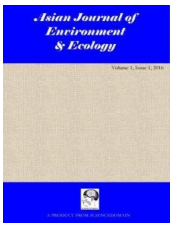

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Rubber Effluent Bio-Analyses and Its Impacts on the Microbial Community Structure of the Soil in Calabar, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AAB and MLI designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AAU and BEA managed the analyses of the study and prepared the final manuscript. All the authors managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The study was carried out by artificially polluting an agricultural soil in Calabar with varying concentrations (0 ml, 250 ml, 500 ml, 1000 ml and 2000 ml) of rubber effluent, in which 0 ml served as control, with the aim of assessing their effect on soil microflora and fertility. The polluted soil was analysed in terms of the following parameters; microbial population, soil pH organic matter, total nitrogen, available phosphorus, electrical conductivity, calcium, magnesium, potassium, sodium, effective cation exchange capacity, exchangeable acidity and base saturation. In the polluted soils, the total heterotrophic bacteria, total heterotrophic fungi and total heterotrophic actinomycetes increased significantly ($p<.05$) with a decrease in the concentration of pollutants. The total heterotrophic bacteria and total heterotrophic actinomycetes showed significant reduction with an increase in the length of pollution while total heterotrophic fungi did not show difference ($p>.05$) over the duration of pollution. Microbial species isolated from the polluted soil were *Pseudomonas* sp.,

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Bacillus sp., *Staphylococcus* sp., *Micrococcus* sp. *Flavobacterium* sp., *Mucor* spp., *Fusarium* spp., *Penicillum* spp., *Aspergillus* spp., *Rhizopus* spp., and *Streptomyces* spp. In the polluted soil, pH, organic matter, total nitrogen, potassium, available phosphorus, magnesium and sodium, showed significant differences ($p<.05$) in their values with the control, while calcium, electrical conductivity, base saturation, effective cation exchange capacity, and exchangeable acidity did not show significant difference ($p>.05$) with that of the control. The results of this study revealed that light application of rubber effluent could enhance microbial proliferation and thus, increases soil fertility, while a heavy application inhibits the same.

Keywords: Rubber effluent; bio-analyses; microbial community; soil; impacts.

1. INTRODUCTION

Various waste materials including solid and liquid wastes and volatile organic gases produced in rubber processing industries are being continuously released into the environment without or in few cases with partial treatment and affect the basic environmental components such as, soil, water and air [1]. Soil and environment are under tremendous pressure due to industrial expansion and discharge of effluents. The industrial effluents and water drainage from spoil and rubbish heaps either washes direct to nearby fields and enter the local streams, river and ultimately into the soil. Once pollutants enter and are incorporated into the soil, their concentration continuously increases and accumulates, and become toxic to all forms of life like plants, microorganisms and human being [2].

Soil is an efficient purifying medium with a great capacity to receive and decompose wastes and matter by its microflora and precipitate out nutrients [3-4]. However, if the input of the pollutants exceeds the soil purifying limit, the effectiveness of soil microorganism activity is reduced considerably. As a result, there occurs an adverse change in the soil physico-chemical properties which consequently affect the growth and development of the crop plants [1]. Industrial effluents as pollutants contain a large number of both known and unknown substances formed during the production process. There is a direct impact of pollutants on minerals, organic matter and microbial community of soil [3-4]. The discharge of industrial effluents especially without treatment may have profound influence on physico-chemical and biological properties of soil related to soil fertility.

Rubber effluent is known to contain a large amount of non-rubber substances in addition to traces of various processing chemicals. The controlled applications of rubber effluent on land have been reported to cause changes in soil properties [5]. Rubber latex processing for example, involves sequential immersion in various chemicals before the final products are ready for the market. This process leaves behind toxic and concentrated aqueous solution with obnoxious odour [6-9]. The discharge of such mixture may give rise to various types of harmful effects or outright pollution in the receiving environment [7]. Pollution action of rubber effluents is due to the presence of large amounts of dissolved organic and inorganic solids which create a high oxygen demand [3,10-13].

2. MATERIALS AND METHODS

2.1 Experimental Design

The soil sample used in this study was an agricultural soil collected from the botanical garden in the University of Calabar. The pollution of the soil was performed artificially with four concentrations (0 ml, 250 ml, 500 ml, 1000 ml and 2000 ml) of rubber effluent in a completely randomized manner. And the duration of this study was sixteen weeks.

2.2 Collection of Samples

2.2.1 Soil sample

Soil samples of 0-15 cm depth were collected (5 kg) from five locations on an agricultural soil within University of Calabar by excavation using spade. The collected samples from all locations were thoroughly mixed on the spot in order to obtain composite sample. 5 kg of this soil was weighed into four different polythene bags [3].

2.2.2 Rubber mill effluents samples

Rubber effluents were collected from Pamol (Nigeria) Limited Estate, Odukpani, in Cross River State. The samples were collected with clean plastic containers rinsed several times with the same sample and transported within 24 hours to the Department of soil science,

University of Calabar for physicochemical analysis and pollution of the soil.

2.2.3 Pollution of the soil sample

Pollution of the soil was achieved by employing the method of Orhue et al. [5]. 0 ml, 250 ml, 500 ml, 1000 ml and 2000 ml of the samples was added to 2 kg of already dried soil in each polyethylene and mixed thoroughly for even distribution. These polluted soils and the unpolluted soil (control) were left outside under normal environmental condition for the duration of 16 weeks (Four months).

2.3 Microbial Analyses

Microbial analyses were carried out before and after pollution. 10 g of Soil sample was collected aseptically, labelled and store in ice packed plastic coolers and transported to the Microbiology Department Laboratory University of Calabar where microbial analysis was carried out within 24 hours of sampling so as to maintain the stability of the sample without significant alteration in the microbial population.

2.4 Dilution

Serial dilution was carried out by weighing 10 g of soil in to 90 ml of sterile water contained in a stoppered 200 ml volumetric flask and agitated to dislodge the microorganisms from the soil particles. From this initial dilution, a ten-fold serial dilution was prepared [3].

2.5 Enumeration of Heterotrophic Bacteria

The counts of total heterotrophic bacteria in the soil samples was determined by pour plating 1 ml of desired dilutions into nutrient agar (NA). The medium was incorporated with antifungal agent (50 µg/ml Nystatin), in order to prevent the growth of fungal contaminants. Bacterial colonies were counted after 24 hours of incubation at 37°C and reported as a number of colony forming units (cfu) per gram of soil [3,9-12].

2.6 Enumeration of Heterotrophic Fungi

The total heterotrophic fungi count was measured by pour plating 1 ml of 10^{-3}dilution into Sabouroud dextrose agar (SDA) supplemented with antibacterial agents (50 µg/ml of streptomycin and 30 µg/ml of penicillin) to inhibit the growth of bacterial contaminants. Fugal counts were reported after 72 hours of incubation at room temperature [3,9-12].

2.7 Enumeration of Heterotrophic Actinomycetes

Enumeration of total actinomycetes was achieved by pour plate technique. 1 ml of 10^{-2}dilution was plated unto sodium caseinate agar, 50 µg/ml of nystatin and 30 µm/ml of tetracycline was added to inhibit fungal and bacterial growth. An actinomycetes count was reported 7 days after incubation at room temperature [3].

2.8 Maintenance of Pure Isolates

Bacterial colonies were repeatedly transferred to freshly prepared nutrient agar plates by the streak-plate method and allow growing for 48 hours before stocking. Similarly, distinct fungal and actinomycetes colonies were subculture repeatedly on freshly prepared Sabouroud dextrose agar plates and sodium caseinate agar, respectively. Pure isolates of the microorganisms were maintained on agar slants as stock, which were preserved in the refrigerator for further use.

2.9 Characterization and Identification of Isolates

Various methods were used to characterize and identify the isolates. The test results for bacteria were evaluated using characteristics presented in *Bergy's Manual of Determinative Bacteriology* [14].

Representative colonies of fungal isolates were characterized and identified based on their cultural and morphological features as described by Barnett and Hunter [15]. The characterizations were achieved through staining techniques-using lactophenol in cotton blue.

2.10 Soil Physicochemical Analysis

Physicochemical analysis of the pristine soil was carried out before pollution. And after pollution with varying concentrations of the effluent on the soils, physiochemical analysis of each soil sample polluted with different concentrations of each effluent was also carried out bimonthly (every 8 weeks) [3,9-12].

2.11 Particle Size and Textural Class Analysis

In carrying out this test, the Bouyoucos-type hydrometer method described by Day [16] was used.

2.12 Soil pH

Soil pH was determined in water 1:2 soils: water ratio using pH meter with glass electrode. 20 g of air-dried soil was weighed into a 50 ml beaker, and 20 ml of distilled water was added and allowed it to stand for 30 minutes. The electrode of the pH meter was inserted into the 1:2.5 soil /water partly settled suspension and measured the pH. The result was recorded as soil pH measure in water [3].

2.13 Electrical Conductivity (EC)

In the same soil solution (1:2.5 soil /water solution) for pH determination, electrical conductivity electrode was inserted into the partly settled suspension and the EC was measured [3, 9-12].

2.14 Organic Matter

This was determined by the dichromate wet-oxidation method as described by Nelson and Summers [17].

2.15 Total Nitrogen

Total nitrogen was determined by the micro-Kjeldahl method as described by Bremmer [18].

2.16 Available Phosphorus

Available phosphorus was extracted with acid fluoride using Bray P-1 method described by Bray and Kurtz [19].

2.17 Exchangeable Cations

The bases were extracted with neutral NH_4OAC. Calcium and magnesium were determined in the extract by EDTA titration, and potassium and sodium by the use of flame photometer [20].

2.18 Exchangeable Acidity

A+ and H+ were obtained by leaching the soil with INKCl solution and the and the extract titrated with standard NaOH. [3,20].

Exchangeable acidity (Al + H) – Exchangeable Al = Exchangeable H

2.19 Effective Cation Exchange Capacity

This was determined by calculation. That is, total exchangeable bases (Ca+ Mg + Na) + Exchangeable acidity (EA) [3].

2.20 Percentage Base Saturation

This will be achieved by dividing the total exchangeable bases by exchangeable cation capacity and multiplied by 100 [6].

$$\% \text{ base saturation} = \frac{\text{Summation of exchangeable bases} \times 100}{\text{ECEC}}$$

2.21 Statistical Analysis

All statistical analysis of data from various treatments was carried out using analysis of variance (ANOVA) test using factorial experiment. Means were separated using least significance difference (LSD).

3. RESULTS

3.1 Microbial Analysis

Table 1 shows the enumeration of total heterotrophic bacteria (THB), total heterotrophic fungi (THF) and total heterotrophic actinomycetes (THA) in the pristine soil. The counts obtained were as follows 1.90 ±1.41 x 10^7 Cfu/g, 1.29 ± 1.25 x 10^5 Cfu/g and 9.2 + 1.25 x 10^3 cfu/g respectively.

Table 1. Total heterotrophic bacteria, total heterotrophic fungi and total actinomycetes counts of the soil before the commencement of the study

THB (cfu/g)	$1.90 \pm 1.41 \times 10^7$
THF (cfu/g)	$1.29 \pm 1.25 \times 10^5$
THA (cfu/g)	$9.2 \pm 1.25 \times 10^3$

Key: THB = Total heterotrophic bacteria, THF= Total heterotrophic fungi, THA= Total heterotrophic actinomycetes, CFU/g = Colony forming unit/gram

Table 3 shows the effects of rubber effluent and duration of pollution on microbial populations. There was a significant reduction ($P< 0.05$) in THA counts obtained after 16 weeks of pollution as compared to 8 weeks of pollution.

3.2 Organic Matter Content

The values of organic matter in control (0 ml) soils were in the range of 2.41 ± 0.23% and 2.41 ± 0.09%. The organic matter content of the soils treated with varying concentrations of rubber effluent treated soils ranged from 2.68 ± 0.18% to 4.24 ± 0.26% (Fig. 2).

Table 2. Effects of concentrations of pollution on microbial population in rubber effluent polluted soil

	0 ml	250 ml	500 ml	1000 ml	2000 ml
THB (cfug^{-1})	$1.87^{a}\pm2.08x10^{7}$	$1.47^{a}\pm1.95x10^{7}$	$1.23^{a}\pm1.26x10^{7}$	$7.8^{b}\pm1.48x10^{6}$	$5.3^{b}\pm1.31x10^{6}$
THF (cfug^{-1})	$1.28^{c}\pm3.06x10^{5}$	$2.58^{a}\pm3.80x10^{5}$	$1.93^{b}\pm2.84x10^{5}$	$1.05^{c}\pm2.26x10^{5}$	$9.4^{c}\pm2.04x10^{4}$
THA (cfug^{-1})	$9.3^{b}\pm2.04x10^{3}$	$1.25^{a}\pm2.34x10^{4}$	$9.9^{b}\pm1.64x10^{3}$	$6.9^{b}\pm1.34x10^{3}$	$5.9^{b}\pm1.20x10^{3}$

Key: means with the same letter along the horizontal arrays indicates significant difference (P< 0.05), and means with different letter along the column indicates no significance difference (P>0.05)
THB = Total heterotrophic bacteria, THF= Total heterotrophic fungi, THA= Total heterotrophic actinomycetes, CFU/g = Colony forming unit/gram

Table 3. Effects of duration of pollution on microbial population in rubber effluent polluted soils

	8 weeks	16 weeks	LSD
THB(CFUg^{-1})	$1.64 \pm 2.42 \times 10^{7\ a}$	$7.11\pm 1.04 \times 10^{6\ b}$	2.69
THF (CFU g^{-1})	$1.67 \pm 3.02 \times 10^{5\ a}$	$1.44\pm 2.86 \times 10^{5\ a}$	3.38
THA (CFU g^{-1})	$1.09 \pm 2.73 \times 10^{4\ a}$	$6.9 \pm 0.86 \times 10^{3\ b}$	4.04

Key: means with the same letter along the horizontal arrays indicates significant difference (P< 0.05), and means with different letter along the column indicates no significance difference (P>0.05)
THB = Total heterotrophic bacteria, THF= Total heterotrophic fungi, THA= Total heterotrophic actinomycetes, CFU/g = Colony forming unit/gram, LSD= Least significant difference

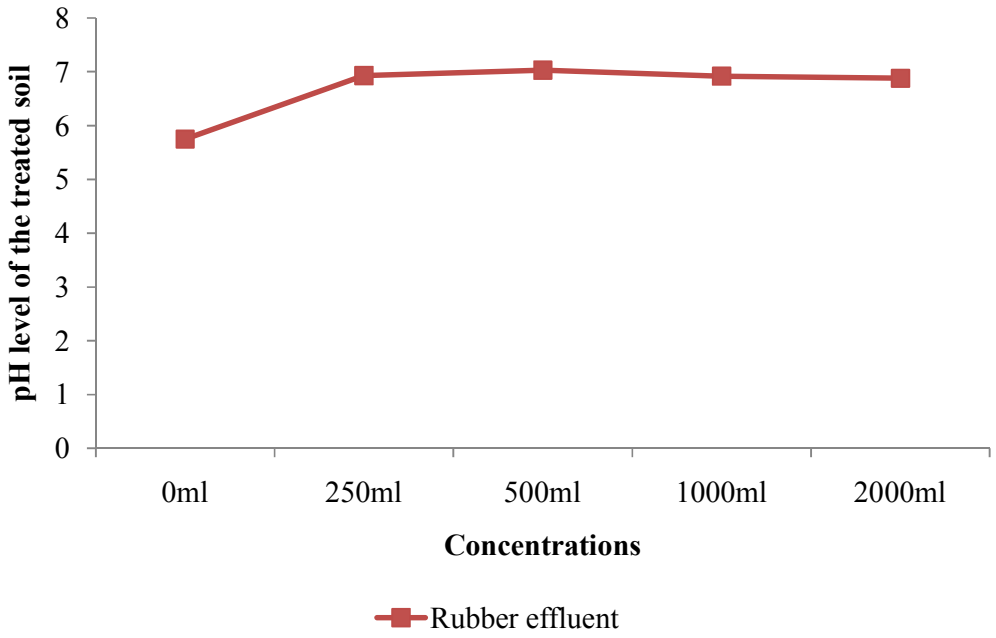

Fig. 1. Effects of concentrations of rubber effluent on soil pH

4. DISCUSSION

The pollution index of rubber effluent varies with the quality of the raw materials and production process used in manufacturing the latex. In this study, following pollution of soil with varying concentrations of rubber effluent, the soil displayed profound changes in the microbial populations and physicochemical characteristics.

In assessing the effects of different concentrations of the effluents on microbial population, the result showed no significant difference ($p > 0.05$) in THB mean count in 250 ml and 500 ml of the polluted soils over the control. This implies that the THB mean counts in 250 ml and 500 ml of polluted soils were in the same range with the control. There was a significant reduction ($p \leq 0.05$) in THB in 1000 ml

and 2000 ml compared to the control. Also, the result showed that there was a significant increase ($p \leq 0.0\ 5$) in THF and THA in 250 ml and 500 ml of polluted soil (Table 2).

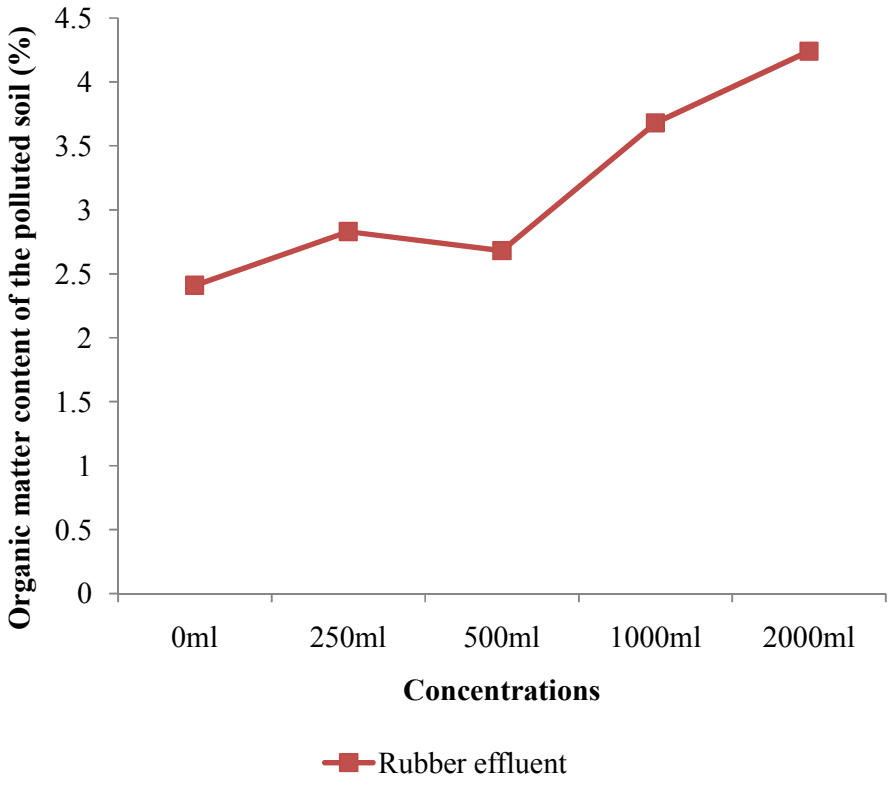

Fig. 2. Effects of concentrations of rubber effluent on soil organic matter

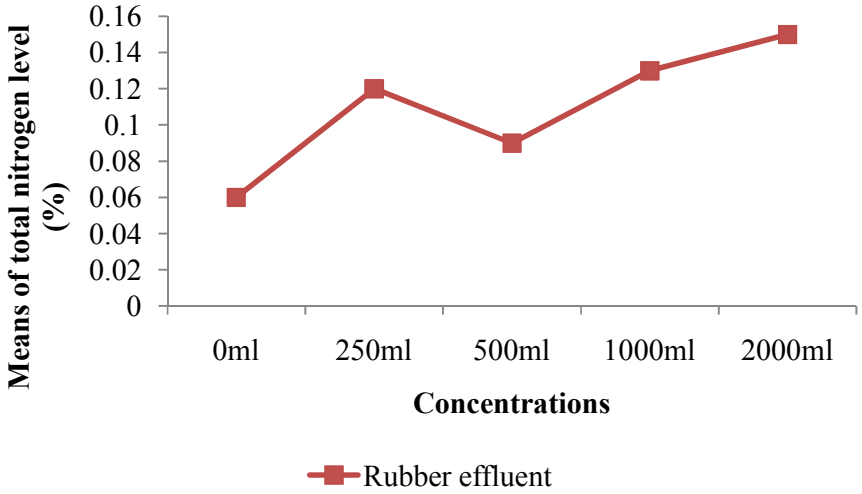

Fig. 3. Effects of concentrations of rubber effluent on total nitrogen content of the polluted soils

Table 4. Physicochemical properties of the soil before the commencement of the study from an agricultural soil in University of Calabar

Parameters	Values
pH	5.60
Electrical conductivity (ds/m)	0.055
Organic matter (%)	2.41
Total nitrogen (%)	0.06
Available Phosphorus (mg/kg)	31.54
THC (mg/kg)	BDL
Ca (cmol/kg)	4.00
Mg (cmol/kg)	2.10
Na (cmol/kg)	0.06
K (cmol/kg)	0.21
EC (cmol/kg)	1.11
ECEC (cmol/kg)	8.82
Base saturation (%)	85.16
Sand (%)	75.80
Silt (%)	12.60
Clay (%)	11.60
Lead (mg/kg)	6.20
Nickel (mg/kg)	9.44
Cadmium (mg/kg)	4.32
Chromium (mg/kg)	5.60
Iron (mg/kg)	919.66

Significant increase ($p \leq 0.05$) in THA count was also observed in 250 ml and 500 ml of polluted soil with a characteristic higher THA count than the control. This was attributed to the acidic nature of the effluent and the soil. Soil actinomycetes can tolerate a pH level of up to 6.80; this was the pH level in most of the treated soils in this study. In assessing the concentrations of pollution, 250 ml of effluents was found to be most favourable for the proliferation of microbial populations, followed by 500 ml. Similar result had earlier been reported by Nguago et al. [22].

Table 5. Effects of duration of pollution on physico- chemical properties of the soil polluted with rubber mill effluent

	8 weeks	16 weeks	LSD
pH	6.96^{a}±0.03	6.45^{c}±0.05	0.13
Organic matter	3.31^{c}±0.11	3.03^{d}±0.06	0.11
Avail. P	19.28^{a}±0.18	17.93^{b}±0.10	0.54
Total N	0.11^{a}±0.03	0.11^{a}±0.01	0.01
Mg	2.66^{b}±0.07	1.85^{d}±0.08	0.06
Na	0.10^{c}±0.03	0.23^{a}±0.02	0.02
Ca	5.46^{a}±0.08	4.24^{b}±0.10	0.38
Ec	0.08^{b}±0.01	0.09^{b}±0.02	0.01
Bs	78.80^{a}±1.83	73.78^{b}±0.98	0.86
K	0.22^{b}±1.04	$0.18.18^{c}$±0.06	0.01
ECEC	10.71^{a}±0.16	8.81^{c}±0.18	0.44
EA	2.27^{a}±0.09	2.31^{a}±0.08	0.13

Key: means with the same letter along the horizontal arrays indicates significant difference (P< 0.05), and means with different letter along the column indicates no significance difference (P>0.05)

Table 6. Effects of concentrations of pollution on the physiochemical properties of the soil polluted

	0 ml	250 ml	500 ml	1000 ml	2000 ml	LSD
pH	5.75^{c}±0.21	6.93^{a}±0.14	7.03^{a}±0.09	6.92^{a}±0.10	6.88^{a}±0.08	0.20
Organic matter	2.41^{e}±0.09	2.83^{d}±0.20	2.68^{d}±0.18	3.68^{c}±0.22	4.24^{b}±0.26	0.18
Avail. P	31.54^{a}±2.50	13.53^{e}±1.09	14.73^{d}±1.18	17.09^{c}±0.98	16.16^{d}±0.5	0.85
Total N	0.06^{c}±0.01	0.12^{b}±0.03	0.09^{b}±0.02	0.13^{b}±0.01	0.15^{b}±0.04	0.02
Mg	2.40^{d}±0.06	2.15^{e}±0.10	2.08^{e}±0.07	2.31^{d}±0.12	2.33^{d}±0.08	0.09
Sodium (Na)	0.09^{b}±0.01	0.10^{b}±0.02	0.11^{b}±0.01	0.45^{a}±0.03	0.10^{b}±0.01	0.04
Calcium (Ca)	4.93^{a}±0.04	4.73^{a}±0.03	4.75^{a}±0.03	4.80^{a}±0.08	4.89^{a}±0.04	0.61
Ec	0.05^{b}±0.01	0.10^{b}±0.02	0.07^{b}±0.01	0.9^{b}±0.01	0.09^{b}±0.01	0.02
Base saturation	78.66^{b}±0.58	75.61^{b}±1.03	74.53^{b}±1.11	76.28^{b}±0.98	77.18^{b}±0.84	1.35
K	0.21^{b}±0.06	0.15^{b}±0.04	0.19^{b}±0.03	0.19^{b}±0.02	0.29^{a}±0.20	0.02
ECEC	9.7^{b}±0.06	9.43^{b}±0.11	9.54^{b}±0.18	10.16^{b}±0.20	9.86^{a}±0.26	0.69
EA	2.07^{a}±0.05	2.30^{a}±0.08	2.43^{a}±0.01	2.41^{a}±0.06	2.25^{a}±0.04	0.20

Key: means with the same letter along the horizontal arrays indicates significant difference (P< 0.05), and means with different letter along the column indicates no significance difference (P>0.05)

Also in assessing the effect of duration of pollution and different concentrations of pollutant on microbial population, THB showed significant increase ($p < 0.01$) with decrease in concentrations of pollutant and significant reduction with increase in the duration of pollution (Table 3). This result implies that there was a steady reduction in THB with increase in concentrations and duration of pollution (Table 3). Also, the THF mean counts at 250 ml of the polluted soil showed significant increase ($p<0.05$) after 8 weeks of pollution and a significant reduction was observed in the same concentration of pollution after 16 weeks of pollution and while there was no significant difference in THF count at 500 ml, 1000 ml after 8 weeks and 16 weeks of pollution (Table 3). Similarly, there was significant increase ($p<0.05$) in THA at 250 ml, 500 ml, after 8 weeks of pollution and 250 ml after 16 weeks of pollution, and no significant difference was also observed in THA in 1000 ml, 2000 ml after 8 weeks of pollution and 500 ml, 1000 ml and 2000 ml after 16 weeks of pollution.

This result implies that, there was a significant increase ($p < 0.05$) in THB, THF and THA in all the treated samples after 8 weeks of pollution, followed by a reduction in THB and THF after 16 weeks of pollution. Furthermore, THA did not show reduction after 16 weeks of pollution (Table 3).

There were significant changes in physicochemical properties. These changes have earlier been reported by Russell et al. [23] that continual applications of effluents on the soil can change soil properties, e.g. pH and nutrient concentrations.

5. CONCLUSION

This study revealed that rubber effluents could be harmful to the soil if not properly managed and also useful if properly discharged since light application of the effluent could enhance microbial proliferation which enhances soil fertility. Therefore, government should create awareness to those involved in small and large scale rubber latex processing on the need for proper disposal of effluent.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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