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EFFECTS OF FLOWING RUBBER EFFLUENT ON PHYSICOCHEMICAL AND BACTERIOLOGICAL PROPERTIES OF SOIL IN CALABAR, NIGERIA

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ABSTRACT

A study was carried out to determine the extent of impact of flowing rubber effluent on physicochemical and bacteriological properties of an area through which it flows. Rubber effluent samples were collected for analysis. For soil analysis, three impact points (25 metres apart) were created along the flow channel of the effluent, and three sample points spaced 5m apart were created on both sides of each impact point. Top and subsoil samples were collected from the impact points and sample points for physicochemical and bacteriological analysis. A pristine (control) soil sample was also analysed similarly. Correlation analysis was carried out between distance and the parameters to discover trends. Single-sample t-test was used to compare study soil parameters with that of pristine soil to spot differences. Results indicated that only temperature, sulphate and chloride conformed to FEPA (1991) standards and the rubber effluent impacted the soil though parameters still recorded low values which is likely an indication of low levels of impact of effluent. In conclusion, the effluent should be treated before discharge into the environment as it contains pathogenic species and many parameters did not conform to standards.

KEYWORDS: *Rubber effluent, physicochemical, bacteriological properties*

1) INTRODUCTION

Although, there has been a general decline in rubber production in Nigeria over the decades both in the area under cultivation and total rubber output due to a variety of factors, Nigeria still remains a major producer and exporter in Africa. Natural rubber is used in a large variety of products due to its flexibility, resistance, plasticity, impermeability and insulating properties (Mooibroek & Cornish, 2000). The latex from rubber is a vital material in the automobile industry as it is used in the manufacture of tyre, car bumpers, transmission belt, car mat, seats etc. The latex is also used for the manufacture of adhesive, baby feeding bottle teat, condom, domestic and industrial gloves, balloons, balls, eraser among others (Abolagba *et al.*, 2003).

The rubber industry generates large amounts of effluent. The volume of effluent discharged relates to the size and capacity of the rubber factory. According to Nordin *et al.* (1989) an average of 45,000 litres of effluent is discharged from a single 20-30 metric tonnes of rubber per rubber factory daily. The release of untreated effluent into the environment has been found to pose harmful and undesirable effects to man and the environment. For instance, the high level of

phosphate and ammonia in rubber effluent makes it a good medium for algal growth and can result in eutrophication of surface waters if discharged without proper treatment (Iyagba *et al*., 2008). Water contaminated by rubber effluent is rendered unsuitable for domestic or industrial purposes. People living near rubber-processing factories often complain about the foul-smelling odour from the factories. Soil physicochemical and microbiological characteristics can become altered when exposed to effluent. These alterations can cause toxicity problems and nutrient imbalance in the soil.

Various researchers have analysed rubber effluent in Nigeria (Asia & Akporhonor, 2008; Omorusi, 2013; Iyagba *et al*., 2008). However, there has been no published research work on the peculiar physicochemical and bacteriological properties of this particular rubber effluent and the soil it impacts, despite the existence of this rubber factory for many decades.

2) MATERIALS AND METHODS

Study area:-

The study area, Pamol (Nigeria) Limited Rubber Estate, is located on the outskirts of Calabar, which is the capital of Cross River state, Nigeria, a city that lies between longitudes $8^0$17'00''E and $8^0$20'00''E latitudes $4^0$50'00''N and 5^0 10'00''N. The rubber factory currently produces only crepe; but had also produced latex concentrate. The factory has been releasing untreated effluent indiscriminately into the environment for decades. Overtime, a channel (near the factory) of an average height of about one metre deep developed through which the wastewater now continuously flows, with rainfall sometimes causing the flooding of the surrounding soil (within the plantation) with the effluent.

Sample collection:-

Water samples:-

Rubber effluent samples were collected at the discharge point into sterile plastic bottles. Samples used for dissolved oxygen (DO) and biochemical oxygen demand (BOD) analyses were collected in dark glass bottles. Parameters such as pH, conductivity, and dissolved oxygen were analysed immediately. When the need arose, samples were preserved at 4^0C until required (usually for 24 hours).

Soil samples:-

The experimental layout for soil sample collection on the surrounding soil around the factory is as shown in Figure 1. The larger stars represent the impact points spaced 25 metres from each other and created along the flow channel of the effluent. Other sample points (smaller stars) were created on both sides of each impact point and spaced five (5) metres from each other. From each impact and sample point, two samples representing topsoil (0-15cm) and subsoil (15-30 cm) collected and stored in sterile bags. Soil sampling was done using a cylindrical T-shaped probe with a length of 110 cm and an internal diameter of 3.2 cm. A circle of diameter (30 cm) was created at each sampling point and from within each a decontaminated probe was vertically-driven randomly into the soil three (3) times for collection of sample for bacteriological analysis and another three (3) times for sample for physicochemical analysis. Subsurface samples were collected by driving probe into the holes created during collection of surface samples. A pristine soil sample was collected from the vertices of an equilateral triangle (length = 5m) created 100 metres away (measured diagonally from second impact point through the rightmost sample point of the first impact point).

PHYSICOCHEMICAL ANALYSIS

Rubber effluent samples:-

Temperature was determined by dipping a mercury-in-glass thermometer into the sample immediately after collection. pH, conductivity, dissolved oxygen and biochemical oxygen demand (BOD) were measured using digital pH meter (HI9813; Hanna Instruments; Rhode Island, USA), conductivity meter (HI9813, Hanna Instruments, Rhode Island, USA), dissolved oxygen meter (HI76408; Hanna Instruments; Rhode Island, USA), dissolved oxygen meter (HI76408; Hanna Instruments; Rhode Island, USA), respectively. Total suspended solid (TSS) and total dissolved solids (TDS) was determined by gravimetry, chemical oxygen demand (COD) by open reflux method, ammonia by phenate spectrophotometry, nitrate by colorimetric method, phosphate by vanado-molybdate method, sulphate by turbidimetry and chloride by silver nitrate titration method (APHA, 1999).

Soil samples:-

Soil samples were air-dried, lightly crushed, passed through a 2-mm sieve, and labeled before analysis. All analysis were carried out according to Udo *et al.* (2009). Soil pH was determined potentiometrically using a glass electrode pH meter (HI9813; Hanna Instruments; Rhode Island, USA) in 1:2.5 soil to water ratio. The organic carbon content of the soil sample was determined by Walkey and Black wet oxidation method, after which each value obtained was multiplied by 1.72 (Nelson & Sommers, 1996) to obtain organic matter. Total nitrogen was determined using macro-Kjeldahl method. Extraction of available phosphorus was carried out using Bray and Krutz method and phosphorus in the extract was determined by colorimetry with an UV/VIS

Spectrophotometer (Labtech Singlebeam—295, India). Exchangeable aluminium was carried out using extraction and titration method.

BACTERIOLOGICAL ANALYSIS

Rubber effluent samples:-

To perform serial dilution, ten (10) millimetres of rubber effluent was added to 90 ml of distilled water for the first tenfold dilution. Subsequent tenfold dilutions were carried out by adding one (1.0) millimetre of already diluted sample to nine (9.0) millimetres of distilled water. For heterotrophic bacteria count, nutrient agar (Oxoid CM 003, UK) was supplemented with 50 µg/ml of nystatin as the antifungal agent. One (1.0) ml of 10^{-4} to 10^{-7} dilutions were each pour-plated out in triplicates. The colony forming units (cfu/ml) were determined after incubation at room temperature for 24 hours. For rubber effluent utilising bacteria, rubber effluent was added to mineral salts agar (Zajic & Supplison, 1972) at 1% (third rubber effluent sample analysed was used) concentration and incorporated with 50 µg/ml of nystatin as the antifungal agent. One (1.0) millimetre of 10^{-2} to 10^{-4} dilutions were each pour-plated out in triplicates. The colony forming units (cfu/ml) were determined after incubation at room temperature for 4-5 days.

Soil samples:-

For enumeration of heterotrophic bacteria (HBC), nutrient agar (Oxoid CM003, UK) was supplemented with 50 µg/ml of nystatin as the antifungal agent. One (1) millimetre of 10^{-3} to 10^{-5} dilutions (topsoil) and 10^{-2} to 10^{-4} dilutions (subsoil) were each pour-plated out in triplicates. The colony forming units (cfu/g) was determined after incubation at room temperature for 24 hours. For enumeration of rubber effluent utilising bacteria (RUBC), rubber effluent was added to mineral salts agar (Zajic & Supplison, 1972) at 1% (third rubber effluent sample analysed was used) concentration and incorporated with 50 µg/ml of nystatin as the antifungal agent. One (1) ml of 10^{-2} to 10^{-4} dilutions (top soil) and 10^{-2} to 10^{-3} dilutions (subsoil) were each pour-plated out in triplicates. The colony forming units (cfu/g) were determined after incubation at room temperature for 4-5 days.

Isolation, preservation, identification and characterization:-

Nutrient agar (Oxoid CM 003, UK) was used. Using a sterile inoculated loop, each morphologically distinct colony from water and soil samples were subcultured twice and incubated for 24 hours before being transferred to agar slant for preservation. Inocula were obtained from the respective tubes, subcultured on the respective media for 18 hours before identification and characterization. Characterization of bacterial isolates was based on morphological and biochemical tests. Identification of bacterial isolates carried out as described in Bergey's Manual of Systematic Bacteriology (Garrity *et al.*, 2001).

Statistical analysis:-

Data were subjected to techniques of descriptive statistics such as mean and standard error of the mean. Inferential statistics were carried out using Pearson's correlation, single-sample. Microsoft Excel 2013 (Microsoft Inc.) and R Statistical Software (R Foundation) were software used for analysis. The following includes definition of terms and how statistical techniques were employed: Sample point: refers to any point of soil sample collection minus impact points. Impact point: refers to any soil sample collection point along the flow channel of effluent only. Sample line: refers to all sample points on both sides of an impact point minus the impact point. Correlation (Pearson's): between successive values of a parameter on both sides of an impact point and sampling distance (excluding distance 0 cm). One-sample t-test: between each impact point and its sample line for each parameter. One-sample t-test: to compare pristine soil and sample point values of study soil for each parameter.

3) RESULTS

Physicochemical analysis of the effluent revealed that only temperature, sulphate and chloride conformed to FEPA standards (1991) (Table 1). The bacteria isolated from the rubber effluent were identified as *Pseudomonas* spp, *Micrococcus* spp, *Bacillus* spp, *Staphylococus* spp, *Proteus* spp, *Klebsiella* spp, *Escherichia coli, Enterobacter* spp and *Aeromonas* spp.

Figure 2-8 present graphical representations of values of impact points and their respective sample lines for the parameters. Table 2 shows the overall, topsoil and subsoil means for impact points, sample points and pristine soil.

Significant correlations were recorded for all parameters except HBC (Table 3). Single sample t-test results for study soil and pristine soil comparison for the parameters are presented in Table 4. For topsoil, significant results were recorded for pH, organic matter, total nitrogen, available phosphorus and RUBC. For subsoil, significant results were recorded for pH, organic matter, total nitrogen and RUBC. Table 5 shows the results of comparisons between values of impact point and their respective sample lines for the parameters. Significant results were recorded to varying degrees for all parameters.

The bacteria isolated from the soil were identified as *Micrococcus* spp, *Bacillus* spp, *Staphylococus* spp, *Alkaligenes* spp, *Serratia* spp,

Klebsiella pneumoniae, Escherichia coli, Pseudomonas spp, *Acinetobacter* spp, *Proteus* spp, *Enterobacter* spp, *Salmonella* spp and *Citrobacter* spp.

4) DISCUSSIONS

The mean temperature was 26^{o}C which falls below the permissible limit of 40^{o}C (FEPA, 1991). Thus, the effluent cannot be associated with thermal pollution. Thermal pollution can alter the temperature of a water body leading to reduced dissolved oxygen levels and fish kills.

The mean value of the rubber effluent was 5.8, indicating slight acidity. This value falls slightly outside the range of 6-9 set by FEPA (1991). An increase in pH of water can increase the solubility of phosphorus and other nutrients—making them more accessible for plant growth (Washington State Department of Ecology, 1991). This could lead to algal bloom causing decreased oxygen levels. Low pH levels leads to the solubility of heavy metals. Although, effluent limit standard does not exist for conductivity, a sudden increase or decrease in conductivity in a body of water can be indicative of pollution (Kemker, 2014).

TDS can affect water taste, and often indicates a high alkalinity or hardness (Thompson, 2006). Evans and Prepas (1996) reported decreased nitrogen fixation in blue-green bacteria exposed to approximately 2,450 mg/l TDS. Total suspended particles can contribute to shallower, lakes and streams. These settleable solids can suffocate benthic organisms and fish eggs (EPA, 2012). Organic suspended solids, such as decomposing matter or sewage effluent often naturally include high levels of microorganisms such as protozoa, bacteria and viruses (Osmond, 1995).

Dissolved oxygen is an important ecological parameter because its presence allows breathing of living organisms in water. If dissolved oxygen levels reach hypoxic or anoxic levels in rivers and streams the production and diversity of aquatic organisms will be reduced. In addition to this life-sustaining aspect, oxygen is important because the end products of chemical and biochemical reactions in anaerobic systems often produce aesthetically displeasing colours, tastes and odours in water (Hach *et al.*, 1997). Mean dissolved oxygen (DO) of 3.1 obtained in this study is relatively low.

The mean value of BOD is higher than the BOD limit of 30 mg/l set by FEPA (1991). The latex particles, proteins, sugars, and other organic matter were likely responsible for this large BOD value. If this effluent with a high BOD level is discharged into a stream or river, it could lead to higher levels of microbial growth thereby lowering the amount of dissolved oxygen available to other aquatic organisms in the water. The high COD value indicates that in addition to the organic matter contributed to BOD; the waste also contains a substantial amount of inorganics.

Ammonia (NH_3) is extremely toxic to aquatic organisms, and as pH increases, the mortality rates rise with the NH_3 concentration (Kemker, 2013). In the environment, ammonia at, neutral pH or slightly less, is oxidized to nitrate by nitrification, creating an oxygen demand and low dissolved oxygen in surface waters (Sabalowsky, 1999). The relatively low ammonia value was likely due to fact that ammonia was not used to preserve the field latex.

Nitrate is important for the growth of plant and aquatic organisms such as algae. Excess nitrate concentrations in aquatic systems is called eutrophication and can lead to algae blooms. This can result in reduced productivity, toxin poisoning, and ecological imbalance. Levels over 30 ppm of nitrate can inhibit growth, impair the immune system and cause stress in some aquatic species (Sharpe, 2013). In the blood, nitrate convert hemoglobin to methemoglobin, where it does not carry oxygen to the body cells, a condition called methemoglobinemia (Manassaram *et al.*, 2010).

High concentration of phosphate can indicate the presence of pollution and is the main cause of eutrophication in water bodies (Chapman & Kimstach, 1992), especially freshwater. An excess content of phosphorus in receiving waters usually leads to extensive algal growth. Algal blooms shade submerge aquatic vegetation, reducing or eliminating photosynthesis and productivity (Dennison *et al.*, 1993). Chronic exposure to such toxins produced by these organisms can cause gastroenteritis, liver damage, nervous system impairment, skin irritation and liver cancer in animals (WHO, 2006). An average mean value of 71.98 mg/l was recorded which exceeds the 5 mg/l set by FEPA (1991).

Sulphates are generally not believed to be toxic to aquatic life except at very high concentrations. One problem associated with sulphate-enrichment is that dissolved sulphate may be reduced to sulphide, and volatilized to air as hydrogen sulphide. This can cause noxious odours where sulphate concentrations are high and dissolved oxygen levels are low (EPA Ireland, 2001). Brouwer *et al.* (1999) suggested that increased sulphate loading in sediments assists with phosphorus release by stimulating mineralization by way of bacterial sulphate reduction. Han *et al.* (2007) stated that one of the key factors that affect the rates of mercury methylation in sediments is sulphate concentration

and the rate of microbial sulphate reduction. Methyl mercury can biomagnify in the food and can cause severe health problems in humans.

The results revealed high and diverse counts for both HBC and RUBC. All bacteria isolated in HBC were also isolated in RUBC. The high bacterial count could be due to a number of factors. Rubber is rich in nutrients required for microorganisms to flourish, so delay in processing can give rise to excessive growth of microorganisms. The use of unsuitable water can add to the microbial load of effluent. Poor sanitary practices by the factory workers can also increase the number of microorganisms in the effluent discharged. Some of the microorganisms obtained in this study have been isolated in previous studies (Girish, 2014; Senthil, 2012). Since many of the microorganisms identified in this study are pathogenic, this could potentially lead to great health concerns if effluent is discharged untreated in the environment.

Soil pH is a measure of the soil solution's acidity and alkalinity. Soil pH is fundamental to the understanding of soil systems, because it is an indicator of many reactions in the soils (Moore & Loeppert, 1987). The overall mean pH of sample points, according to Landon (1991), indicates very strongly acidic. The acidic nature of the study soil can be attributed to the high rainfall resulting in the leaching of some basic cations from the surface horizons of the soils (Iwara *et al*., 2011). The overall mean pH of sample points falls within the range of 4.0-5.5 prescribed by Pusharajah (2005) for optimal rubber growth.

Soil fertility is closely linked to soil organic matter. Organic matter provides a source of energy and food for microorganisms that are essential to biological processes in the soil; serves as large reservoir of nutrients (especially nitrogen but also phosphorus and sulphur, and the micronutrients) which are released to the plant-available pool by decomposition processes essential to the recycling of nutrients. The overall mean of sample points for organic matter, according to Landon (1991), indicates a low content. The sampled area has low organic matter content because, according to Foth (2006), areas enjoying high precipitation and temperature, have a quick rate of decomposition of biomass, hence the subsequent disappearance of organic matter.

Soil organic matter holds 90 to 95% of nitrogen held in soils and nitrogen nutrient cycle is intimately tied in with soil organic matter and soil microbial population (Murphy, 2014). Soil nitrogen is greatly influenced by various populations of microorganisms, soil organic matter, soil pH, the climate of the soil and textural classes of soil (Amin and Flowers, 2004). The overall mean of sample points, according to Landon (1991), indicates a low mean content. The low TN content is not unusual since nitrogen is intimately tied in with soil organic matter and the study soil has a low organic matter content. Low nitrogen content is a characteristic feature of tropical soils with high temperature due to fast loss of nitrogen owing to volatilization, crop removal, erosion and leaching (Landon, 1991).

The overall mean of sample points for available phosphorus, according to Landon (1991), indicates a medium mean content. This relatively high content can be attributed to root exudates like citrate acid and oxalate of the rubber tree which blocked adsorption sites of phosphate thereby increasing its availability. Organic acids and soluble humic and fulvic acids released during the decomposition of organic matter increases desorption of added phosphate by competing for binding sites of soil colloids (Gourango, 2007). Also, exchangeable acidity was low and calcium was the main cation on exchange sites. Field studies have generally shown that phosphate availability increases as calcium saturation of the exchange complex increases (Pierre, 1948). Organic matter can enhance the availability of soil phosphorus and even fixed phosphorus can be made available to plants after solubilization by soil microorganisms as well as complexation of soluble aluminium and iron by organic molecules (Ano & Ubochi, 2007). The increase in litter inputs and in situ decomposition increased the pH content, which also favoured increase in available phosphorus.

High exchangeable aluminium is common in all regions where precipitation is high enough to leach appreciable amounts of exchangeable bases from the soil surface (Achalu, *et al.,* 2012). Aluminium is not a plant nutrient. In the root, aluminium has been shown to interfere with many physiological processes including the uptake and transport of calcium and other essential nutrients, cell division, cell wall formation, and enzyme activity (Rout *et al*., 2001). The overall mean of sample points, according to Landon (1991), indicates a low mean content. This could be attributed to the presence of organic molecules that complexed aluminium present.

The significant negative correlations of physicochemical parameters indicates the receding effect of the rubber effluent on the surrounding soil, as one moves away from the impact points. These significant correlations also suggest that other potential correlations were probably blurred out by processes like leaching, erosion or rubber root chemistry. However, there were no significant correlations for exchangeable aluminium because

rubber effluent is typically not a component of rubber effluent.

The significant single-sample t-test results between pristine soil and study soil comparison for the physicochemical parameters is an indication of the likely effect of the rubber effluent on study soil. However, texture, erosion, leaching, and rubber root chemistry might also have led to these significant differences.

It was a mixed bag of results for single-sample t-test in the comparison of values of impact points with their respective sample lines for physicochemical parameters. The very weakly acidic rubber effluent flowing almost daily ensured weakly acidic pH values for the impact points, causing their values to be higher than for their respective sample lines. For organic matter and total nitrogen, there was a predominance of significant single-sample t-test results because erosion annulled the deposition of organic material by effluent, with impact point values decreasing. There was a general trend of non-significant single-sample t-test results for available phosphate because of the effect of the flowing effluent. It is rich in phosphate which it continuously deposit along its route, thereby cancelling out the effect of erosion caused by precipitation, since phosphate is also not easily washed out of the soil. For exchangeable aluminium, it was a mixed result created mainly by erosion since rubber effluent typically contains little or no aluminium.

Soil is a habitat for large populations of microorganisms. Although the microbial biomass is only a small amount of the soil organic matter (less than 5%), it is an important and dynamic fraction of soil organic matter. Although in the soil only 2-30% of the microbial mass is living biomass (Anderson & Domsch, 1978), it has profound effects on the soil. Soil bacteria and fungi play important roles in various biogeochemical cycles.

RUBC indicates the presence of organism that can utilize rubber effluent. The RUBC were lower than HBC due to the toxicity of the effluent to some organisms, lack of suitable substrates or nutrients for others. No significant correlations were observed for HBC since the media used were not selective. The significant negative correlations for RUBC highlights the receding effect of the effluent on the study soil. The sample points closer to channels were impacted more, leading to stimulation of capable organisms. These significant correlations also indicates that other potentially significant correlations were cancelled out by leaching, erosion and rubber root chemistry.

The HBC was not significantly different from that of pristine soil. This means that stimulation of rubber effluent utilizing microorganisms did not lead to increases in total number of organism in the study soil, even when RUBC increased. RUBC was significantly different from that of pristine soil due to stimulation of capable organisms by the effluent in the study soil, leading to their increase. This stimulation was near-absent in pristine soil causing smaller RUBC. The significant results of single-sample t-test in the comparison of impact points with sample lines were mainly due to washing away of microorganisms at impact points, thereby annulling the deposition of microorganisms by the effluent.

5) CONCLUSIONS

The effluent should be treated before discharge into the environment as many parameters violated standards. The study revealed that physicochemical and bacteriological investigations can be employed to study the impact of the flowing rubber effluent on the surrounding soil. Particularly, the bacteriological investigations added more weight to the body of evidence in support of the impact of the wastewater on the environment, since the stimulation of rubber-utilising microorganisms in a receding manner points to an impact decreasing with increasing distance from the flow channel of the wastewater. This implies that bacteriological investigations using a selective substrate can be used to augment or properly interpret results obtained from physicochemical studies especially in a situation where pollution is not obvious or where factors like root uptake, leaching and erosion, as in this study, can potentially affect physicochemical analysis results. Results also imply that the impact of the effluent is generally of a low nature since parameters like organic carbon, total nitrogen still recorded low values. The rubber effluent and soil could also be used to screen for microorganisms with the necessary capabilities for biodegradation of the effluent as they contains a large and diverse group of capable microorganisms.

6) Acknowledgments:-

I wish to express my deepest gratitude to Professor S. P. Antai who diligently provided guidance for this research.

7) TABLES

Table 1: Results of physicochemical analysis of rubber effluent and compared to FEPA (1991) standards

Parameters	First sample	Second sample	Third sample	Mean ± SEM	FEPA standards
Temperature (^oC)	26	25	26	26±0.33	40
pH	5.6	5.8	6.1	5.8±0.14	6-9
Conductivity (µS/cm)	6,075	4,245	3,050	4,457±880	-
DO (mg/l)	1.7	3.4	4.2	3.1±0.737	-
BOD (mg/l)	4,504	2,900	1,710	3,038±810	30
COD (mg/l)	6,200	4,749	2,643	4,531±1,033	-
TSS (mg/l)	2,164	1,550	1,200	1,638±282	30
TDS (mg/l)	3,874	2,635	1,898	2,802±576	2000
Phosphate (mg/l)	95.92	73.28	46.73	71.98±14.21	5
Nitrate (mg/l)	52.60	40.11	27.68	40.13±7.19	20
Ammonia (mg/l)	1.22	0.90	1.32	1.15±0.12	-
Sulphate (mg/l)	27.70	16.42	16.33	20.15±3.78	500
Chloride (mg/l)	59.4	39.5	32.7	43.87±8.0	600
HBC (CFU/ml)	5.50±3.60 x 10^8	1.66±5.90 x 10^7	9.60±4.81 x 10^5	1.89±4.77 x 10^8	-
RUBC (CFU/ml)	2.17±5.29 x 10^6	1.11±3.79 x 10^5	3.90±1.86 x 10^4	7.73±3.65 x 10^5	-

KEY: DO = Dissolved oxygen, BOD = Biological oxygen demand, COD = Chemical oxygen demand, TSS = Total suspended solids, TDS = Total dissolved solids, HBC = Heterotrophic bacteria count, RUBC = Rubber effluent utilising bacteria, µS/cm = MicroSiemens per centimeter, mg/l = Milligram per litre, SEM = Standard error of the mean, FEPA = Federal Environmental Protection Agen

TABLE 2: Means of physicochemical/bacteriological analysis of study soil and pristine soil

Parameters	Impact points means			Sample points means			Pristine soil	
	Overall	Topsoil	Subsoil	Overall	Topsoil	Subsoil	Topsoil	Subsoil
pH	5.9±0.19	6.1±0.03	5.8±0.38	5.0±0.05	5.0±0.09	4.9±0.07	4.7	4.6
Organic matter (%)	1.72±0.26	1.8±0.38	1.7±0.43	2.2±0.06	2.4±0.07	2.0±0.06	2.2	1.9
Total nitrogen (%)	0.09±0.01	0.09±0.02	0.09±0.02	0.10±0.00	0.11±0.00	0.10±0.00	0.10	0.09
Available phosphorus (mg/kg)	36.80±0.42	36.93±0.70	36.67±0.62	36.50±0.41	36.92±0.37	36.08±0.73	33.75	35.20
Exchangeable aluminium (cmol/kg)	0.26±0.05	0.23 ±0.04	0.30 ±0.10	0.450±0.024	0.45± 0.03	0.45± 0.04	0.41	0.39
Heterotrophic bacteria count (CFU/g)	3.38±5.77 x 10^5	6.18±6.13 x 10^5	5.75± 5.41 x 10^4	3.45±4.45 x 10^5	6.00±4.40 x 10^5	8.99±4.50 x 10^4	4.90±3.46 x 10^5	1.0±4.37 x 10^5
Rubber effluent utilising bacteria count (CFU/g)	6.30±5.37 x 10^4	1.13±4.75 x 10^5	1.31±5.99 x 10^4	6.31 ±5.61 x 10^4	1.08±4.92 x 10^6	1.75±6.30 x 10^4	8.00±4.33 x 10^4	1.23±4.26 x 10^4

Table 3. Correlation coefficient (r) relating sampling distance with each parameter

Parameters	Topsoil sample lines			Subsoil sample lines		
	1st	2nd	3rd	1st	2nd	3rd
pH	-0.05	-0.43	-0.61	-0.79	-0.94**	-0.39
Organic matter	-0.93**	-0.13	0.23	-0.22	0.46	0.80
Total nitrogen	-0.76	0.48	-0.18	0.22	0.20	-0.82*
Available phosphorus	-0.70	-0.45	-0.94**	-0.64	-0.71	-0.57
Exchangeable aluminium	-0.57	-0.29	< 0.01	-0.33	-0.32	0.81
Heterotrophic bacteria count	0.11	-0.06	-0.08	0.63	0.31	-0.11
Rubber effluent utilising bacteria count	-0.83*	-0.10	-0.83*	-0.10	0.19	-0.96**

*Correlation is significant at 0.05 alpha level (two-sided)
**Correlation is significant at 0.01 alpha level (two-sided)

Table 4. Results of one-sample t-test comparing physicochemical/bacteriological parameters of study soil with pristine soil

Parameters	Topsoil/topsoil (*p*-values)	Subsoil/subsoil (*p*-values)
pH	0.005764**	0.0002261**
Organic matter	0.003755**	0.04982*
Total nitrogen	0.00743**	$< 2.2 \times 10^{-16}$**
Available phosphorus	1.289×10^{-7}**	0.2476
Exchangeable aluminium	0.2872	0.09448
Heterotrophic bacteria count	0.1601	0.1094
Rubber effluent utilising bacteria count	0.007049**	0.02097*

*Correlation is significant at 0.05 alpha level (two-sided)
**Correlation is significant at 0.01 alpha level (two-sided)

Table 5. Results of one-sample t-test comparing the physicochemical/bacteriological parameters of three impact points with their respective sample lines

Parameters	Topsoil			Subsoil		
	1st IM/1st SL	2nd IM/2nd SL	3rd IM/3rd SL	1st IM/1st SL	2nd IM/2nd SL	3rd IM/3rd SL
pH	0.001972**	7.494×10^{-6}**	0.001559**	0.001032**	1.373×10^{-5}**	0.2722
Organic matter	0.02614*	0.04783*	1.86×10^{-5}**	0.1051	0.01388*	4.5×10^{-5}**
Total nitrogen	0.1412	0.07983	0.0001187**	0.01082*	0.07559	4.802×10^{-5}**
Available phosphorus	0.1063	0.01805*	0.5548	0.5305	0.9378	0.09985
Exchangeable aluminium	0.03324*	0.05746	0.0001564**	0.2403	0.002412**	0.8985
HBC	0.1073	0.2494	0.000149**	0.0425*	0.547	6.808×10^{-6}**
RUBC	0.001337**	0.04454*	7.256×10^{-5}**	0.004183**	0.03932*	9.134×10^{-5}**

*Correlation is significant at 0.05 alpha level (two-sided)
**Correlation is significant at 0.01 alpha level (two-sided)
KEY: HBC = Heterotrophic bacteria count, RUBC = Rubber effluent utilising bacteria

8) FIGURES

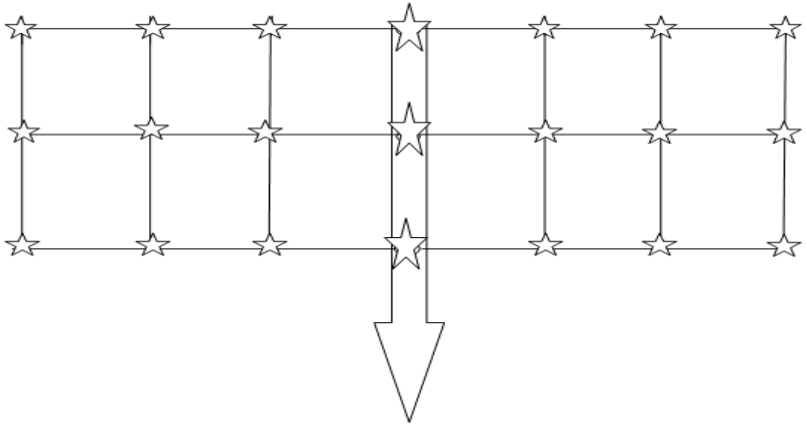

FIG 1. Experimental layout

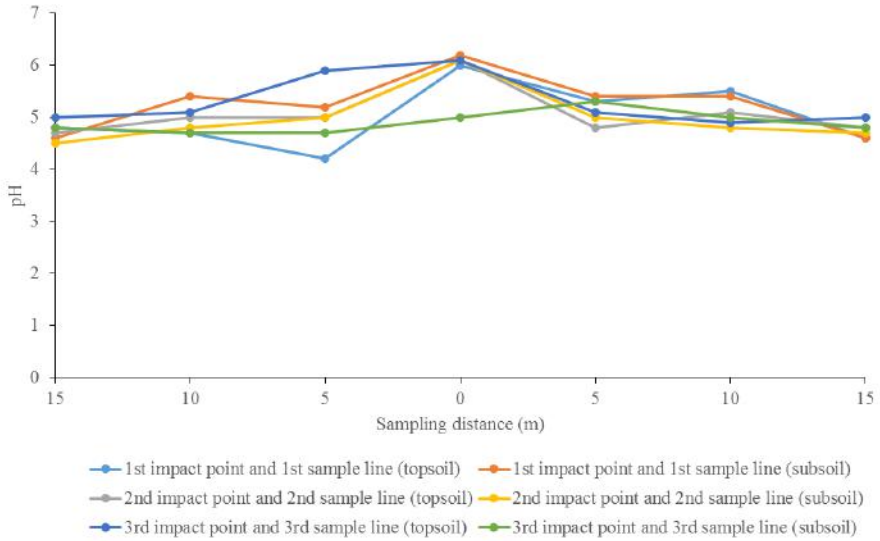

FIG 2. Graphical representation of pH values of study soil

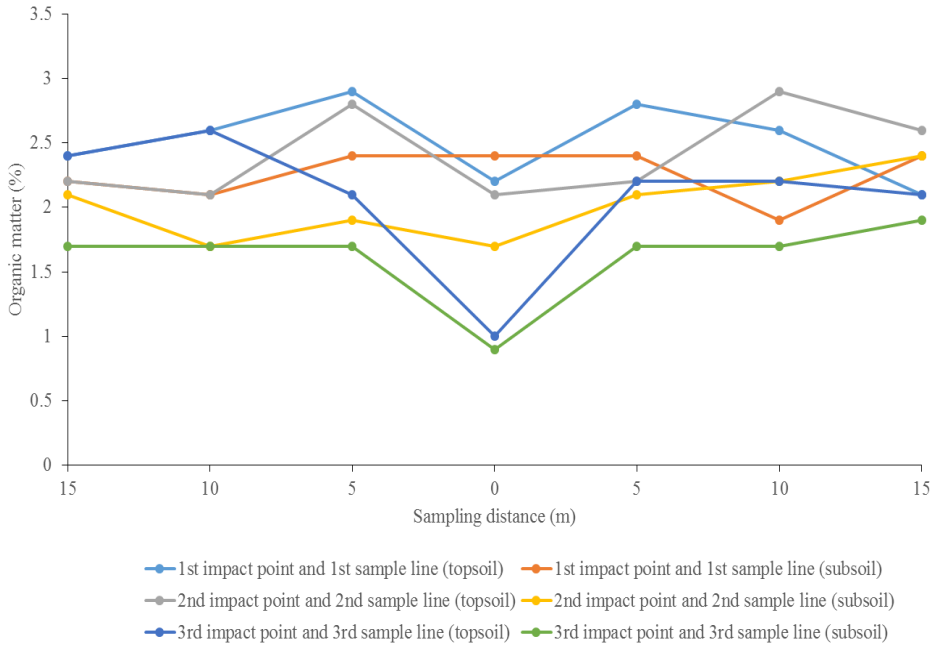

FIG 3. Graphical representation of organic matter values of study soil

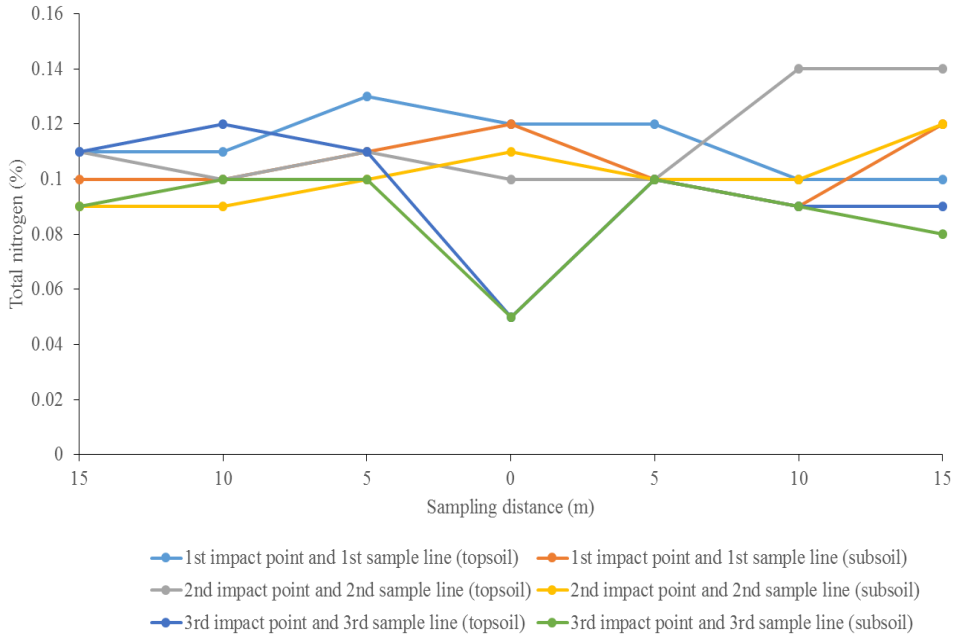

FIG 4. Graphical representation of total nitrogen values of study soil

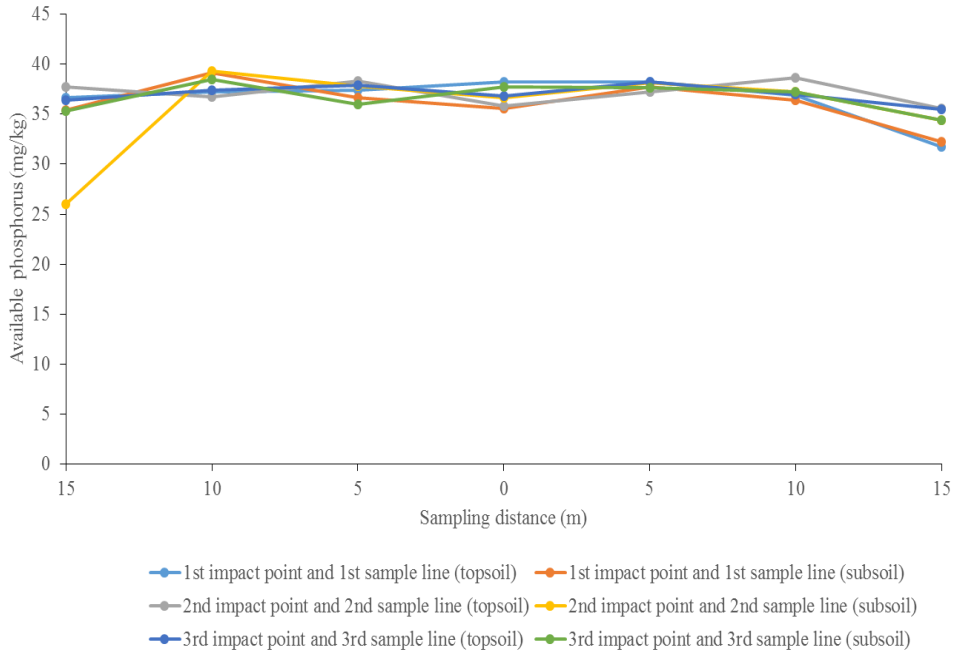

FIG 5. Graphical representation of available phosphorus values of study soil

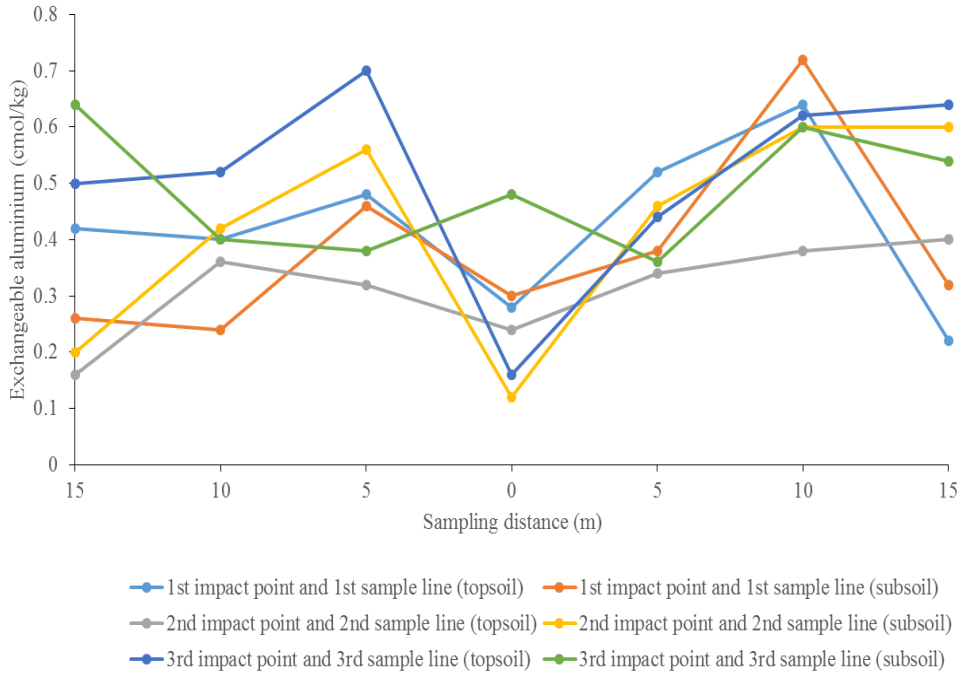

FIG 6. Graphical representation of exchangeable aluminium values of study soil

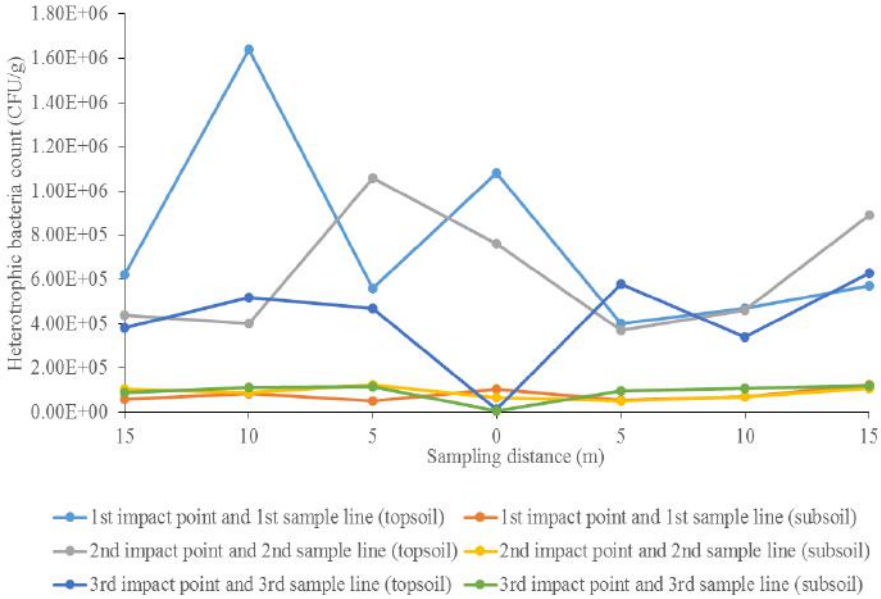

FIG 7. Graphical representation of HBC values of study soil

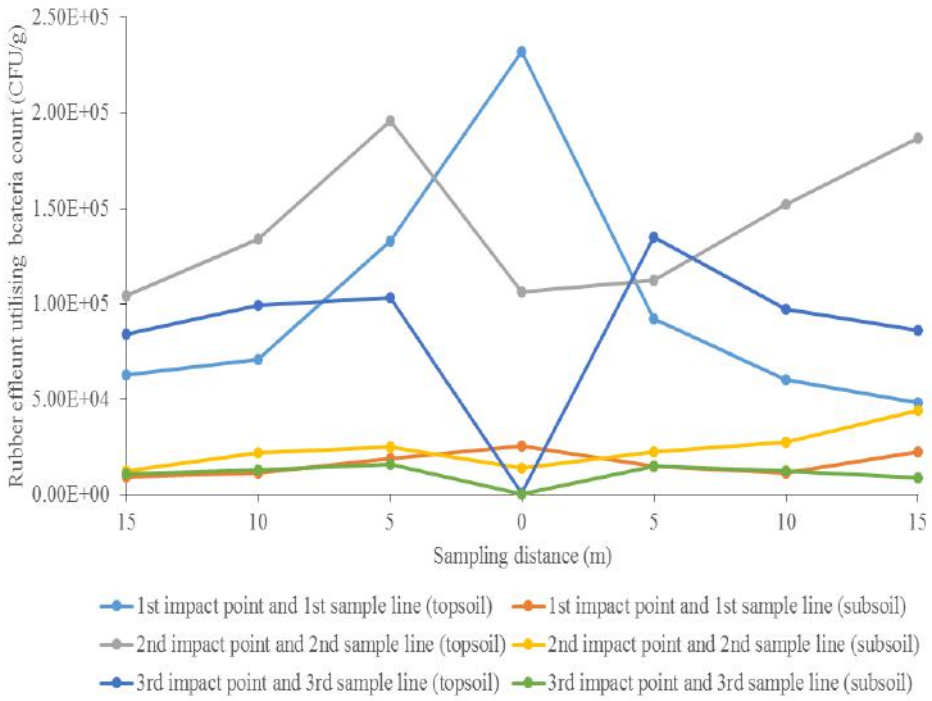

FIG 8. Graphical representation of RUBC values of study soil

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