Physico-chemical, biological and heavy metal status of spent oil-contaminated soils in the vicinity of garages in and around Guwahati city, Assam, India

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In this study, we analyse the impact of the indiscriminate spilling of spent oil by garages on the surrounding soils. Physico-chemical, biological and heavy metal (HM) profiles of the spent oil-contaminated soils were compared with control samples in Guwahati city, Assam, India. The results revealed that the spent oil-contaminated soils show an increase in the abundance of HMs (varying from 58 to 18,400 mg/kg), total oil and grease (77,000–161,000 mg/kg) and a decrease in bacterial load (68.2–76.2%) and enzymatic activities (18.02–98.4%) when compared with control samples. Site-specific remediation strategies are needed to mitigate this problem.

Keywords: Bacterial load, contaminated soil, garage, heavy metals, spent oil.

OIL contamination in the soil is a major problem due to rapid industrialization and urbanization across the globe[1]. With the population explosion, there has been an enormous increase in the use of motor vehicles during the past decades. For repair and maintenance of these vehicles, there has been a simultaneous increase in the number of garages too. These are some of the most polluting sources that discharge spent oil, petroleum, petrochemicals, and organic and inorganic pollutants[2]. Normally, oil products contain several toxic materials. In particular, engine oil used extensively in vehicles consists of several hydrocarbons and heavy metals (HMs). Engine oil contamination and associated pollutants change the nutrient composition available to the soil organisms. In addition, the pollutants are mutagenic and carcinogenic to humans[3].

The spilling of used engine oil has gained serious attention as it can spread to the surrounding regions through rainwater run-off and seep into the groundwater. Besides, it has been reported that HMs, in the spent oil are toxic not only to microbes, plants, animals and humans, but also to agriproducts that contribute to the food chain[4]. Negative impacts of HMs on soil health have been widely reported in the literature[5–7].

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The physico-chemical properties, microbial population and related enzyme activities reflect the ecological health of the soil[8]. The concentration of pollutants such as HMs and changes in physico-chemical properties affect soil enzyme activities. Moreover, soil enzyme activities are considered an indicator of soil quality due to rapid response to changes in the soil environment[4]. Therefore, analysis of enzyme activities is vital to understand the state of soil health before undertaking any remediation strategies. Studies pertaining to adverse effects of spent oil and other associated pollutants on the biological health of soils are still limited in India. The present study has been conducted in Guwahati city, Assam, India, to understand the impact of spent oil contamination on the physico-chemical, biological and HM profiles of the soil.

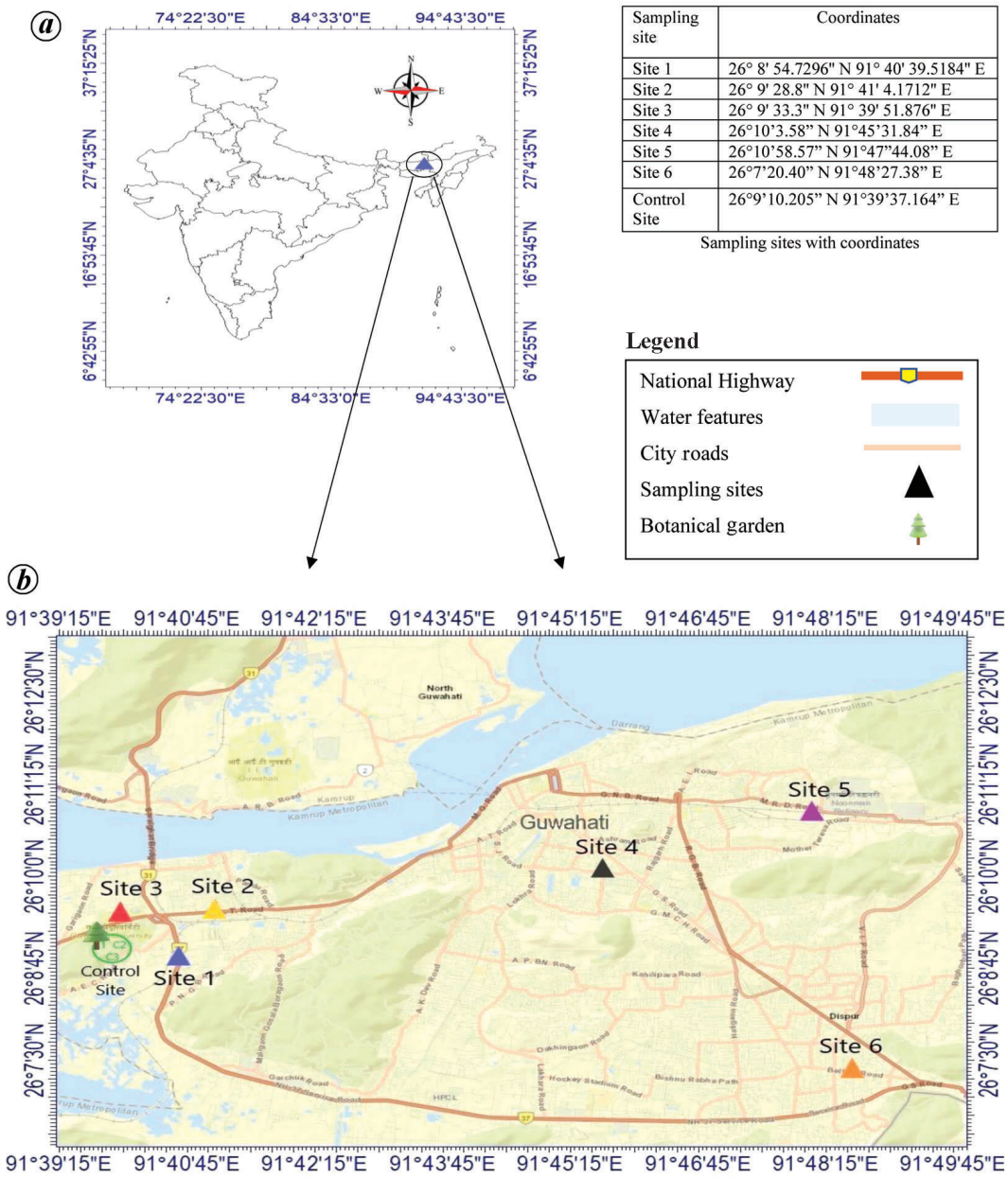
Materials and methods

Collection of soil samples

The soil contaminated by oil from garages was collected from different sites in Guwahati city (Figure 1). For comparison, control soil samples were collected from three different sites (c1–c3) where contamination was not evident. The collected soil samples were dried and processed for further analysis.

Analysis of soil samples

The pH, conductivity, water holding capacity (WHC) and total organic carbon (TOC) of the soil samples were determined using standard methods[9]. The conductivity and pH were measured in 1 : 5 (w/v) soil and water suspension (with the help of Systronics 304 conductivity meter and Biochem PM79 digital pH meter respectively). WHC of the soil samples was measured using the method outlined by Piper[10]. TOC content of the samples was determined following the Walkley and Black titration method, as described by Jackson[11]. Total oil and grease contents of the studied soil samples were extracted using a Soxhlet extractor with dichloromethane



Sampling site	Coordinates
Site 1	26° 8' 54.7296" N 91° 40' 39.5184" E
Site 2	26° 9' 28.8" N 91° 41' 4.1712" E
Site 3	26° 9' 33.3" N 91° 39' 51.876" E
Site 4	26°10'3.58" N 91°45'31.84" E
Site 5	26°10'58.57" N 91°47"44.08" E
Site 6	26°7'20.40" N 91°48'27.38" E
Control Site	26°9'10.205" N 91°39'37.164" E

Sampling sites with coordinates

Figure 1. ***a***, Map of India. ***b***, Map showing study sites in the greater Guwahati area, Assam, with sampling sites of oil-contaminated soils. (Site 1 – Sundarbari (26°8′54.7296″N, 91°40′39.5184″E); site 2 – Adabari (26°9′28.8″N, 91°41′4.1712″E); site 3 – Jalukbari (26°9′33.3″N, 91°39′51.876″E); site 4 – Ulubari (26°10′3.58″N, 91°45′31.84″E); site 5 – Bamunimaidan (26°10′58.57″N, 91°47′44.08″E); site 6 – Khanapara (26°7′20.40″N, 91°48′27.38″E).) Control soil sample site – Botanical garden, Gauhati University (26°9′10.205″N, 91°39′37.164″E). Map source: ArcGIS.

Table 1. Analysis of soil enzyme activities

Enzyme	Reference
Alkaline phosphatase (mg PNP kg^{-1} soil h^{-1})	14
Amylase (mg glucose kg^{-1} soil 24 h^{-1})	15
Catalase (mmol H_2O_2 kg^{-1} soil min^{-1})	16
Cellulase (mg glucose kg^{-1} soil 24 h^{-1})	17
Dehydrogenase (mg TPF kg^{-1} soil 24 h^{-1})	18
Polyphenol oxidase (mmol purpurogallin kg^{-1} soil h^{-1})	19
Peroxidase (mmol purpurogallin kg^{-1} soil h^{-1})	19
Urease (mg NH_4^+–N kg^{-1} soil h^{-1})	20

(DCM) as the solvent and measured gravimetrically[12]. HM analysis of the soil samples was done following the nitric acid digestion method proposed by Zeheljazkov and Nielsen[13]. The metal profiles of the samples were analysed using an atomic absorption spectrometer (AAS) (Shimadzu model no. AA 7000).

Analysis of soil enzymes was carried out following the standard methods (Table 1)[14–20].

The pour plate method and serial dilatation technique determined the total bacterial population along with the

Table 2. Physico-chemical properties of garage oil-contaminated soil

Study site	Latitude and longitude	pH	Conductivity (mS/cm)	TOC (%)	WHC (%)	TOG (mg/kg)
Site 1	26°8′54.7296″N, 91°40′39.5184″E	7.78 ± 0.1a	0.29 ± 0.005a	14.27 ± 0.98b	13.43 ± 0.76a	153000a
Site 2	26°9′28.8″N, 91°41′4.1712″E	7.83 ± 0.032a	0.31 ± 0.032a	15.83 ± 1.13a	12.67 ± 0.83a	161000b
Site 3	26°9′33.3″N, 91°39′51.876″E	7.59 ± 0.05bc	0.32 ± 0.02a	14.59 ± 0.62ab	17.27 ± 1.9b	147000c
Site 4	26°10′3.58″N, 91°45′31.84″E	7.61 ± 0.05b	0.31 ± 0.01a	13.37 ± 0.78b	16.38 ± 1.07b	134000d
Site 5	26°10′58.57″N, 91°47′44.08″E	7.49 ± 0.08c	0.3 ± 0.02a	8.59 ± 1.07c	25.21 ± 1.35c	77000e
Site 6	26°7′20.40″N, 91°48′27.38″E	7.53 ± 0.13bc	0.33 ± 0.04a	11.23 ± 0.93d	21.33 ± 0.91d	117000f
Control soil	26°9′10.205″N, 91°39′37.164″E	6.2 ± 0.04d	1.22 ± 0.015b	2.21 ± 0.09e	51.51 ± 1.81e	ND

Mean ± SD, $n = 3$. Different letters within the same column indicate significant differences in the values (ANOVA, LSD test, $P < 0.05$). TOC, Total organic carbon; ND, Not determined; WHC, Water-holding capacity; TOG, Total oil and grease.

HM degrader bacteria. The nutrient agar medium was used for total bacterial population estimation. Similarly, mineral salt medium (MSM) was utilized for enrichment culture to detect HM degrader bacterial population. All plates were incubated at 37°C in an incubator. Then the colonies were counted using a colony counting software (open CFU) and expressed as colony forming unit/gram (CFU g^{-1}).

Statistical analysis

For statistical analysis, SPSS software (2018 version) was used. Significant differences in the values of the contaminated soil and control soil samples for different parameters were determined by one-way ANOVA and LSD test.

Results and discussion

Physico-chemical properties of the soil

Table 2 shows the physico-chemical properties of soil samples contaminated by garage oil and the control soil samples.

Soil pH is a measure of H^+ ion activity and is responsible for the abundance of soil microbial population and availability of nutrients. The pH in all the contaminated soil samples was found to be alkaline, ranging from 7.49 to 7.83. However, the pH of the control soil sample was a little acidic (6.2). Contamination of the soil with HMs can increase soil pH levels. The soil pH depends upon the biological properties of the soil[21]. The presence of HMs in the spent oil-contaminated soil might have increased the pH by inhibiting the growth of microbes, resulting in low microbial respiration and hydrogen ions (H^+) concentration in the soil. This finding is backed by a previous study where alkaline pH was reported in engine oil-contaminated soils[3].

Soil conductivity is a measure of the soluble salt content in the soil. It is an excellent indicator of the availability of nutrients in the soil. The electrical conductivity (EC) of the contaminated soil samples was in the range 0.29 ± 0.005–0.33 ± 0.04 mS/cm, much lower than that of the control soil sample (1.22 ± 0.015 mS/cm). This result conforms with the previous studies which reported that oil contamination in the soil changes its texture and creates a non-polar environment, which reduces ionic movement in the soil by immobilization and reduction in velocity, consequently lowering the conductivity[8,22].

TOC in the range 8.59–15.83% was higher in the contaminated soil samples than in the control soil sample. An increase in TOC may be due to high concentration of carbon in oil and grease[23].

WHC of a soil is the amount of water that the soil can retain against gravity. It nourishes organic matter in the soil and is particularly important in nutrient management. WHC of the contaminated soil was greatly reduced compared to the control soil sample. It was 12.67–25.21%, as against 51.51% in the control soil sample. The reduction in WHC may be due to a reduction in the absorption capacity of the soil. The spent oil might have changed the hydrophilic nature of the soil to hydrophobic, consequently lowering the wettability surface and reducing the absorption capacity[24].

A significant amount of total oil and grease was detected in the contaminated soil samples, with a maximum of 161,000 mg/kg and a minimum of 77,000 mg/kg. No oil and/or grease was found in the control soil sample. The high oil and grease concentration might be due to the release of spent oil waste from automobile garages, as reported in a previous study[25].

Heavy metals content

Table 3 presents the HM concentration in the soil samples. The permissible limits of HM concentration in the soils, as given by the World Health Organization (WHO)[26] were used as a reference for the contamination level. Excluding nickel (Ni) and chromium (Cr), in the contaminated soil samples, the concentration of other HMs, namely iron (Fe), copper (Cu), zinc (Zn) and manganese (Mn), was found to be higher than the permissible limits given by WHO (Table 3). The highest concentration (18,400 mg/kg) of Fe was found in site 2, as against the minimum value of 4440 mg/kg in the control soil sample. Similarly, the highest concentrations of Cu (149 mg/kg), Zn (1455 mg/kg) and Mn (235.5 mg/kg) was detected in site 2, while it was below permissible limits in the control soil sample. Although the concentration of the nickel (Ni) and chromium (Cr) was below the limit prescribed by WHO, it was found to be high compared to that

Table 3. Heavy metals concentration in the soil samples contaminated with spent oil from garages

Study site Permissible limit (WHO) (mg/kg)	Latitude and longitude	Fe (mg/kg) 20	Cu (mg/kg) 36	Ni (mg/kg) 35	Cr (mg/kg) 100	Zn (mg/kg) 50	Mn (mg/kg) 12
Site 1	26°8′54.7296″N, 91°40′39.5184″E	12600 ± 390d	140 ± 2.5a	11 ± 0.075a	9.65 ± 0.09bc	1050 ± 32a	232 ± 4.8a
Site 2	26°9′28.8″N, 91°41′4.1712″E	18400 ± 700a	149 ± 0.8b	15.3 ± 0.2b	10.95 ± 0.25a	1455 ± 74b	235.5 ± 2.25a
Site 3	26°9′33.3″N, 91°39′51.876″E	13100 ± 530cd	118.5 ± 1.35c	10.45 ± 0.18c	9.85 ± 0.55b	977 ± 44a	223.5 ± 5.5b
Site 4	26°10′3.58″N, 91°45′31.84″E	15700 ± 430b	132.7 ± 1.17d	10.31 ± 0.09c	9.01 ± 0.41d	1137 ± 57c	229.09 ± 4.3ab
Site 5	26°10′58.57″N, 91°47″44.08″E	12000 ± 310e	58 ± 2.5e	7.61 ± 0.07d	8.33 ± 0.1e	575 ± 1.9d	173.79 ± 6.1d
Site 6	26°7′20.40″N, 91°48′27.38″E	13700 ± 630c	125.3 ± 1.63f	8.33 ± 0.11e	9.11 ± 0.36cd	832 ± 61e	196.33 ± 2.78c
Control soil	26°9′10.205″N, 91°39′37.164″E	4440 ± 9f	4.65 ± 0.07g	4.3 ± 0.18f	7.1 ± 0.24f	20.4 ± 0.185f	9.55 ± 0.07e

Mean ± SD, $n = 3$. Different letters within the same column indicate significant differences in the values (ANOVA, LSD test $P < 0.05$); WHO, World Health Organization.

Table 4. Beneficial bacterial population in crude oil-contaminated soil and control soil

Study site	Latitude and longitude	TBP ($\times 10^6$ CFU g^{-1} soil)	HMs DBP ($\times 10^6$ CFU g^{-1} soil)
Site 1	26°8′54.7296″N, 91°40′39.5184″E	58 ± 6.5a	11.34 ± 3.78a
Site 2	26°9′28.8″N, 91°41′4.1712″E	52.34 ± 5.03ab	12.34 ± 2.3a
Site 3	26°9′33.3″N, 91°39′51.876″E	48.34 ± 8.08ab	10.34 ± 1.15a
Site 4	26°10′3.58″N, 91°45′31.84″E	55.67 ± 4.16a	8.67 ± 0.57ab
Site 5	26°10′58.57″N, 91°47″44.08″E	57.34 ± 3.51a	6.34 ± 1.52bc
Site 6	26°7′20.40″N, 91°48′27.38″E	43.34 ± 5.68b	5.67 ± 2.08c
Control soil	26°9′10.205″N, 91°39′37.164″E	182.34 ± 6.42c	ND

Mean ± SD, $n = 3$. Different letters within the same column indicates significant differences in the values (ANOVA, LSD test $P < 0.05$). ND, Not detected; TBP, Total bacterial population; HMs DBP, Heavy metals degrading bacterial population.

in the control soil sample. Similar results have been reported in the literature[2,25]. Interestingly, iron concentration in the control soil sample was also higher than the permissible limit. This may be due to the acidic pH of the soil, which usually carries high iron contents[27]. Moreover, iron concentration increases during the dry season in the soil due to the absence of rainwater run-off and leaching[28].

The detected HM concentration in the oil-contaminated soil was above the geochemical baseline data for Guwahati city[29]. The high levels of HMs in the spent oil-contaminated soil might be due to the release of spent oil and other petroleum products into the soil system.

Bacterial population

The total bacterial population, including HM degrader bacteria, are expressed as colony-forming unit per gram soil (CFU g^{-1} soil) (Table 4). The total bacterial population was found to be in the range 43.34–58 $\times 10^6$ CFU g^{-1} soil in the case of contaminated soil samples, whereas it was 182.34 $\times 10^6$ CFU g^{-1} soil in the control soil sample. Thus, the result shows a significant reduction in the total bacterial population in the contaminated soil samples compared to the control soil sample. The results are in accordance with the results of the previous studies[5,7]. HM degrader bacterial population (5.67 ± 2.08–$11.34 \pm 3.78 \times 10^6$ CFU/g^{-1} soil) was also detected in all the contaminated soil samples. It was, however, not detected in the control soil sample. The presence of HM degrader population in the contaminated soil may be due to the ability of some indigenous microbes to resist and adapt to the stress conditions, as reported in a previous study[5].

Soil enzymatic activities

The soil enzyme activities of urease, amylase, catalase, dehydrogenase, cellulase, alkaline phosphatase, peroxidase and polyphenol oxidase were determined to characterize the response of microbial activities to contamination. Figure 2 present the results in a graphical form.

Urease activity is due to an extracellular enzyme responsible for urea hydrolysis to provide accessible nitrogen to soil organisms. It has been reported in previous studies that HMs can affect a coordination reaction with the enzyme and inhibit it by altering its conformation[4]. The present finding where reduced urease activity was detected in spent oil-contaminated soil (0.117–0.236 mg NH_4^+–N kg^{-1} soil h^{-1}) compared to the control soil sample (0.399 mg NH_4^+–N kg^{-1} soil h^{-1}) is consistent with that of Aponte *et al.*[4] and Guo *et al.*[30].

Soil amylase breaks down the complex polysaccharides, such as starch to glucose. The results obtained for the amylase activity were minimum in the contaminated soil sample (0.014 mg glucose kg^{-1} soil 24 h^{-1}) and maximum in the

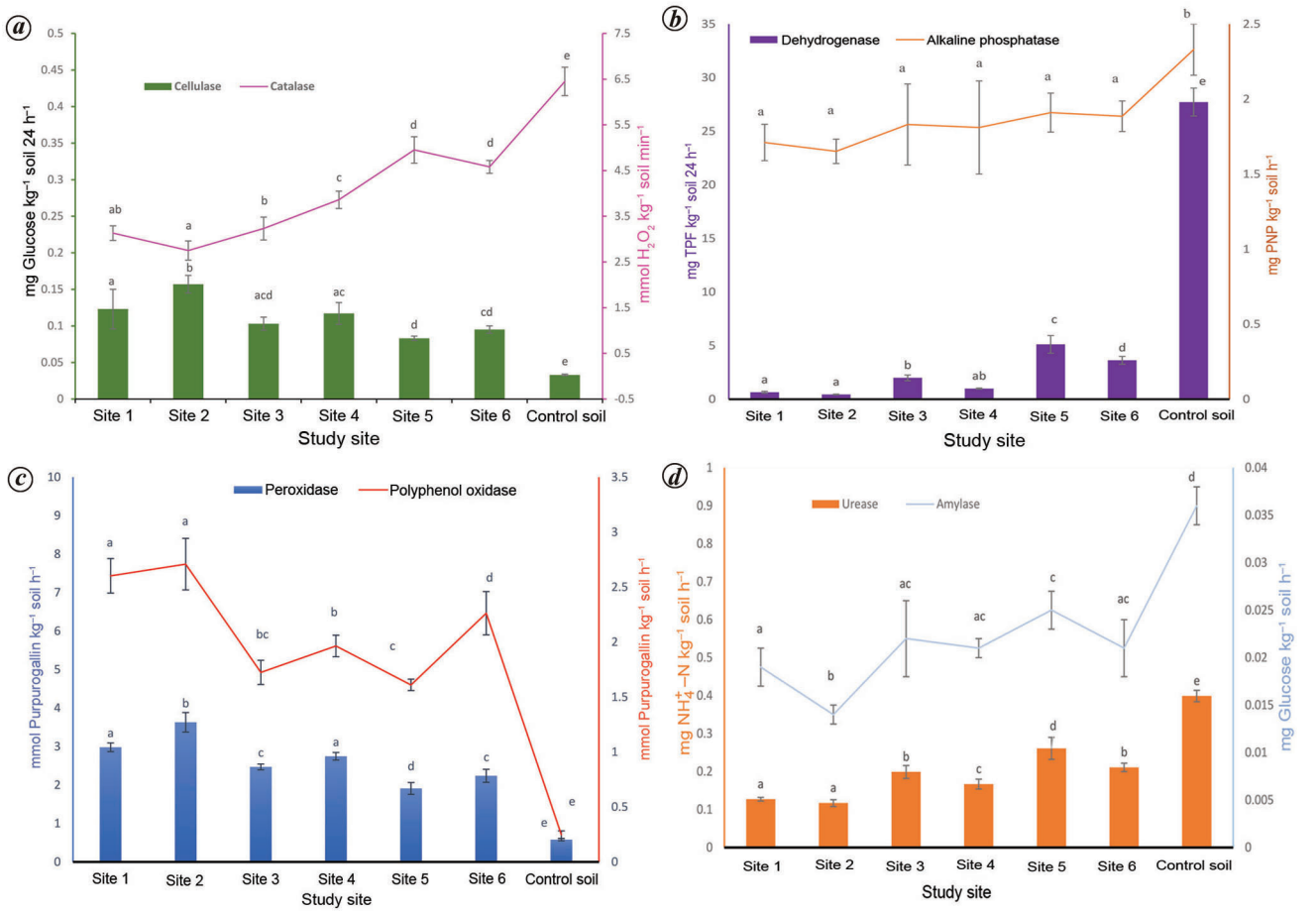


Figure 2. ***a***, Cellulase and catalase enzyme activities in the soil samples. Values are mean of three samples; bars indicate SD. Different letters within the same parameter indicate significant differences in the values (ANOVA, LSD test $P < 0.05$). ***b***, Dehydrogenase and alkaline phosphatase enzyme activities in the soil samples. Values are mean of three samples; bars indicate SD. Different letters within the same parameter indicate significant differences in the values (ANOVA, LSD test $P < 0.05$). ***c***, Peroxidase and polyphenol oxidase enzyme activities in the soil samples. Values are mean of three samples; bars indicate SD. Different letters within the same parameter indicate significant differences in the values (ANOVA, LSD test $P < 0.05$). ***d***, Urease and amylase enzyme activities in the soil samples. Values are mean of three samples, bars indicate SD. Different letters within the same parameter indicate significant differences in the values (ANOVA, LSD test $P < 0.05$).

control soil sample (0.036 mg glucose kg^{-1} soil 24 h^{-1}). The reduction in amylase activity may be due to HM contamination in the soil sample, which can reduce the decomposition of starch by changing the active centre and structure of the enzymes[31].

Dehydrogenase is an intracellular enzyme which is regarded as one of the most sensitive enzymes in response to pollution. It is directly related to the total microbial activity in the soil[17]. The results show a 98.4% reduction in dehydrogenase activity in the contaminated soil sample. This is the maximum reduction among all the studied enzymes. This decrease in activity might be due to excess HMs which can inhibit the enzyme reaction by competing and replacing the essential metals in the enzyme – substrate complex[4]. Similar results were reported by various workers, where enzyme activities were reduced by up to 64% (ref. 4) and 86.46% (ref. 32) in the HM-contaminated soils.

Catalase was also reduced significantly, ranging from 23.25% to 57.51% in the spent oil-contaminated soil samples. Catalase promotes the decomposition of H_2O_2 into oxygen and hydrogen, thereby preventing H_2O_2 toxicity in the soil. This finding is consistent with the previous observation of a reduction in catalase due to the presence of toxic products from oil, such as HMs, which could inhibit enzyme activities by competing with the substrate in its active sites or by chelating with the substrate[33].

Alkaline phosphatase is an extracellular enzyme and functions to cleave phosphate into an assimilable form from its substrate. The alkaline phosphatase activity was reduced by only 18–29% in the contaminated soil sample of the study area. Previous studies have shown low levels of reduction in alkaline phosphatase enzyme activity[4,34]. The reduction in alkaline phosphatase may be due to the cumulative effect of HM stress, while its tolerance level could be attributed to Ni concentration which can increase alkaline phosphatase activity[6].

The peroxidase, polyphenol oxidase and cellulase enzyme activities in the spent oil-contaminated soil samples

showed maximum activities over the control soil sample. The polyphenol oxidase activity was recorded in the range 0.248–2.709 mmol purpurogallin kg^{-1} soil h^{-1}. The highest value was detected in the contaminated soil samples and the lowest in the control soil sample. Similarly, peroxidase activity was highest in the contaminated soil samples (3.63 mmol purpurogallin kg^{-1} soil h^{-1}) and lowest in the control soil sample (0.581 mmol purpurogallin kg^{-1} soil h^{-1}). The results obtained agree with those of a previous study which has shown an increase in enzyme activity in response to certain HMs like Mn(II) and Zn(II) acting as stimulants[35]. Moreover, these enzymes are secreted in response to toxic pollutants such as HMs by microbes to aid them in the antimicrobial defence and remediation process[36]. Since the polyphenol oxidase and peroxidase enzymatic activities are enhanced in response to the toxicity of HMs and pollution, they may be regarded as remediating enzymes.

The cellulase activity was found to be maximum in contaminated soils with (0.157 mg glucose kg^{-1} soil 24 h^{-1}) and minimum in the control soil (0.033 mg glucose kg^{-1} soil 24 h^{-1}). The increase in cellulase activity may be due to the alkaline nature of the contaminated soil. Maximum cellulase activity in alkaline soils has been reported in a previous study[37]. In addition to the alkaline soil, cellulase activity is also linked to the concentration of organic carbon[8].

From the present study, it can be concluded that the spent oil contamination in the soil causes HM accumulation and reduces the soil quality in terms of physico-chemical and biological properties. Several remediation strategies could be adopted to remove the pollutants and improve soil health, as suggested by Rajadurai *et al.*[3].

Conclusion

This study reports elevated levels of HMs and TOG in spent oil-contaminated soils in the vicinity of garages in parts of Guwahati city, which has caused adverse effects on their physico-chemical and biological properties. The spent oil contamination has also caused a significant reduction in urease, dehydrogenase, amylase, catalase and alkaline phosphatase enzyme activities and total bacterial population in the soil. The present study also demonstrates that spent oil contamination is detrimental to soil health. Thus a proper regulation and management strategy should be implemented to control and reduce the indiscriminate contamination of soil near garages. In addition, future studies should focus on site-specific remediation strategies to avoid further soil contamination.

Conflict of interest: The authors declare that there is no conflict of interest.

1. Polyak, Y. M., Bakina, L. G., Chugunova, M. V., Mayachkina, N. V., Gerasimov, A. O. and Bure, V. M., Effect of remediation strategies on biological activity of oil-contaminated soil – a field study. *Inter. Biodeterior. Biodegrar.*, 2018, **126**, 57–68.
2. Gushit, J. S., Oguche, V. F. and Ogbe, I., Treatment of spilled automechanic garage oils in soil using polyethylene terephthalate (PET) waste materials. *Science*, 2020, **8**, 117–121.
3. Rajadurai, M., Karmegam, N., Kannan, S., Yuvaraj, A. and Thangaraj, R., Vermiremediation of engine oil contaminated soil employing indigenous earthworms, *Drawida modesta* and *Lampito mauritii*. *J. Environ. Manage.*, 2022, **301**, 113849.
4. Aponte, H. *et al.*, Meta-analysis of heavy metal effects on soil enzyme activities. *Sci. Total Environ.*, 2020, **737**, 139744.
5. Li, C., Wang, X., Huang, H., Wang, L., Wei, F., Zhang, C. and Rong, Q., Effect of multiple heavy metals pollution to bacterial diversity and community structure in farmland soils. *Hum. Ecol. Risk Assess.*, 2021, **27**, 724–741.
6. Dotaniya, M. L. and Pipalde, J. S., Soil enzymatic activities as influenced by lead and nickel concentrations in a Vertisol of Central India. *Bull. Environ. Contam. Toxical.*, 2018, **101**, 380–385.
7. Abbasian, F., Lockington, R., Megharaj, M. and Naidu, R., The biodiversity changes in the microbial population of soils contaminated with crude oil. *Curr. Microbiol.*, 2016, **72**, 663–670.
8. Chakravarty, P. and Deka, H., Enzymatic defense of *Cyperus brevifolius* in hydrocarbons stress environment and changes in soil properties. *Sci. Rep.*, 2021, **11**, 1–12.
9. X'APHA, *Standard Methods for the Examination of Water and Wastewater*, *19th edn*, American Public Health Association, Washington, USA, 1998, vol. 6, pp. 4145–4146.
10. Piper, C. S., *Soil and Plant Analysis*, Inter Science Publication, Inc., Adelaide, Australia, 1943, **56**, 68.
11. Jackson, M. L., *Soil Chemical Analysis*, Prentice-Hall of India, New Delhi, 1975, pp. 183–226.
12. Patowary, K., Patowary, R., Kalita, M. C. and Deka, S., Development of an efficient bacterial consortium for the potential remediation of hydrocarbons from contaminated sites. *Front. Microbiol.*, 2016, **7**, 1092.
13. Zheljazkov, V. D. and Nielsen, N. E., Effect of heavy metals on peppermint and cornmint. *Plant. Soil*, 1996, **178**, 59–66.
14. Tabatabai, M. A. and Bremner, J. M., Use of *p*-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biol. Biochem.*, 1969, **1**, 301–307.
15. Cole, M. A., Lead inhibition of enzyme synthesis in soil. *Appl. Environ. Microbiol.*, 1977, **33**, 262–268.
16. Johnson, J. L. and Temple, K. L., Some variables affecting the measurement of 'catalase activity' in soil. *Soil Sci. Soc. Am. J.*, 1964, **28**, 207–209.
17. Pancholy, S. K. and Rice, E. L., Soil enzymes in relation to old field succession: amylase, cellulase, invertase, dehydrogenase, and urease. *Soil Sci. Soc. Am. J.*, 1973, **37**, 47–50.
18. Casida Jr, L. E., Klein, D. A. and Santoro, T., Soil dehydrogenase activity. *Soil. Sci.*, 1964, **98**, 371–376.
19. German, D. P., Weintraub, M. N., Grandy, A. S., Lauber, C. L., Rinkes, Z. L. and Allison, S. D., Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. *Soil Biol. Biochem.*, 2011, **43**, 1387–1397.
20. Hoffmann, G. G. and Teicher, K., A colorimetric technique for determining urease activity in soil. *Dung B.*, 1961, **95**, 55–63.
21. Kazlauskaitė-Jadzevičė, A., Volungevičius, J., Gregorauskienė, V. and Marcinkonis, S., The role of pH in heavy metal contamination of urban soil. *J. Environ. Eng. Landsc. Manage.*, 2014, **22**, 311–318.
22. Akram, S. and Deka, H., Phytoremediation potential of some abundantly growing indigenous herbs of crude oil contaminated sites. *J. Environ. Biol*., 2021, **42**, 51–61.
23. Wang, X., Feng, J. and Zhao, J., Effects of crude oil residuals on soil chemical properties in oil sites, Momoge Wetland, China. *Environ. Monit. Assess*, 2010, **161**, 271–280.
24. Wei, Y. and Li, G., Effect of oil pollution on water characteristics of Loessial soil. *IOP Conf. Ser: Earth Environ. Sci*., 2018, **170**, 1–7.
25. Khan, S. R., Kumar, J. I., Kumar, R. N. and Patel, J. G., Physicochemical properties, heavy metal content and fungal characterization

of an old gasoline-contaminated soil site in Anand, Gujarat, India. *Environ. Exp. Biol.*, 2013, **11**, 137–143.
26. WHO, Permissible Limits of Heavy Metals in Soil and Plants, World Health Organization, Geneva, Switzerland, 1996.
27. Mahender, A., Swamy, B. P., Anandan, A. and Ali, J., Tolerance of iron-deficient and toxic soil conditions in rice. *Plants*, 2019, **8**, 31.
28. Oluyemi, E. A., Feuyit, G., Oyekunle, J. A. O. and Ogunfowokan, A. O., Seasonal variations in heavy metal concentrations in soil and some selected crops at a landfill in Nigeria. *Afr. J. Environ. Sci. Technol.*, 2008, **2**, 089–096.
29. Govil, P. K., Keshav Krishna, A. and Dimri, V. P., Global geochemical baseline mapping in India for environmental management using topsoil. *J. Geol. Soc. India*, 2020, **95**, 9–16.
30. Guo, H. *et al*., Effects of petroleum contamination on soil microbial numbers, metabolic activity and urease activity. *Chemosphere*, 2012, **87**, 1273–1280.
31. Kujur, M. and Kumar Patel, A., Kinetics of soil enzyme activities under different ecosystems: An index of soil quality. *Chil. J. Agric. Res.*, 2014, **74**, 96–104.
32. Ofoegbu, C. J., Akubugwo, E. I., Dike, C. C., Maduka, H. C. C., Ugwu, C. E. and Obasi, N. A., Effects of heavy metals on soil enzymatic activities in the Ishiagu mining area of Ebonyi State-Nigeria. *J. Environ. Sci., Tox. Food Technol.*, 2013, **5**, 66–71.
33. Tang, J. *et al.*, Physicochemical features, metal availability and enzyme activity in heavy metal-polluted soil remediated by biochar and compost. *Sci. Total Environ.*, 2020, **701**, 134751.
34. Wyszkowska, J., Kucharski, J. and Lajszner, W., The effects of copper on soil biochemical properties and its interaction with other heavy metals. *Pol. J. Environ. Stud.*, 2006, **15**, 927–934.
35. Carine, F., Enrique, A. G. and Stéven, C., Metal effects on phenol oxidase activities of soils. *Ecotoxicol. Environ. Saf.*, 2009, **72**, 108–114.
36. Sinsabaugh, R. L., Phenol oxidase, peroxidase and organic matter dynamics of soil. *Soil Biol. Biochem.*, 2010, **42**, 391–404.
37. Sethi, S., Datta, A., Gupta, B. L. and Gupta, S., Optimization of cellulase production from bacteria isolated from soil. *Int. Scholar. Res. Noti.*, 2013, **2013**, 1–7.

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