Physicochemical, biological and heavy metal (HM) status of spent oil contaminated soils in the vicinity ofgarages in and around Guwahati city, Assam, India

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ABSTRACT

This work studies the impact of indiscriminate spilling of spent oil by the garages on surrounding soils. Physicochemical, biological and heavy metal (HMs) profiles of the spent oil contaminated soils are compared with control samples in Guwahati city, Assam, India. The results show that the spent oil contaminated soils show increase in the abundance of heavy metals (HMs), abundances varying from 58- 18400 mg/kg, total oil and grease (77000-161000 mg/kg) and 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 the problem.

Keywords: Garage, Spent Oil , Heavy metals, Biological , Soil.

1. INTRODUCTION

Oil contamination in soil is a major problem due to rapid industrialization and urbanization across the globe[1]. Due to population explosion there is an enormous increase in the use of motor vehicles on the roads during the past decades. For repairs and maintenance of the vehicles there has been simultaneous increase in the number of garages too. Garages 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. Engine oil contamination and associated pollutants change the nutrient composition available to the soil organism. In addition, the pollutants are proven to be mutagenic and carcinogenic to humans[3]. Spilling of used engine oil has gained serious attention as it can spread to the surrounding regions through rainwater run-off and subsequently seep into groundwater. Besides, it has been reported that the heavy metals present in spent oil are toxic not only to microbes, plants, animals, and humans but also to agriproducts that contribute to food chain [4]. Negative impacts of heavy metals on soil health have been widely reported in literature [5,6,7].

The physicochemical properties, microbial population and related enzyme activities reflect the ecological health of soil[8]. The concentration of pollutants such as heavy metals and changes in physicochemical properties affect soil enzyme activity. Moreover, soil enzymatic activities are considered as an indicator of soil quality due to rapid response to changes in soil environment[4]. Therefore, measurement of enzyme activities is of great importance 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 biological health of soils is still limited in India. The present study has been conducted in Guwahati city, India, to understand the impact of spent oil contamination on soil's physicochemical, biological, and heavy metal

profiles.

2.MATERIALS AND METHODS

Collection of soil samples:

The soil contaminated by oil from garages was collected from different sites in the Guwahati city,Assam, India (Figure 1). For comparison, control soil samples were collected from three different sites (c1, c2, c3) where contamination was not evident. The collected soil samples were dried and processed for further analysis.

Analysis of the soil samples:

pH, conductivity, water holding capacity (WHC) and total organic carbon (TOC) of the soil samples were determined by 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. The WHC of the soil samples was measured using the method as outlined by Piper[10]. The TOC content of the samples was determined following the Walkley and Black titration method as described by Jackson (1975)[11]. Total oil and grease contents of the studied soil samples were extracted using Soxhlet extractor using dichloromethane (DCM) as the solvent and measured gravimetrically[12]. Heavy metal analysis of the soil samples was determined following the nitric acid digestion method given by Zeheljazkov and Nielson [13]. The metal profiles of the samples were analyzed using Shimadzu. Model no. AA 7000 Atomic Absorption Spectrometer (AAS).

Analysis of soil enzymes was carried out by following the standard methods as given in Table2.

Table 2: Showing methods for analysis of soil enzyme activities

Enzyme	Unit	References
Alkaline phosphatase	mg PNP kg^{-1} soil h^{-1}	Tabatabai & Bremner (1969)[14]
Amylase	mg glucose kg^{-1} soil $24h^{-1}$	Cole (1977)[15]
Catalase	mmol H_2O_2 kg^{-1} soil min^{-1}	Johnson & Temple (1964)[16]
Cellulase	mg glucose kg^{-1} soil 24 h^{-1}	Pancholy & Rice (1973)[17]
Dehydrogenase	mg TPF kg^{-1} soil 24 h^{-1}	Casida et al; (1964)[18]
Polyphenol oxidase	mmol purpurogallin kg^{-1} soil h^{-1}	German et al; (2011)[19]
Peroxidase	mmol purpurogallin kg^{-1} soil h^{-1}	German et al; (2011)[19]
Urease	mg NH_4^+-N kg^{-1} soil h^{-1}	Teicher & Hoffmann (1961)[20]

Total bacterial population along with the heavy metal degrader bacteria was determined by the pour plate method and serial dilatation technique. Media used for total bacterial population estimation was nutrient agar. Similarly, mineral salt media (MSM) was utilized for enrichment culture to detect heavy metal (HMs) degrader bacterial population. All plates were incubated at 37^0C in an incubator. After that, colonies were counted in colony counting software (open CFU) and expressed as 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 analysis and LSD test.

2.RESULTS AND DISCUSSIONS

Physico chemical properties of the soil

The physico-chemical properties of the soil samples contaminated by the garage oil and the control soil samples are presented in Table 3.1.

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. As against this, the pH of the control soil sample is a little acidic with a pH of 6.2. Contamination of soil with heavy metals can stimulate rise of soil pH levels. The soil pH depends upon the biological properties of the soil[21]. The presence of heavy metals in the spent oil contaminated soil might have elevated the pH by inhibiting the growth of microbes, resulting in low microbial respiration in soil and H^+ concentration. This finding is backed by a previous study where alkaline pH was reported in engine oil contaminated soil[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 is in the range 0.29±0.005 mS/cm –0.33±0.04 mS/cm much lower than that of control soil sample which has an EC value of 1.22±0.015 mS/cm. This result is in conformity with the previous work where it was reported that oil contamination in the soil changes its texture and creates a non-polar environment, which reduces the ionic movement in soil by immobilization and reduction in velocity, consequently lowering the conductivity[8,22].

The total organic carbon (TOC) in the range 8.59%-15.83% was found to be higher in the contaminated soil samples relative to that in control soil sample. Increase in TOC may be due to high concentration of carbon in oil and grease[23].

Water holding capacity of the soil is the amount of water that soil can retain against gravity. It provides nourishment to the organic matter in the soil and is particularly important in nutrient management. Water holding capacity (WHC) of the contaminated soil is greatly reduced compared to the control soil sample. It is 12.67% - 25.21% as against 51.51% in control soil sample. The reduction in WHC may be due to reduction of 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 (O/G) was detected in the contaminated soil samples with a maximum of 161000 mg/kg and a minimum of 77000 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 the automobile garages as reported in a previous study[25].

Content of Heavy metals (HMs)

The contents of heavy metals (HMs) are presented in the Table 3.2. The permissible limits of heavy metal concentrations in soils as given by the World Health Organization (WHO)[26] are used as a reference for understanding the contamination level. Excluding nickel (Ni) and chromium (Cr), in the contaminated soil samples, the concentrations of other heavy metals namely iron (Fe), copper (Cu), zinc (Zn), and manganese (Mn) were found to be higher than the permissible limits given by the WHO (Table 4.2). The highest concentration (18400 mg/kg) of Fe was found in site No.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) were 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) is below the limit prescribed by WHO, it wasfound to be high compared to that in control soil samples. Similar results have been reported heavily in literature[2,25]. Interestingly the iron concentration in the control soil sample was also detected to be higher than the permissible limit. This may be due to the acidic pH of the soil which usually carries high iron contents[27]. Moreover, the iron concentration increases during the dry season in the soil due to the absence of rainwater run-off and leaching[28].

In addition, the detected heavy metal concentration in the oil contaminated soil, were all above the geochemical baseline data for the Guwahati city[29]. These high levels of heavy metals in the spent oil contaminated soil might be due to release of spent oil and other petroleum products into the soil system.

Bacterial population

The total bacterial population including the heavy metals (HMs) degrader bacteria are expressed as CFU g^{-1}soil (colony-forming unit per gram soil). The results are presented in the Table 3.3. The total bacterial population was found in the range 43.34 – 58 x 10^6 CFU g^{-1} soil in case of contaminated soil samples, whereas it is 182.34 x 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 compared to the control soil sample. The result of this finding is in accord with the results of previous studies[5,7]. Heavy metal degrader bacterial population (5.67±2.08 - 11.34±3.78 ×10^6 CFU/g soil) was also detected in all the contaminated soil samples. It was, however, not detected in the control soil sample. The presence of heavy metal's (HMs) degrader population in the contaminated soil may be due to the ability of some indigenous microbes whichcan resist and adapt to the stress conditions as reported in previous studies[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. The results are presented in graphical form in Fig. 2.1 to 2.4.

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

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 samples with

0.014mg glucose kg^{-1} soil $24h^{-1}$and maximum in the control soil sample 0.036 mg glucose kg^{-1} soil $24h^{-1}$. The reduction in the amylase activity may be due to the heavy metal contamination present in the soil sample which can reduce the decomposition of starch by changing the active center and structure of the enzymes[31].

Dehydrogenase is an intracellular enzyme and is regarded as one of the most sensitive enzymes in response to pollution. It is directly related to the total microbial activity in soil[17]. The results show as much as 98.4% reduction in dehydrogenase activity in the contaminated soil samples. This is the maximum reduction among all the studied enzymes. The decrease in activity may be due to the presence of excess heavy metals which can inhibit the enzyme reaction by competing and replacing the essential metals in enzyme substrate complex[4]. Similar results were reported by various workers where enzyme activities were reduced by up to 64%[4] and 86.46%[34] in the heavy metal contaminated soils.

Catalase also got reduced significantly ranging from 23.25% - 57.51% in the spent oil contaminated soil samples. Catalase promotes 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 reduction of catalase due to the presence of toxic products from oil such as heavy metals which could inhibit the enzyme activities by competing in its active sites with the substrate or by chelating with substrate[32].

Alkaline phosphatase is an extracellular enzyme and functions to cleave phosphate into an assimilable form from its substrate. The alkaline phosphatase activity is reduced by only 18% to 29% in the contaminated soil samples of the study area. Studies by Aponte et al[4] and Wyszkowska et al[33] have shown such low-level reduction of alkaline phosphatase enzyme activity. The reduction in alkaline phosphatase may be due to the cumulative effect of the heavy metal stress while its tolerance level could be attributed to nickel (Ni) concentration which can elevate the alkaline phosphatase activities[6].

The peroxidase, polyphenol oxidase, and cellulase enzyme activities in the spent oil contaminated soil show maximum activities over the control soil samples. The polyphenol oxidase activities were recorded in the range 0.248-2.709 mmol purpurogallin kg^{-1} soil h^{-1}. The highest value is detected in the contaminated soil sample and the lowest in the control soil sample. Similarly, peroxidase activity is detected to be highest in the contaminated soil samples with 3.63 mmol purpurogallin kg^{-1} soil h^{-1}while it is lowest in the control soil sample with a value of 0.581 mmol purpurogallin kg^{-1} soil h^{-1}. The results obtained are in agreement with the previous work which has shown an increase in the enzyme activity in response to certain heavy metals Mn (II) and Zn (II) acting as stimulants [35]. Moreover, these enzymes are secreted in response to toxic pollutants such as heavy metals by microbes to aid them in the antimicrobial defense and remediation process[36]. Since the polyphenol oxidase and peroxidase enzymatic activities are enhanced in response to the toxicity of heavy metals and pollution, they may beregarded as remediating enzymes.

The cellulase activity has also been detected to be maximum in contaminated soil with a value of 0.157 mg glucose kg^{-1} soil 24 $h^{-1}$and minimum in control soil with 0.033 mg glucose kg^{-1}soil 24 h^{-1}. The increase in cellulase activities 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, the cellulase activity is also linked to the level of concentration of organic carbon[8].

Based on the present study described in the foregoing it can be stated that the spent oil contamination in the soil causes heavy metal accumulation in soil and reduces the soil quality in terms of physicochemical and biological properties. Several remediation strategies could be adopted to remove the pollutants and improve the soil health as suggested by Rajadurai et al.,[3].

3. CONCLUSION

This study reports elevated level of HMs and TOG in spent oil contaminated soils in the

vicinity of garages in parts of Guwahati City which evidently has caused adverse effects on their physico-chemical and biological properties. The spent oil contamination has caused significant reduction in urease, dehydrogenase, amylase, catalase and alkaline phosphatase enzyme activities and total bacterial population in soil. The present study also demonstrates that spent oil contamination is detrimental to soil health and a proper regulation and management strategy should be evolved and 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 contamination.

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Conflict of Interest:

The authors declare no conflict of interest.

Figure Captions:

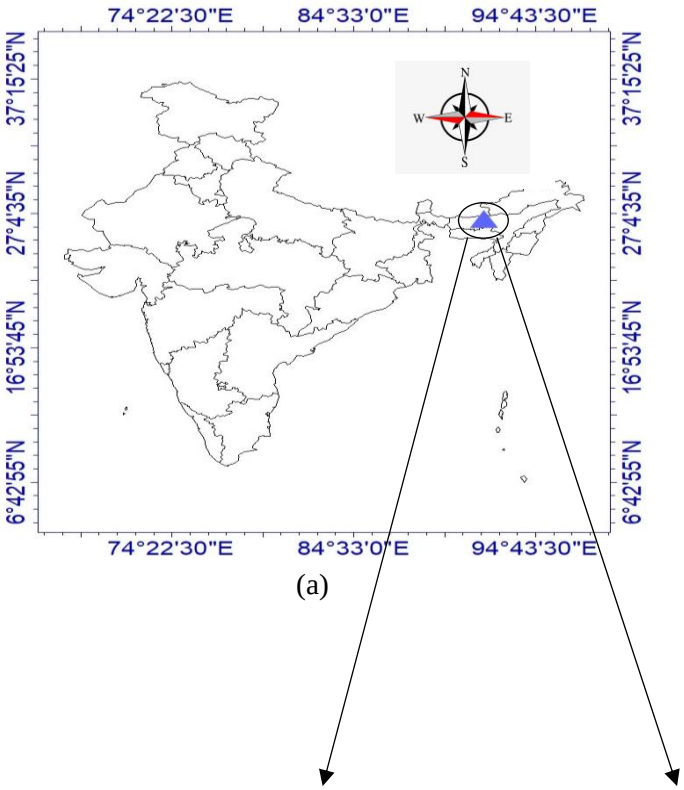
Figure 1: Maps of study area: (a) Map of India, (b) Map showing study sites in greater Guwahati Area showing the sampling sites of oil contaminated soils. [Site 1-Sundarbari (26° 8' 54.7296" N 91° 40' 39.5184" E) Site 2-Adabari (26° 9' 28.8" N 91° 41' 4.1712" E) Site 3-Jalukbari (26° 9' 33.3" N 91° 39' 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

Figure 2.1: Cellulase and catalase enzyme activities in the soil samples. Values are mean of 3 samples, bars indicate SD. Different letters within the same parameter indicate significant differences of the values (ANOVA, LSD test $p<0.05$)

Figure 2.2: Dehydrogenase and alkaline phosphatase enzyme activities in the soil samples. Values are mean of 3 samples; bars indicate SD. Different letters within the same parameter indicate significant differences of the values (ANOVA, LSD test $p<0.05$)

Figure 2.3: Peroxidase and polyphenol oxidase enzyme activities in the soil samples. Values are mean of 3 samples; bars indicate SD. Different letters within the same parameter indicate significant differences of the values (ANOVA, LSD test $p<0.05$)

Figure 2.4: Urease and Amylase enzyme activities in the soil samples. Values are mean of 3 samples, bars indicate SD. Different letters within the same parameter indicate significant differences of the values (ANOVA, LSD test $p<0.05$)



(a)

Sampling sites	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

Legend

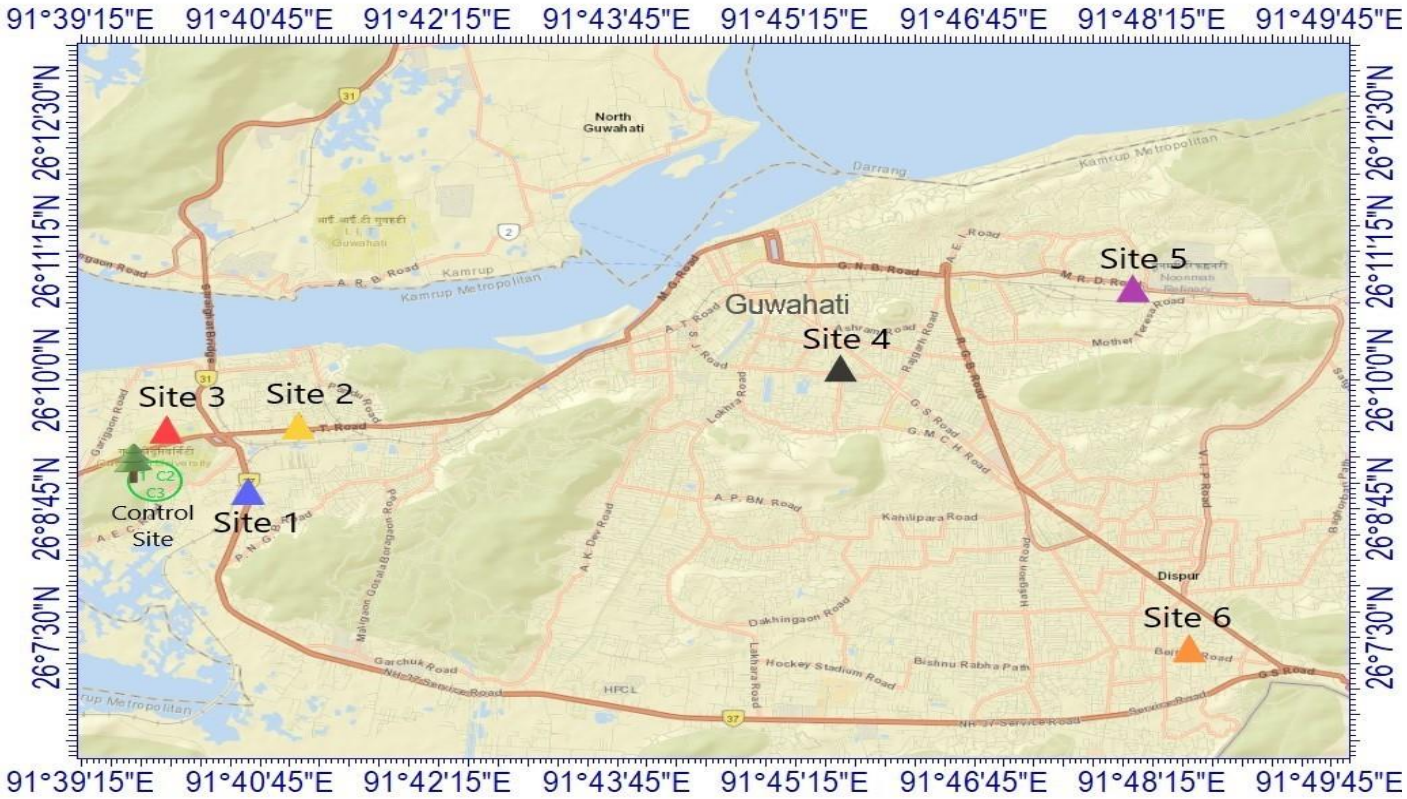
National Highway

Water features

City Roads

Sampling sites

Botanical Garden



(b)

Figure 1

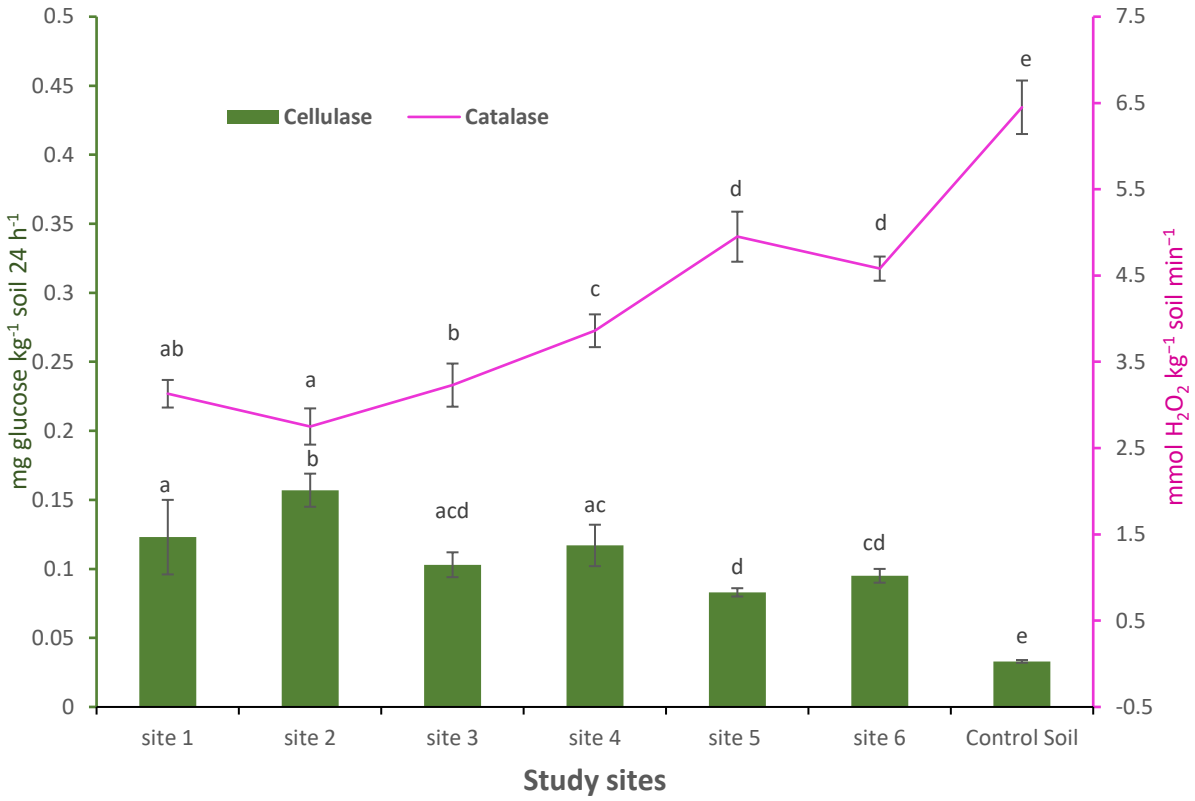

Cellulase
Catalase
mg glucose kg-1 soil 24 h-1
mmol H2O2 kg-1 soil min-1
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
7.5
6.5
5.5
4.5
3.5
2.5
1.5
0.5
-0.5
ab
a
b
acd
c
ac
d
d
d
cd
e
e
site 1
site 2
site 3
site 4
site 5
site 6
Control Soil
Study sites

Figure 2.1

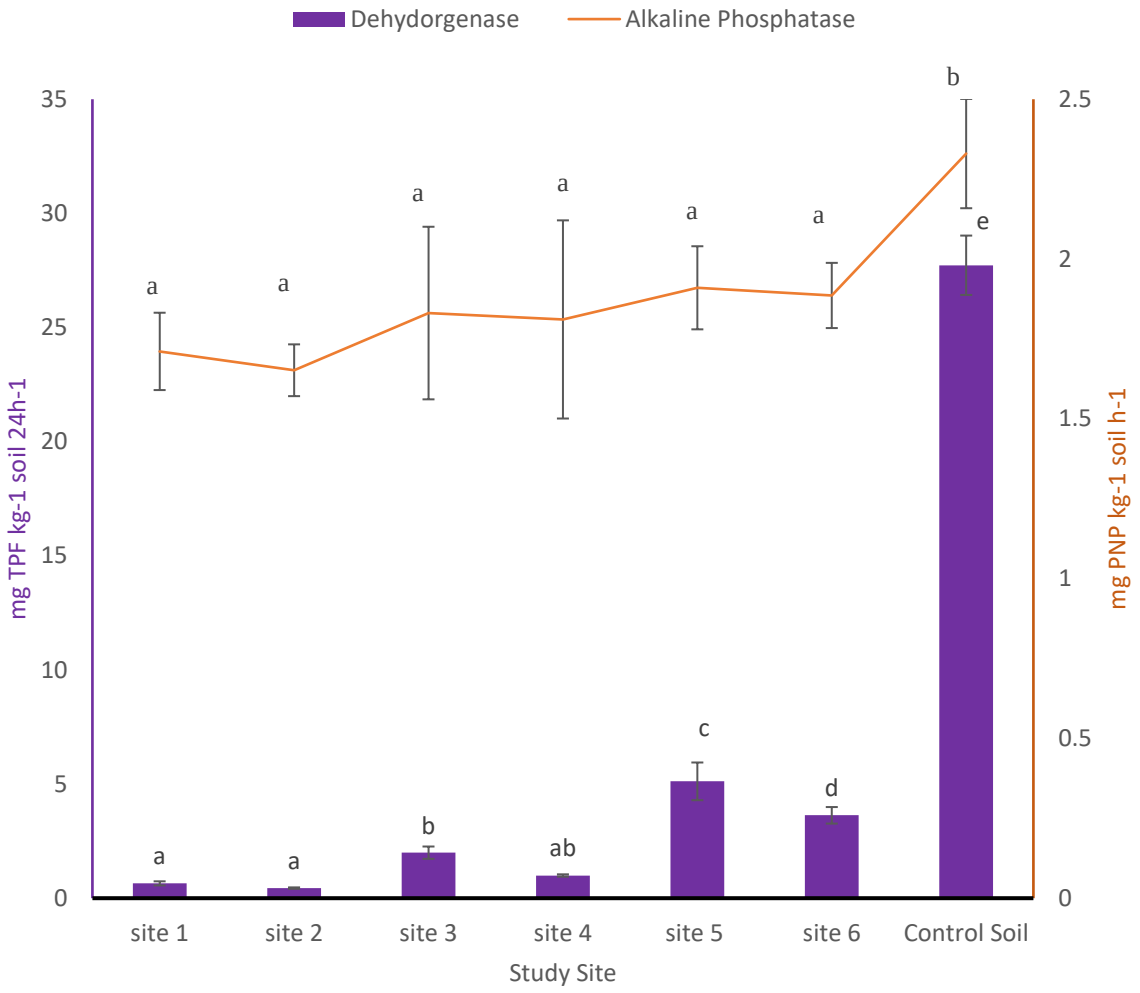

Dehydorgenase
Alkaline Phosphatase
mg TPF kg-1 soil 24h-1
mg PNP kg-1 soil h-1
35
30
25
20
15
10
5
0
2.5
2
1.5
1
0.5
0
a
a
a
a
a
a
b
a
a
b
ab
c
d
e
site 1
site 2
site 3
site 4
site 5
site 6
Control Soil
Study Site

Figure 2.2

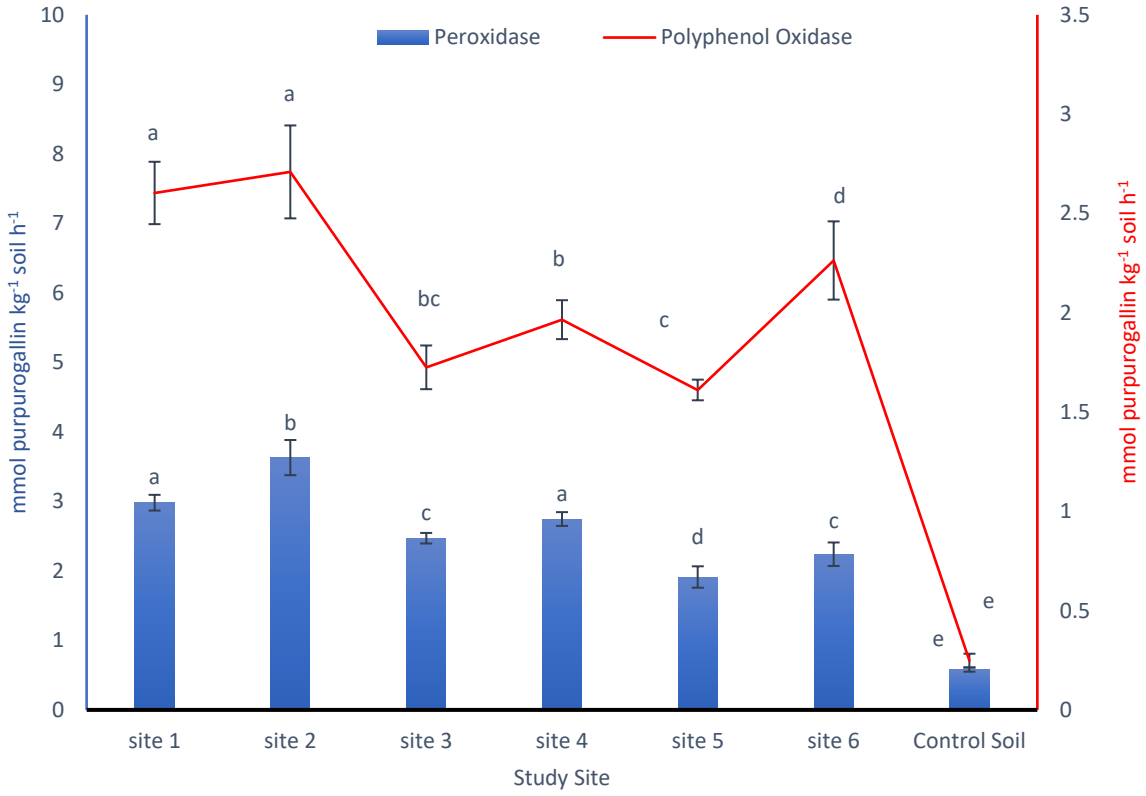

Peroxidase
Polyphenol Oxidase
mmol purpurogallin kg-1 soil h-1
mmol purpurogallin kg-1 soil h-1
10
9
8
7
6
5
4
3
2
1
0
3.5
3
2.5
2
1.5
1
0.5
0
a
a
bc
b
c
d
e
b
c
a
d
c
e
site 1
site 2
site 3
site 4
site 5
site 6
Control Soil
Study Site

Figure 2.3

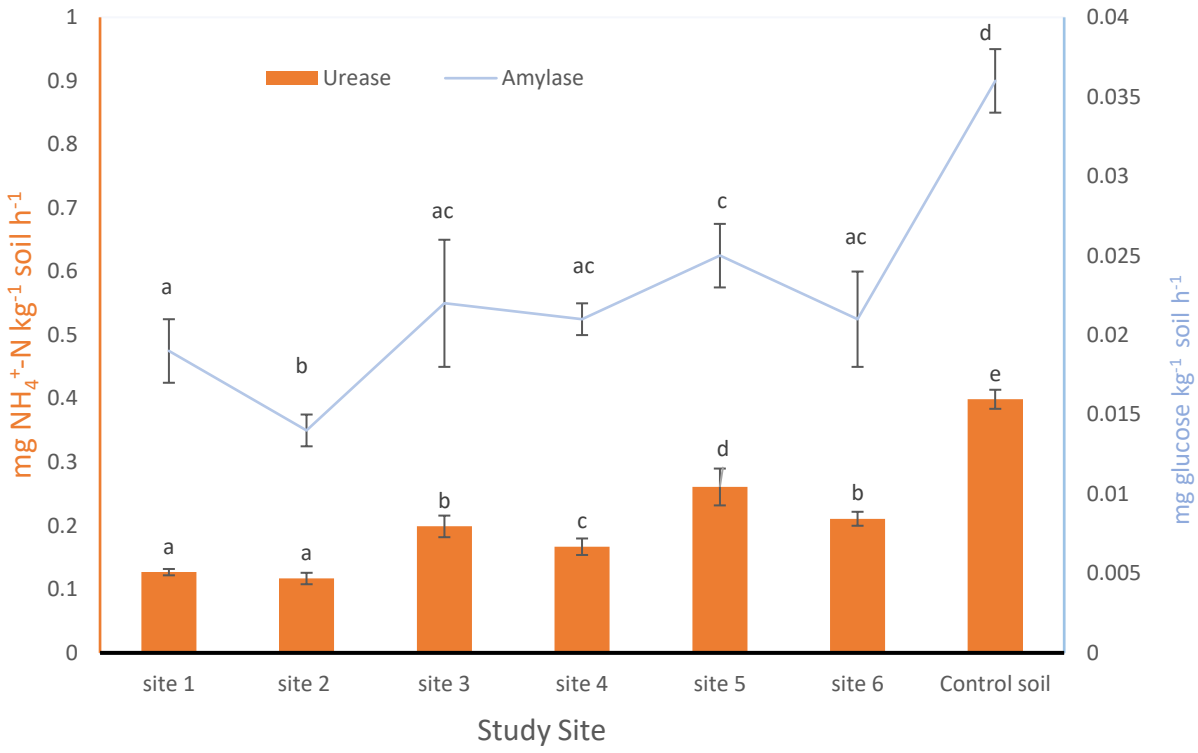

Urease
Amylase
mg NH_4^+-N kg^{-1} soil h^{-1}
mg glucose kg^{-1} soil h^{-1}
Study Site
site 1
site 2
site 3
site 4
site 5
site 6
Control soil
a
b
ac
ac
c
ac
d
a
a
b
c
d
b
e

Figure 2.4

Table 3.1: Physicochemical properties of garage oil contaminated soils.

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 indicates significant differences of the values (ANOVA, LSD test, $p<0.05$)
TOC -Total Organic Carbon ND- Not Determined
WHC- Water Holding Capacity TOG- Total oil and grease

Table 3.2: Heavy metal's (HMs) concentrations in the soil samples contaminated with spent oil of garages.

Study site	Latitude and Longitude	Fe (mg/kg)	Cu (mg/kg)	Ni (mg/kg)	Cr (mg/kg)	Zn (mg/kg)	Mn (mg/kg)
Permissible Limit (WHO) (mg/kg)		20	36	35	100	50	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 indicates significant differences of the values (ANOVA, LSD test, $p<0.05$)
WHO- World Health Organization

Table 3.3: Beneficial bacterial population in crude oil contaminated soil and control soil.

Study sites	Latitude and Longitude	TBP	HMs DBP
		($\times 10^6$ CFU/g 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 of the values (ANOVA, LSD test $p<0.05$) ND- Not Detected TBP- Total Bacterial Population
HMs DBP- Heavy metals degrading bacterial population