basha, K., & Asif Jamal, G. A. (2023). A manual on waste management audit. *Laser Park Publish House, Coimbatore, Tamil Nadu, India*. 163p. <https://www.nsfonline.org.in/wp-content/uploads/2023/04/A-Manual-of-Waste-Management-Audit-by-Nature-Science-Foundation-2023-1.pdf>
71. Rauckyte, T., Hargreaves, D. J., & Pawlak, Z. (2006). Determination of heavy metals and volatile aromatic compounds in used engine oils and sludges. *Fuel*, 85(4), 481–485.
72. Ren, L., Zhang, J., Geng, B., Zhao, J., Jia, W., & Cheng, L. (2025). Ecological Shifts and Functional Adaptations of Soil Microbial Communities Under Petroleum Hydrocarbon Contamination. *Water*, 17(8), 1216.
73. Rhead, M. M., & Hardy, S. A. (2003). The sources of polycyclic aromatic compounds in diesel engine emissions☆. *Fuel*, 82(4), 385–393.
74. Sabir, R. M., Sarwar, A., Shoaib, M., Saleem, A., Alhousain, M. H., Wajid, S. A., Rasul, F., Adnan Shahid, M., Anjum, L., Safdar, M., Muhammad, N. E., Waqas, R. M., Zafar, U., & Raza, A. (2024). Managing Water Resources for Sustainable Agricultural Production. In S. Kanga, S. K. Singh, K. Shevkani, V. Pathak, & B. Sajan (Eds.), *Transforming Agricultural Management for a Sustainable Future* (pp. 47–74). Springer Nature Switzerland. https://doi.org/10.1007/978-3-031-63430-7_3
75. Santa Maria, M. P., Hill, B. D., & Kline, J. (2019). Lead (Pb) neurotoxicology and cognition. *Applied Neuropsychology: Child*, 8(3), 272–293. <https://doi.org/10.1080/21622965.2018.1428803>
76. Sbardella, L., Comas, J., Fenu, A., Rodriguez-Roda, I., & Weemaes, M. (2018). Advanced biological activated carbon filter for removing pharmaceutically active compounds from treated wastewater. *Science of the Total Environment*, 636, 519–529.
77. Shukla, S. K., Mangwani, N., & Rao, T. S. (2019). Bioremediation approaches for persistent organic pollutants using microbial biofilms. *Microb Biofilms Bioremed Wastewater Treat*, 179, 179–206.
78. Sokolo, R. S., Atagana, H., & Akani, N. P. (2018). Molecular Characterisation of Culturable Aerobic Hydrocarbon Utilising Bacteria and Fungi in Oil Polluted Soil at Ebubu-Ejama Community, Eleme, Rivers State, Nigeria. *Journal of Advances in Biology and Biotechnology*, 18(4), 1–7.
79. Sonone, S. S., Jadhav, S., Sankhla, M. S., & Kumar, R. (2020). Water contamination by heavy metals and their toxic effect on aquaculture and human health through food Chain. *Lett. Appl. NanoBioScience*, 10(2), 2148–2166.
80. Tan, X. (2023). Remediation of PAHs contaminated soil enhanced by nano-zero-valent iron combined with white rot fungi *Peniophora incarnata*. *Alexandria Engineering Journal*, 83, 85–91.
81. Tan, Y.-S., Zhang, R.-K., Liu, Z.-H., Li, B.-Z., & Yuan, Y.-J. (2022). Microbial adaptation to enhance stress tolerance. *Frontiers in Microbiology*, 13, 888746.
82. Tang, C., Lv, X., Zou, L., Rong, Y., Zhang, L., Xu, M., Li, S., & Chen, G. (2025). Cadmium exposure and osteoporosis: Epidemiological evidence and mechanisms. *Toxicological Sciences*, 205(1), 1–10.
83. Terry, N., & Banuelos, G. S. (2020). *Phytoremediation of contaminated soil and water*. CRC press. https://books.google.com/books?hl=en&lr=&id=N2eYJIC4_5kC&oi=fnd&pg=PP18&dq=phytoremediation+into+the+contaminated+soil&ots=jCZSbWjeMa&sig=GzhdY4I3-xu18YNRY2gLPCXV_WY
84. Thakur, D., Dubey, N. P., & Singh, R. (2024). A Review on Spike and Recovery Method in Analytical Method Development and Validation. *Critical Reviews in Analytical Chemistry*, 54(7), 2053–2071. <https://doi.org/10.1080/10408347.2022.2152275>
85. Thakur, M., Rachamalla, M., Niyogi, S., Datusalia, A. K., & Flora, S. J. S. (2021). Molecular mechanism of arsenic-induced neurotoxicity including neuronal dysfunctions. *International Journal of Molecular Sciences*, 22(18), 10077.

86. Truskewycz, A., Gundry, T. D., Khudur, L. S., Kolobaric, A., Taha, M., Aburto-Medina, A., Ball, A. S., & Shahsavari, E. (2019). Petroleum hydrocarbon contamination in terrestrial ecosystems—Fate and microbial responses. *Molecules*, *24*(18), 3400.
87. Wang, C., & Kuzyakov, Y. (2024). Soil organic matter priming: The pH effects. *Global Change Biology*, *30*(6), e17349. <https://doi.org/10.1111/gcb.17349>
88. Wang, L. K., & Wang, M.-H. S. (2025). Environmental Management of Electroplating and Metal-Finishing Operations. In *Control of Heavy Metals in the Environment* (pp. 325–378). CRC Press. <https://www.taylorfrancis.com/chapters/edit/10.1201/9781003451754-13/environmental-management-electroplating-metal-finishing-operations-lawrence-wang-mu-hao-sung-wang>
89. Wang, Z., Sun, X., Song, K., Lu, J., & Wu, M. (2025). Combined Optimization of Petroleum Hydrocarbon Degradation Rate Prediction Model Using Response Surface Methodology and Artificial Neural Networks. *Journal of the Indian Chemical Society*, 101937.
90. Yanagisawa, R., Koike, E., & Takano, H. (2021). Benzo[a]pyrene aggravates atopic dermatitis-like skin lesions in mice. *Human & Experimental Toxicology*, *40*(12_suppl), S269–S277. <https://doi.org/10.1177/09603271211036123>
91. Zhang, D., Hu, Q., Wang, B., Wang, J., Li, C., You, P., Zhou, R., Zeng, W., Liu, X., & Li, Q. (2023). Effects of single and combined contamination of total petroleum hydrocarbons and heavy metals on soil microecosystems: Insights into bacterial diversity, assembly, and ecological function. *Chemosphere*, *345*, 140288.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.