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An assessment of the Physiochemical Factors, Heavy Metal Limitations and Microbial Diversity in the Water and Soil Sediments of Automobile Work Station in Monday market, Kanyakumari District, Tamil Nadu

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ABSTRACT: Water and soil property was assessed with the estimation of physiochemical constituents and heavy metal contaminations, both are responsible for serious environmental and health related issues. In this investigation, the analysis focuses on the estimation of physiochemical parameters and quantification of heavy metals (Cd and Pb) in water and soil samples from automobile service stations (S1N1, S2V2 and S3X3). The temperature of soil and water ranged from 28°C to 29 °C depending on depth of soil sampling and due to the continuous flow of vehicle washing water discharges, while pH was measured as 7.6 to 8.5 due to release of battery effluent and hydrocarbons. EC was recorded as 201 to 305 (µScm-1) in three stations of automobile soil and water effluents. One-way ANOVA showed the significance of 0.002164 < 0.05 P-value on temperature of soil samples and 0.081887 > 0.05 P-value explained the variability in water samples of three automobile workstations. The pH of soil sediments showed insignificance (0.075972 >0.05 P-value) and significant in water samples (0.01541 < 0.05 P-value). EC of soil and water demonstrated the significance at < 0.05 P-value. Heavy metals such as Cadmium and Lead was also estimated in soil and water samples of all three automobile workstations and expressed as ppm/ mL. Both the metals were an evident of insignificance at < 0.05 P-value by one-way ANOVA among the data sets of soil and water collected from three stations. The result of Cadmium was above the permissible limits set by WHO due to the automobile discharges and conclusively it is hereby recommended that a separate portion of land be set apart for automobile workshops which can be called mechanic village as it is in some cosmopolitan areas in this library. In addition to this, microbial diversity was also examined in study samples of soil and water from automobile work stations. *Bacilli sps* was a predominant in soil samples and major screened species in water samples were *Bacilli* and *Diplobacilli sps* which further employed for pure culture and characterized cultural prospective.

Keywords: Automobile soil and Water effluents, Physiochemicals characteristics, Heavy metal, microbial diversity and environmental pollution.

INTRODUCTION

In past centuries, people have coexisted harmoniously with the environment. Nonetheless, the present, fast-paced pace of urbanisation and industrialization (Jiang *et al*., 2013), mining activities (Navarro *et al*., 2008), agriculture (Vaalgamaa and Conley 2008; Syed *et al*., 2012), industries and transportation (Jaradat *et al*., 2005; David and Sunday 2012) leads severe environmental contamination. Soil physiochemical properties determine nutrient content, but heavy metal pollution is a significant issue due to toxicity, persistence, and non-degradability, affecting soil processes and microbial composition (Tyler *et al*., 1989). About 80 % of country's pollution driven from industrial maintenance and automobile service, with automotive emissions and Lead, zinc, cadmium, and nickel are the main contributors Heavy metal pollution in Nigeria (Onianwa and Fakayoda 2000; Adriano, 2001). Xenobiotic substances and expansion of human activities are responsible for another way of soil contamination (Badejo *et al*., 2013).

The most lethal contaminants from automobile workshops generates waste water PAH, PCB, heavy metals, and detergents. Discharging of this waste water contaminates surface and ground water hence causing disturbance of flora and fauna ecosystem. An auto repair facility leads the possibility of causing kidney damage and cancer by car wash water contains debris, brake lining solids, and dust. Detergents also harm fish populations, with concentrations affecting fish populations. Recycling and reuse are crucial for water conservation. Soil quality is influenced by chemical and physical properties, with physiochemical properties determining heavy metal pollution (Okoye and Agbo (2011). Soil quality, influenced by physical and chemical factors, plays a crucial role in water retention, carbon sequestration, plant productivity, and waste remediation (Nelson and Sommers 1982).

The global environmental problem of heavy metal contamination disrupts ecosystems by changing the characteristics of soil and limiting ecosystem functions and production (Osakwe, 2012). Polluted sites frequently include lead, chromium, arsenic, zinc, cadmium, copper, mercury, and nickel among other heavy metals (Oguntimehin and Ipinmoroti K., 2007). These metals are frequently added to petrol and lubricants, hence non-biodegradable (Campbell, 2006). According to Ingole (2015); Sde *et al*. (2000), the presence of heavy metals in soil and water is a serious problem for ecological stability, agricultural productivity, and human health. The study aims to quantify the heavy metals (Cd and Pb) and assess the physiochemical properties of soil and water samples from automobile work stations in Kanyakumari district as designated priority pollutants by the US Environmental Protection Agency.

MATERIAL AND METHODS

Collection of Soil Samples. Soil samples were collected from three different automobile contaminated stations S1N1, S2V2 and S3X3 by digging the soil for 10 to 30 cm depth in sterile polypropylene containers and water samples were collected by dipped into the small water discharges in automobile workstations with polypropylene tubes. The samples were immediately transferred analytical workstation laboratory and processed for the analysis of physiochemicals and heavy metal quantification. crucible, pestle, mortar and some other tools for sample collection were washed with liquid soap rinsed with distilled water and then soaked in 10% HNO_3 solution for 24 hrs (Todorovic *et al*., 2001). They were then washed with distilled water and stored in drying oven at 80 Degree C for 5 hrs.

Analysis of physiochemical parameters of collected water and soil sediments. The physico chemical parameters (appearance, turbidity, odor, temperature, electrical conductivity, and dissolving oxygen, pH, Total Alkalinity as $CaCO_3$, Calcium, Sodium, Iron, Nitrite, Chloride, Phosphate, and Dissolved Oxygen 4 hrs) of water and soil were evaluated by employing the standard operating protocol followed by the Tamil Nadu agriculture sector and Tamil Nadu water supply and Drainage Board (TWAD). The estimated data was interpreted using the Bureau of Indian Standards (BIS) – India's standard acceptable range.

Analysis of Toxic Heavy metal trace in Water and Soil Sediments. Three distinct automobile service stations (S1N1, S2V2 and S3X3) provided the chosen water and soil samples, which were then processed for further heavy metal analysis. Transferred to the lab, grade 1 Whatman filter paper was used to filter the obtained water samples. After being sun-dried, the soil samples were weighed at 1 g in 10 ml of distilled water. The components were well combined before being centrifuged at 200 rpm. It was decided to collect the supernatant for heavy metal analysis. The following is a description of the experimental variables.

Heavy metal analysis using AAS. Using an Atomic absorption spectrophotometer (Type Model Name ROM Version S/N) with parameters of flow rates of fuel gas (L/min) of 1.8, support gas flow rate (L/min) of 15.0, Air-C2H2 (Acetylene) flame type, and wavelength for cadmium (Cd) of 228.8nm, the study water and soil samples were collected from three different sites of the automobile workshops. While the support gas flow rate (L/min) was 15.0 and the support gas flow rate (L/min) was 2.0, lead (Pb) had a wavelength of 283.3 nm.

Screening of Heavy metal resistant bacteria from study samples. For the selective screening of heavy metal resistant bacteria, the selective media was prepared with the following composition; 300µg/mL of heavy metal (Lead) incorporated LB (Luria Bertani) agar plates (Peptone 10.00 g/L, yeast extract, 5.00 g/L, NaCl 5.00 g/L, dextrose anhydrate 10.00 g/L and agar 30.00 g/L: pH 7.00) were used and water and soil samples of site-I, II and III was inoculated by standard pour plate method observed at 37°C. After 24 h of incubation the plates were observed for any kind of development on the culture medium. After preliminary screening of effluent samples containing heavy metal degrading isolates, serial dilution was done as Azad *et al*. (2013) to isolate desired bacteria. Streak plate technique was followed during isolation. Control plates also prepared with LB media without including any heavy metal to make comparison. Colonies differing in morphological characteristics were selected, picked, purified by quadrant streaking and then preserved on slands for further studies.

Statistical Interpretation. The obtained data were meticulously analyzed using one way ANOVA for the significance of physical and bacterial abundance in experimental samples. In addition, the bacterial isolates were identified by genus level and calculate the heavy metal resistance bacterial diversity using Shannon Wiener Diversity Index.

$$H' = -\sum_{i=1}^{s} pi \ln pi$$

RESULTS

Physiochemical Parameters and heavy metals analysis. The soil and water samples from the study stations of automobile workshop were assessed with the parameters of temperature, pH, EC and heavy metals such as cadmium and lead. The analytical data verified that the color and appearance was seemed turbid and brownish in color while slight oil mixed odour was noted in all samples. The temperature vary from 28°C to 29 °C in soil samples while pH was measured as 7.6 to 8.5 and EC was recorded as 201 to 305 (μScm^{-1}) accordingly in three stations of automobile soil in Table 1a. In other hand, water samples were recorded as 28.4 to 29.8 °C temperature and pH was 7.9 to 8.6. The electrical conductivity (EC) of the water ranged from 200.2 to 420.8 (μScm^{-1}). One-way ANOVA showed the significance of 0.002164 < 0.05 P-value on temperature of soil and 0.081887 > 0.05 P-value explained the variability in water samples of three automobile workstations. The pH of soil sediments showed insignificance (0.075972 >0.05 P-value) and significant in water samples (0.01541 < 0.05 P-value). EC of soil and water demonstrated the significance at < 0.05 P-value. Heavy metals such as Cadmium and Lead was also estimated in soil and water samples of all three automobile workstations and expressed as ppm/ mL. In table: elaborated that both the metals were an evident of insignificance at < 0.05 P-value by one-way ANOVA among the data sets of soil and water collected from three stations.

In Table 1b described the detection of chemical constituents in collected water samples in three different automobile service stations and among them iron (Fe) and free ammonia (NH3) seems to be higher in all three station compared with the standards (BSI: 10500: 2012) regulations. This due to the excretion of automobile effluents to the nearby water bodies and therefore turned into unfavorable to human purposes and the overall significance was expressed by one-way ANOVA, the p-value of three stations was 0.1351 (F=2.1167) > 0.05 α value. The overall concentration was represented in Fig. 1.

According to the AAS analysis, lead (Pb) metal has comparatively increased 0.125667 ± 0.001528 ppm/mL in site-II of water sample than 0.006533 ± 0.000351 ppm/mL in site-III water sample described in Table 2 and Fig. 2. The quantification data of heavy metals such as Cd and Pb insignificant P-value 0.9879 (Cd) and P-value 0.999973 (Pb) > 0.05 α level using one-way ANOVA.

Assessment of Heavy Metal Resistant Bacterial Diversity of Soil and Water samples from three Automobile stations

The collected soil and water samples were further analyzed and serially diluted, plated and sorted by dominancy of the colony into pure bacterial isolates for characterization. The emerged bacterial isolated from Cd incorporated culture plates were further examined and sorted by the morphological characters in nutrient agar media with Cd. Selected isolates were screened through gram's staining and concluded with the genus of staining properties. Table 3 showed the morphological characters of isolated bacteria from soil and the data were interrelated statistically expressed with diversity indices in Table 4, however the higher diversity (0.9946) of *Bacillus sps* in station 2 followed by other stations. The predominated genus was *Bascili* sps in soil whereas in water was observed as *Bacilli sps* and *Diplobacillia* sps accordingly. Fig. 3a and b. showed the heavy metal resistance bacterial diversity in soil and water samples of automobile workshops.

DISCUSSION

An anoxic-aerobic sequential reactor system was used to cleanse the sewage from an auto service shop so that it could be recycled. Phosphate, metals, hydrocarbons, and phenol were all present in the wastewater that was collected. Over 99% of the phenol and hydrocarbons were removed by the system, and over 99% of the remaining NH4+-N was oxidised. *Pseudomonas aeruginosa* was found in the anoxic biomass, and Subrat Kumar Mallick and Saswati Chakraborty (2019) described *Lysinibacillus* sp., *Stenotrophomonas* sp., *Pseudomonas aeruginosa*, and *Pseudomonas aeruginosa* ISB4 for possible NH_4+-N exploitation. Nguegang *et al*. (2019) investigated at the microbiological, physicochemical, and toxicological properties of carwash effluents. Danio rerio and Daphnia pulex were used in toxicity tests, and samples were examined for heterotrophic bacteria Martins *et al*. (2007). All automobile effluents were acutely hazardous, with fatality rates of 75% within 24 hours, according to the results. The range of heterotrophic bacterial counts was 2800–4600 CFU/100 ml. Following a sequence analysis, it was shown that 43% of the isolates belonged to Pseudomonas species and 57% to *Aeromonas* sps. Effluents from automobiles have the potential to include hazardous chemicals as well as microbiological pollutants. Poonkothai and Parvatham (2005) have Studied on the physico-chemical and microbiological characteristics of automotive wastewater in Nammakkal, Tamilnadu, India, revealed that the BIS allowed limits were exceeded in terms of pH, EC, TDS, TH, BOD, COD, Calcium, Chloride, Sulphate, Bicarbonate, Oil, and Greases. Studies in microbiology demonstrated the existence of microorganisms *like Pseudomonas aeruginosa*. At high concentrations, *Bacillus subtilis*, *Micrococcus* sps, *Klebsiella sp*s and fungal species like *Rhizopus*, *Mucor*, *Fusarium*, *A. niger*, *A. terreus*, *Curvularia*, and *Pencilliwn sps*. function as pollutants' indicators. The MPN test that was performed on these samples came out positive.

Both dangerous chemicals and microbiological contaminants may be present in carwash effluents. The likelihood that increased network complexity is brought on by metabolic pathways and microbial interactions Jakovljević *et al*. (2022) observed the impact of automotive industry pollutants on microbial strains' biofilm growth, matrix protein content, and hydrolytic enzyme activity. Results showed that pollutants inhibited or stimulated single cultures and consortia. The study highlights the positive correlation between ALP and biofilm biomass, providing insights into extracellular hydrolytic activity in auto paints and paving the way for future research. The study analyzed bacterial diversity, organic pollutants, and metabolites in tannery wastewater treatment at a common effluent treatment plant (CETP). Results showed *Escherichia sp.*, *Stenotrophomonas* sp., *Bacillus* sp., *and Cronobacter* sp. dominate in aeration lagoon-I, while *Stenotrophomonas and Burkholderiales* dominated in aeration lagoon-II (Chandra *et al*., 2011).

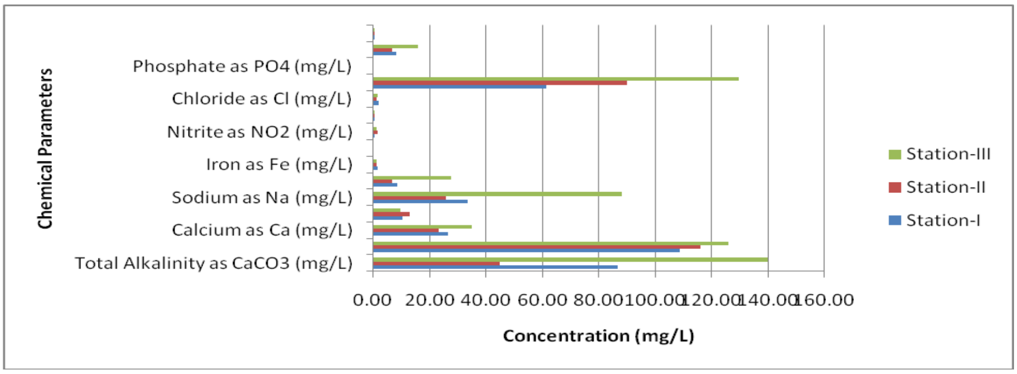

Fig. 1. Determination of Chemical Parameters in Collected Water Samples from Automobile Service Station.

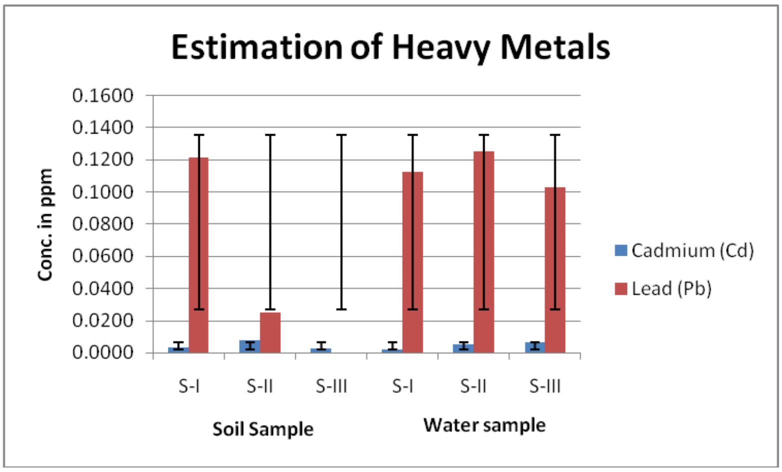

Fig. 2. Bar Representation of Heavy Metals Concentration from Collected Automobile Effluent Samples.

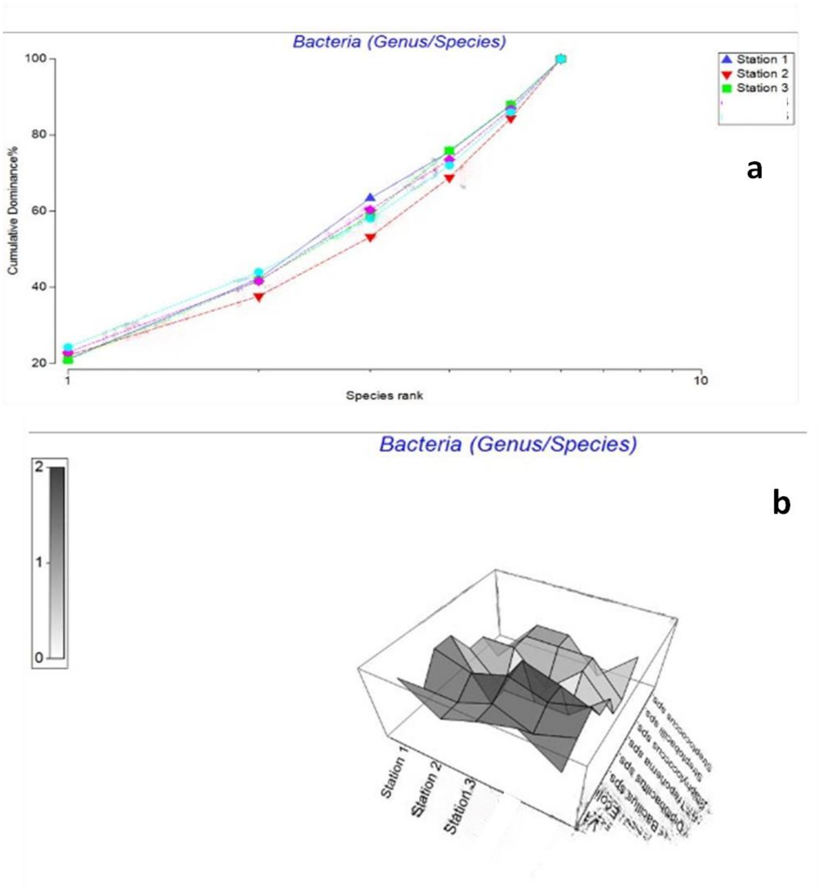

Fig. 3a. Bacterial Diversity by Shannon Wiener Indices, b. Surface Representation of Bacterial sps. Diversity in Five Different Automobile Workstations.

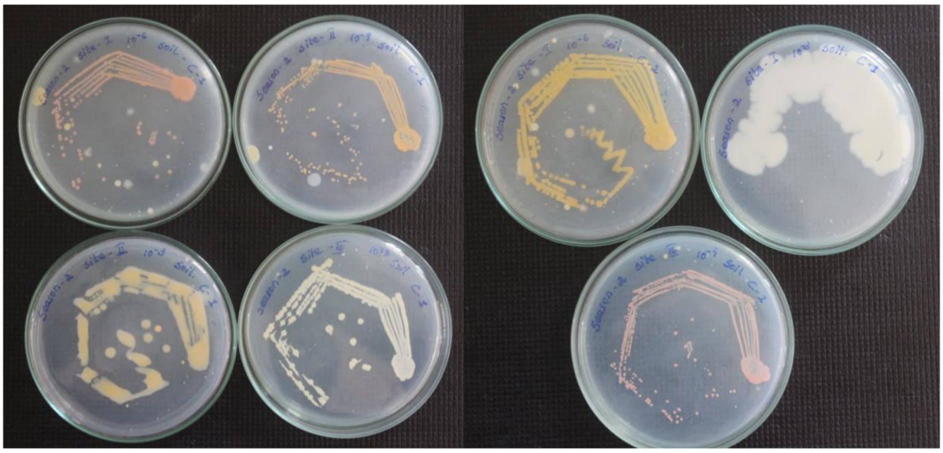

Fig. 4. Isolated Bacterial Strains from the Soil Sediment Effluent of Automobile Service Station.

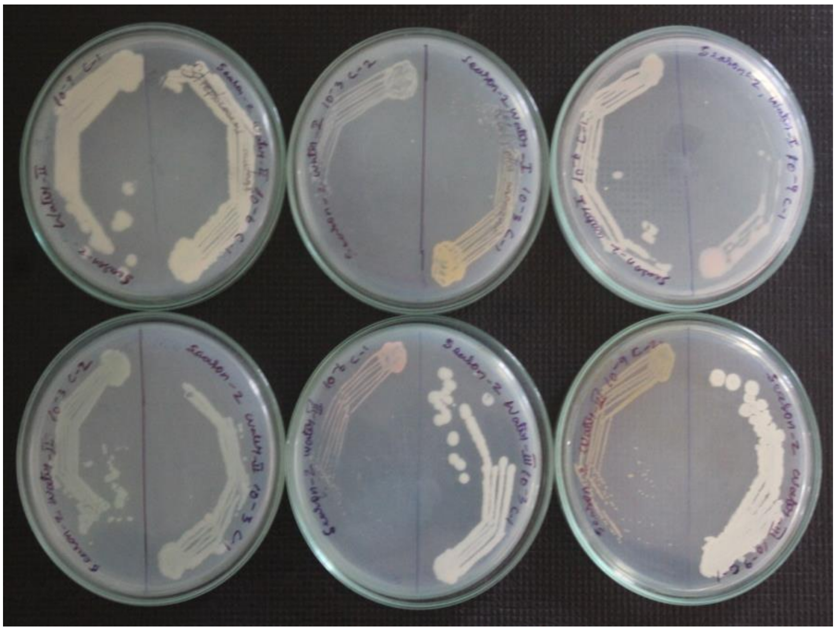

Fig. 5. Isolated Bacterial Strains from the Water Effluent of Automobile Service Station.

Table 1a: Physical Parameters and Heavy metals Analysis of Soil and Water samples from Automobile Workstations.

Soil Sediments				
Parameters	**Station-I**	**Station-II**	**Station-III**	**One-Way ANOVA (P-value)**
Temp	28.27 ± 0.15	28.93 ± 0.15	29.1 ± 0.20	0.002164*
pH	7.87 ± 0.06	8.03 ± 0.12	8.13 ± 0.15	0.075972**
EC	205.17 ± 21.19	232.07 ± 17.69	303.07 ± 13.54	0.0013189*
Color	Slight cloudy	Slight Cloudy	More Cloudy	-
Appearance	Turbid	Turbid	Turbid	-
Odour	Oil and Fuel smell	Oil and Fuel smell	Oil and Fuel smell	-
Water samples				
Parameters	**Station-I**	**Station-II**	**Station-III**	**One-Way ANOVA (P-value)**
Temp	28.3 ± 0.36	29.07 ± 0.87	29.6 ± 0.31	0.081887**
pH	7.63 ± 0.31	8.37 ± 0.35	8.57 ± 0.15	0.01541*
EC	201.83 ± 14.73	238.73 ± 17.46	305.73 ± 6.56	0.000257*
Color	Slight cloudy	Slight Cloudy	More Cloudy	-
Appearance	Turbid	Turbid	Turbid	-
Odour	Oil and Fuel smell	Oil and Fuel smell	Oil and Fuel smell	-

* Significant p-value at 0.05 α-value, ** not significant p-value at 0.05 α-value

Table 1b: Analysis of Chemical Parameters in Water samples from Automobile Workstations.

Chemical Parameters	Season-I	Season-II	Season-III
Total Alkalinity as $CaCO_3$ (mg/L)	86.67 ± 36.95	44.67 ± 16.77	140 ± 135.11
Total Hardness as $CaCO_3$ (mg/L)	108.67 ± 61.72	116 ± 31.75	126 ± 86.69
Calcium as Ca (mg/L)	26.33 ± 15.01	23 ± 12.53	35 ± 29.44
Magnesium as Mg (mg/L)	10.33 ± 5.69	12.67 ± 2.08	9.67 ± 3.06
Sodium as Na (mg/L)	33.33 ± 18.48	25.67 ± 15.04	88 ± 93.55
Potassium as K (mg/L)	8.33 ± 1.15	6.67 ± 4.04	27.67 ± 34.08
Iron as Fe (mg/L)	1.49 ± 2.07	0.98 ± 1.11	1.26 ± 1.01
Free Ammonia as NH_3 (mg/L)	0.3 ± 0.20	1.64± 1.89	0.96 ± 0.71
Nitrite as NO_2 (mg/L)	0.03 ± 0.03	0.04 ± 0.01	0.01 ± 0.02
Nitrate as NO_3 (mg/L)	1.67 ± 2.12	1 ± 0.71	1.33 ± 0.00
Chloride as Cl (mg/L)	61.33 ± 39.26	90 ± 42.76	129.67 ± 125.96
Sulphate as SO_4 (mg/L)	8 ± 4.36	6.67 ± 6.35	15.67 ± 15.89
Phosphate as PO_4 (mg/L)	0.13 ± 0.03	0.11 ± 0.03	0.1
One-Way ANOVA	P- value of three stations was 0.1351, insignificant at 0.05 α level of range**		

* Significant p-value at 0.05 α-value, ** not significant p-value at 0.05 α-value

Table: 2. Quantification of Heavy Metals from Collected Automobile Effluent Samples.

Sample	Stations	Cadmium (Cd)	Lead (Pb)
Soil	**S-I**	0.003033 ± 0.000153	0.121833 ± 0.000306
	S-II	0.0076 ± 0.0003	0.024733 ± 0.000351
	S-III	0.002467 ± 0.000153	-
Water	**S-I**	0.002 ± 0.000361	0.112467 ± 0.000404
	S-II	0.005167 ± 0.000306	0.125667 ± 0.001528
	S-III	0.006533 ± 0.000351	0.102867 ± 0.000252
ANOVA		P-value 0.9879** > 0.05 α	P-value 0.999973** > 0.05 α

* Significant p-value at 0.05 α-value, ** not significant p-value at 0.05 α-value

Table 3: Heavy metal resistant Bacterial Morphological Identification isolated from Automobile Workstations.

Sr. No.	Strain code	Colour	Elevation	Texture	Margin	Shape	Gram Staining	Genus Name
1.	BGMS1	Creamy yellow	Raised	slimy moist	Entire	Circular	Pink (G -ve), Cocci Cluster	*Neisseria sps*
2.	BGMS2	Pinkish	Convex	dry, mucoid	Entire	Circular	Pink (G -ve), Cocci Chain	*Streptobacilli*
3.	BGMS3	Slight Orange	Convex	dry, mucoid	Entire	Punctiform	Purple (G +ve), Cocci Cluster	*Staphylococcus sps*
4.	BGMS4	White	Flat	shiny, viscos	Undulate	Irregular	Purple (G +ve), Rod	*Bacillus sps*
5.	BGMS5	Pinkish	Convex	dry, mucoid	Entire	Punctiform	Purple (G +ve), Cocci chain	*Strepcoccus sps*
6.	BGMS6	Yellowish	Convex	dry, mucoid	Entire	Circular	Pink (G -ve), Rod	*Ecoli*
7.	BGMS7	White	Flat	shiny, viscos	Undulate	Irregular	Purple (G +ve), Rod Chain	*Bacillus sps*
8.	BGMW1	White	Raised	slimy moist	Entire	Circular	Pink (G -ve), Cocci Cluster	*Neisseria sps*
9.	BGMW2	Pale White	Raised	slimy moist	Entire	Circular	Pink (G -ve), Rod	*Ecoli*
10.	BGMW3	White	Flat	shiny, viscos	Entire	Circular	Purple (G +ve), Rod Chain	*Bacillus sps*
11.	BGMW4	White	Flat	shiny, viscos	Undulate	Irregular	Pink (G -ve), Plum	*Treponema sps*
12.	BGMW5	Slight Orange	Convex	dry, mucoid	Entire	Punctiform	Purple (G +ve), Rod	*Bacillus sps*
13.	BGMW6	White	Draughtsman	dry, mucoid	Lobate	Irregular	Pink (G -ve), Cocci Cluster	*Neisseria sps*
14.	BGMW7	White	Raised	slimy moist	Entire	Circular	Purple (G +ve), Rod Chain	*Bacillus sps*
15.	BGMW8	Slight Pink	Convex	dry, mucoid	Entire	Punctiform	Pink (G -ve), Rod Diplobacilli	*Diplobacillus sps*
16.	BGMW9	Creamy White	Raised	slimy moist	Entire	Circular	Pink (G -ve), Rod Diplobacilli	*Diplobacillus sps*
17.	BGMW10	Creamy Rose	Raised	slimy moist	Entire	Circular	Pink (G -ve), Cocci Cluster	*Neisseria sps*
18.	BGMW11	White	Convex	dry, mucoid	Entire	Punctiform	Purple (G +ve), Cocci Cluster	*Staphlococcus sps*
19.	BGMW12	Orange	Convex	dry, mucoid	Entire	Punctiform	Pink (G -ve), Rod Diplobacilli	*Diplobacillus sps*

Table 4: Heavy metal resistant Bacterial isolates of Water samples in Automobile Workstations.

Bacteria (Genus/Species)	Station 1	Station 2	Station 3
Neisseria sps.	1.7321*	1*	1.4142*
E. coli	1*	1*	1.4142*
Bacillus sps.	1.7321*	1.4142*	1.7321*
Diplobacillus sps.	1.7321*	0	1.7321*
Treponema sps.	1	0	0
Staphylococcus sps.	1	1	0
Streptobacilli sps.	0	1	1
Streptococcus sps.	0	1	1
S	6	6	6
N	8	6	8
Diplobacillus sps.	2.377	2.69	2.364
J'	0.9797	0.9946	0.9866
H'(loge)	1.755**	1.782**	1.768**
1-Lambda	0.9355	0.9831	0.9387

* square root value of individual bacterial sps.; ** is natural log value of Shannon diversity indices

CONCLUSIONS

According to the investigation, the water and soil sediments were purely contaminated with various hydrocarbon effluents and Heavy metals as well as the strong bacterial community was noted in the automobile effluent environment. The physiochemical analysis revealed the consistency of soil and water as well as bacterial diversity in the sites of automobile workshop. The bacterial community was further the isolated strains were scrutinized for heavy metals (Pb) resistance in minimal media. From the heavy metal plates, 7 isolates in soil samples and 12 isolates in water samples have been determined for the tolerance of heavy metals. The positive strains were afterward employed for characterizations and hydrocarbon degradation studies.

FUTURE SCOPE

The promising bacterial agents were dominating in such polluted environment has ability to scavenge the toxic heavy metals hence can be used as bioremediation of Heavy metals.

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Conflict of Interest. None.

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