Original Research Article

Assessment of the impact of untreated rubber effluent on the base cationic and mycological properties of rubber plantation soil in Calabar, Nigeria.

ABSTRACT

The study was carried out to determine the impact of rubber effluent on the cationic and mycological properties of soil in a rubber plantation through which it flows. Rubber effluent samples were collected for physicochemical and mycological analysis from the effluent discharge point of a rubber factory in Calabar, Nigeria. 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 base cationic and mycological analysis. A control soil sample was also collected similarly. Correlation analysis, single-sample and two-sample were used to analyse the results. Results revealed that only temperature (26^{o}C), sulphate (20.15mg/l) and chloride (43.87mg/l) conformed to Federal Environmental Protection Agency (FEPA) permissible limits of 40^{o}C, 500mg/l and 600mg/l, respectively. Bacteria isolated from the rubber effluent were identified as *Pseudomonas* spp, *Micrococcus* spp, *Staphylococus* spp, *Proteus* spp, *Klebsiella* spp, *Bacillus* spp, *Escherichia coli*, *Enterobacter* spp and *Aeromonas* spp. Fungi isolated were identified as *Aspergillus* spp, *Penicillium* spp, *Rhizopus* spp, *Mucor* spp and *Sporothrix* spp. Results also revealed that the rubber effluent impacted the soil, but parameters still recorded low values, as the effects of the effluent on the soil were altered by leaching, erosion and rubber root uptake. The study also revealed that microbiological investigation involving the use of a selective substrate can be used to augment or properly interpret results obtained from base cation studies similar to the current study, especially in a situation where pollution is not obvious or where factors like root uptake, leaching and erosion can potentially affect statistical results of base cation analysis.

Keywords: Base cations, Calabar soil, Mycological analysis, Nigeria, Rubber effluent, Rubber plantation

1. INTRODUCTION

Natural rubber is a common and easily available polyisoprenoid (biopolymers produced by living organisms). Although, over 1,500 species across 300 genera and eight families are known to produce latex-containing rubber particles, only a small number produce large quantities of rubber particles of high molecular mass [1]. Currently, natural rubber (*Hevea brasiliensis*) is the most important source of natural rubber.

Natural rubber is extensively used in the production of thousands of products in a variety of areas due to highly desirable qualities like impermeability, plasticity, flexibility, insulating and resistance properties [2]. Natural rubber is an important component of the automobile industry and it is used in the production of tyres, seats, bumpers, transmission belts, car mats, etc. Latex is used for the production of gloves, boots, baby feeding bottle teats, condoms, adhesives, balls, balloons, eraser etc [3]. Natural rubber is a highly valuable biopolymer of strategic importance which, unlike the majority of other biopolymers, cannot be completely substituted by synthetic materials in some applications.

Agro-based industries generate large amounts of effluent and natural rubber processing is a typical example. Natural rubber processing requires large amounts of water and chemicals for its operation, generating large quantities of effluent in the process. Effluent volume generated is related to the size and capacity of the rubber plant. A factory that produces 20-30 metric tonnes of rubber generates an average of 45,000 litres of effluent daily [4].

Rubber effluent, if not properly treated before disposal, can cause severe damage to man and the environment. For instance, rubber effluents usually contain high levels of phosphate and ammonia which makes it a suitable medium for algal growth; therefore, eutrophication of rivers and streams can result if discharged without proper treatment [5]. The presence of suitable substrates and nutrients (from natural latex) also makes it an ideal medium for a variety of microorganisms.

People living close to 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. Pollution of the soil can also be hazardous to man and the environment when toxic chemicals move through the food chain or percolate into groundwater used for drinking purposes [6]. Various researchers have analysed rubber effluent in Nigeria [7, 5, 8]; however, there has been scanty published research work on the peculiar physicochemical and mycological properties of this particular rubber effluent, and its impacts on surrounding soil. Also, the ever-increasing global spotlight on the environment requires that effluent properties and effluent impact be properly monitored.

2. MATERIALS AND METHODS

2.1. Study area

The rubber factory (N 5° 6' 80" and 8° 20' 24" E) is located on the outskirts of Calabar, which is the capital of Cross River state, Nigeria. For soil samples, the study area (8^0 20' 24.5" E and N 5^0 6' 6.2", geocoordinates for the second impact point) lies just outside the rubber factory. The factory used to produce latex concentrate, however currently produces mainly crepe rubber. The factory has been releasing untreated effluent indiscriminately into the environment for decades. Over time, a channel (near the factory) of an average depth of about one metre developed, through which the wastewater flows, with rainfall sometimes causing flooding of the surrounding soil. The soils sustaining the rubber plantation are classified as Ultisols [9].

2.2 Sample collection

2.2.1 Rubber effluent samples

Rubber effluent samples were collected once per week consecutively (three times) at the discharge point into sterile plastic bottles. Samples used for dissolved oxygen (DO) and biochemical oxygen demand (BOD_5) analyses were collected in dark glass bottles. Parameters such as pH, conductivity, and dissolved oxygen were analysed immediately. Samples were preserved (usually for 24 hours) at 4°C until required.

2.2.2 Soil samples

The experimental layout for soil sample collection 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 effluent flow channel. 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) were collected and stored in sterile bags. Soil sampling was done using a cylindrical T-shaped probe. A circle of diameter (30 cm) was created at each sampling point and from each a decontaminated probe was vertically-driven randomly into the soil three (3) times for collection of samples for mycological analysis and randomly again 3 times for base cation samples. Subsoil samples were collected by driving a decontaminated probe into the holes created during collection of topsoil samples. A control (pristine) soil sample was collected from the vertices of an equilateral triangle (length = 5m) created 100 metres away (measured diagonally from the second impact point through the rightmost sample point of the first impact point).

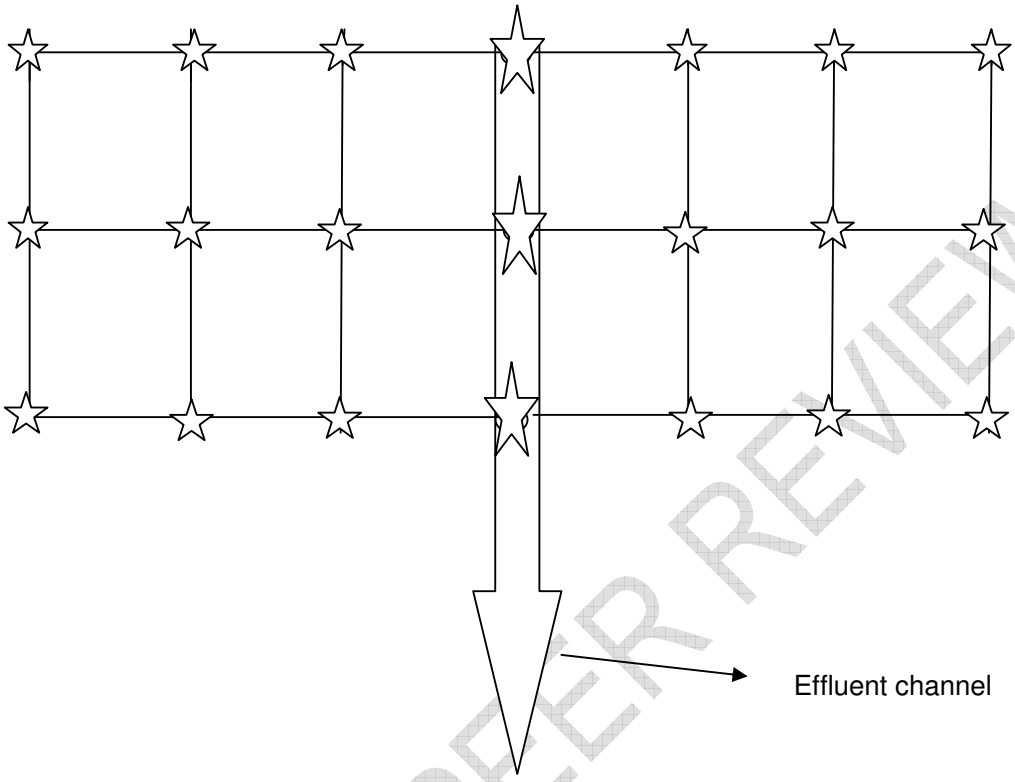

Fig. 1. Experimental layout of study soil

2.3 Physicochemical analysis

2.3.1 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_5) 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. Calcium and magnesium were determined by titrating with 0.1M EDTA while potassium and sodium were determined by flame photometry [10]. Total suspended solids (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 [10].

2.3.2 Determination of exchangeable bases of soil samples

Exchangeable cations (Ca, Mg, K, and Na) were extracted with 1N ammonium acetate (pH 7.0) [11]. Potassium and sodium were determined by flame photometry while calcium and magnesium were determined by titrating with 0.1M EDTA [11].

2.4 Mycological analysis

2.4.1 Rubber effluent

For serial dilution, ten (10) millilitres of rubber effluent was added to 90 ml of distilled water for the first ten-fold dilution. Subsequent ten-fold dilutions were carried out by adding one (1.0) millilitres of an already diluted sample to nine (9.0) millilitres of distilled water.

2.4.1.1 Enumeration of heterotrophic fungi

Potato dextrose agar (Criterion C6621, USA) was prepared according to manufacturer's instructions and supplemented with 100 µg/ml of chloramphenicol to inhibit bacterial growth. Zero-point-one (0.1) ml of 10^{-3} to 10^{-5} dilutions were each spread-plated out in triplicates. The colony forming units (CFU/ml) was determined after incubation at room temperature for 2-3 days.

2.4.1.2 Enumeration of rubber effluent utilizing fungi

Rubber effluent was added to mineral salts agar [12] at 2% (third rubber effluent sample analysed was used) concentration and incorporated with 100 µg/ml of chloramphenicol as the anti-bacterial agent. Zero point one (0.1) millilitres of 10^{-2} to 10^{-4} dilutions were each spread-plated out in triplicates. The colony forming units (CFU/ml) was determined after incubation at room temperature for 4-5 days.

2.4.2 Soil samples

For serial dilution, 10 grams of soil was added to 90 ml of distilled water for the first ten-fold dilution. Subsequent ten-fold dilutions were carried out by adding one (1.0) millilitres to nine (9.0) millilitres of distilled water.

2.4.2.1 Enumeration of heterotrophic fungi

Potato dextrose agar (Criterion C6621, USA) was prepared according to manufacturer's instructions and supplemented with 100 µg/ml of chloramphenicol to inhibit bacterial growth. Zero-point-one (0.1) millilitres of 10^{-2} to 10^{-3} dilutions (topsoil) and 10^{-1} to 10^{-2} (subsoil) dilutions were each spread-plated out in triplicates. The colony forming units (CFU/g) was determined after incubation at room temperature for 2-3 days.

2.4.2.2 Enumeration of rubber effluent utilising fungi

Rubber effluent was added to mineral salts agar [12] at 2% (third rubber effluent sample analysed was used) concentration and incorporated with 100 µg/ml of chloramphenicol as the antibacterial agent. One (1) millilitres of 10^{-1} to 10^{-2} dilutions (topsoil) and 10^{-1} dilution (subsoil) were each spread-plated out in triplicates. The colony forming units (CFU/g) was determined after incubation at room temperature for 4-5 days.

2.5. Isolation and preservation of pure culture

Potato dextrose agar (Criterion C6621, USA) was used. Using a sterile inoculating loop, each morphologically distinct colony from water and soil samples were sub-cultured twice and incubated at 64 hrs, before being transferred to agar slant for preservation. Inocula were obtained from the respective tubes, sub-cultured on potato dextrose agar for 3 days for identification and characterization purposes.

2.6. Identification and characterization of fungal isolates

Characterization of fungal isolates was based on macroscopic and microscopic appearances which comprised pigmentation, colour of aerial and substrate hyphae, shape and kind of asexual spore, presence of special structures, sporangiophore or conidiophores and characteristic of the spore head. Isolates were determined using the scheme of Domsch et al. [13] and Barnett and Hunter [14].

2.7. Statistical analysis

Microsoft Excel 2013 (Microsoft Inc.) and R Statistical Software (R Software Foundation) were used for a variety of statistical analyses which included Pearson's correlation, single-sample and two-sample t-tests. The following includes definitions of terms and how statistical tests were employed. Sample point: refers to any soil sample point collection, excluding impact points. Impact point: refers to any soil sample collection point along the channel of effluent only. Sample line: refers to all sample points on both sides of an impact point excluding the impact point. Correlation (Pearson's): carried out between successive values of a parameter on both sides of an impact point and sampling distance (excluding the particular impact point). One-sample t-test: was carried out between the value of a parameter at a particular impact point and values of its sample line. One-sample t-test was also used to compare control (pristine) soil and study soil parameters. Two-sample independent t-test was used to compare topsoil and subsoil for each parameter.

3. RESULTS AND DISCUSSION

Physicochemical and mycological analysis of the effluent revealed that only temperature, sulphate and chloride conformed to FEPA [15] standards for inland waters (Table 1). The fungi isolated from the effluent were identified as *Aspergillus* spp (33%), *Penicillium* spp (24%), *Rhizopus* spp (20%), *Mucor* spp (14%) and *Sporothrix* spp (9%).

The mean temperature (26^{o}C) falls below the permissible limit (40 ^{o}C) set by FEPA [15]. Similarly, Senthil et al. [16] obtained a mean value of 25.64^{o}C. The mean pH value (5.8) indicates slight acidity. This value falls outside the range of 6-9 set by FEPA [15]. pH values in the range of 5-8.1 have been recorded by other authors [17,18,5,16,7]. Although effluent limit standard does not exist for conductivity, an abrupt change in conductivity of a water body can be indicative of pollution [19]. This study recorded a mean conductivity value of 4,457 µS/cm.

This study recorded an average value of 2,802 mg/l for TDS, which is higher than FEPA (1991) 2,000 mg/l. Non-isoprene constituents such as carbohydrates, sugar, proteins, lipids, carotenoids, inorganic chemicals and a variety of chemicals used during processing make up the effluent from natural rubber processing [20]. The high contents of many of these components likely contributed to the high TDS of this rubber effluent. Similarly, Shruthi et al. [18], Girish [21] and Pillai and Girish [17] recorded mean values of 2,240 mg/l, 2, 397 mg/l and 2,240 mg/l, respectively from their studies. However, Iyagba et al. [5] and Asia and Akporhonor [7] reported mean values of 550 mg/l and 450.0 mg/l, respectively.

The average value of 1,638 mg/l obtained for total suspended solids (TSS) is higher than the 30 mg/l limit set by FEPA [15]. The high mean value recorded can be attributed to the heavy presence of latex particles, microorganisms and inorganic matter in the effluent. Several authors have also recorded high mean values for TSS [16,7,17,21].

The effluent has a low (anoxic) mean dissolved oxygen level (3.1 mg/l). Rubber effluents typically have low DO levels, as revealed by Iyagba et al. [5], 0 mg/l; Asia and Akporhonor [7], 4.70 mg/l; Senthil et al. [16], 1.16 mg/l. The mean BOD_5 value (3,038 mg/l) is higher than the 30 mg/l limit set by FEPA [15]. High BOD values can be attributed to the presence of large amounts of latex particles, proteins, sugars, and other organic matter. Similarly, high values ranging from 1,340-2,610 mg/l have been reported by many researchers [17,7,18,21]. However, Senthil et al. [16] and Iyagba et al. [5] reported low rather low BOD_5 values of 326 and 189 mg/l, respectively. The high mean COD value (4,531 mg/l) indicates that the waste also contains substantial amounts of inert organic matter and inorganics. This high COD result is consistent with the results of other authors [17,7,18,21].

Table 1: Physicochemical and mycological properties of rubber effluent and FEPA 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_5 (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
Calcium (mg/l)	48.50	30.59	22.81	33.97±7.60	200
Magnesium (mg/l)	11.02	7.54	8.44	9.00±1.042	200
Potassium (mg/l)	34.76	29.33	16.42	26.84±5.44	-
Sodium (mg/l)	4.46	1.35	0.89	2.23±1.12	-
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
HFC (CFU/ml)	5.40±2.08 x 10^6	2.20±1.73 x 10^5	1.30±1.15 x 10^5	1.91±1.65 x 10^6	-
RUFC (CFU/ml)	1.70±1.20 x 10^5	4.70±2.18 x 10^4	2.30±1.76 x 10^4	8.00±1.71 x 10^4	-

KEY: DO = Dissolved oxygen, BOD = Biological oxygen demand, COD = Chemical oxygen demand, TSS = Total suspended solids, TDS = Total dissolved solids, HFC = Heterotrophic fungi count, RUFC = Rubber effluent utilising fungi, NTU = Nephelometric turbidity unit, µS/cm = microSiemens per centimeter, mg/l = Milligram per litre, CFU/ml = Colony-forming unit per millilitre, SEM = Standard error of the mean, FEPA = Federal Environmental Protection Agency

Mean values of calcium (33.97 mg/l) and magnesium (9.00 mg/l) were within FEPA [15] limit of 200 mg/l. An average ammonia value of 1.15 mg/l was recorded in this study. The relatively low ammonia value was likely due to the fact that ammonia was not used to preserve the field latex. Similarly, Asia and Akporhonor [7] obtained a low mean of 4.49 mg/l. High ammonia values ranging from 39.3-230 mg/l have been obtained [5,21,18,16,17], pointing to the use of ammonia for preservation.

In this study, a mean nitrate value of 40.13 mg/l was obtained against a limit of 20 mg/l set by FEPA [15]. Iyagba et al. [5] obtained 0.07 mg/l and Asia and Akporhonor [7] recorded 1.36 mg/l. However, Senthil et al. [16] obtained a high value (149 mg/l). A mean phosphate value of 71.98 mg/l, which exceeds the 5 mg/l limit set by FEPA [15] was recorded. This result is consistent with high values (48-94.3 mg/l) recorded by other authors [16,18,21,5,17]. However, Asia and Akporhonor [7] reported a mean of 1.32mg/l. The mean sulphate value was 20.15 mg/l against 500 mg/l set by FEPA [15]. The mean chloride content was 43.87 mg/l against a limit of 600 mg/l set by FEPA [15]; however, Senthil et al. [16] recorded a mean chloride value of 1, 386 mg/l. Differences in the type and quantity of water and chemicals utilised, type of rubber processing or processing conditions are likely responsible for the big variations in physicochemical results obtained by different authors.

This study recorded a high mean TFC of 1.91 x 10^6 CFU/ml. Iyagba et al. [5] also recorded a similarly high value of 3.8 x 10^7 CFU/ml. The high fungal count of this study can be attributed to the nutrient-rich nature of rubber effluent which favoured the proliferation of fungi, the kind of water used in processing, or poor sanitary practices by the factory workers. Some of the fungi obtained in this study have been isolated in previous studies [21,16] and many are pathogenic. Rubber effluent utilizing fungi count (RUFC) indicates the presence of fungi that can utilize the rubber effluent.

Table 2 presents the overall, topsoil and subsoil means for impact points, sample points and control soil for the parameters. The overall means of exchangeable calcium, potassium and sodium, according to the classification of Landon [22], indicates low contents, except for magnesium. The low base contents can be attributed to erosion, leaching, clay fixation of these base cations. Also, rubber plantations can cause base cations values of soil to decline over time [23,24]. The moderate magnesium content of the study soil indicates that the soil is moderately rich in magnesium minerals like dolomite and serpentine. Rubber effluent utilizing fungi count (RUFC) indicates the presence of fungi that can utilize the rubber effluent. The RUFC was lower than HFC due to the probable toxicity of the effluent to some fungi or lack of suitable substrates or nutrients for others.

Table 2: Means of exchangeable bases and mycological properties of study soil and control soil

Parameters[+*]	Impact points means			Sample points means			Control soil	
	Overall	Topsoil	Subsoil	Overall	Topsoil	Subsoil	Topsoil	Subsoil
Ex. Ca	3.90±0.09	3.93±0.18	3.87±0.07	3.97±0.07	3.92±0.07	4.03±0.12	3.8	3.6
Ex. Mg	1.83±0.15	1.9±0.29	1.73±0.13	1.5±0.05	1.57±0.07	1.50±0.07	1.4	1.3
Ex. K	0.11±0.00	0.11±0.01	0.11±0.01	0.11±0.00	0.11±0.00	0.11±0.00	0.11	0.11
Ex. Na	0.07±0.01	0.08±0.01	0.07±0.01	0.06±0.00	0.07±0.00	0.06±0.00	0.07	0.08
HFC	5.90±1.42 x 10^3	8.53±1.25 x 10^3	3.27±1.58 x 10^3	1.33±2.61 x 10^4	2.24±2.30 x 10^4	4.19±2.92 x 10^3	1.90±1.73 x 10^4	4.9±2.03 x 10^3
RUFC	2.73±1.76 x 10^3	3.57±2.16 x 10^3	1.90±1.36 x 10^3	4.32±2.77 x 10^3	3.10±3.28 x 10^3	1.60±2.03 x 10^3	2.70±1.45 x 10^3	1.30±1.20 x 10^3

+Mean±standard error of mean (SEM)

**Units: Ex. Ca, Ex. Mg, Ex. K, Ex. Na = cmol/kg; HFC, RUFC = CFU/g*

KEY: Ex. Ca = Exchangeable calcium, Ex. Mg = Exchangeable magnesium, Ex. Mg = Exchangeable potassium, Ex. Mg = Exchangeable sodium, cmol/kg = centimoles/kg, HFC = Heterotrophic fungi count, RUFC = Rubber effluent utilising fungi

Table 3 presents the results of correlation analysis relating sample lines (distance) to each of the parameters. There were significant negative correlations for sodium (r = -0.97, P < 0.01) at the third sample line of subsoil and for RUFC at first (r = -0.83, P < 0.05) and third (r = -0.95, P < 0.01) sample lines of topsoil; however, there were no significant correlations (P > 0.05) for calcium, magnesium, potassium and HFC. The significant negative correlation for sodium implies that other potentially significant correlations were cancelled out by erosion, leaching and rubber root uptake. No significant correlations were observed for HFC (topsoil and subsoil) since the media used was not selective. The significant negative correlations for RUFC highlights the receding effect of the effluent on the study soil. The sample points closer to impact channels were impacted more, leading to stimulation of metabolically capable fungi. The significant correlation for RUFC also indicates that other potentially significant correlations were cancelled out by leaching, erosion and rubber root uptake.

Table 3: Coefficients of correlation (r) relating sample lines (distance) to each of the parameters

Parameters	Topsoil			Subsoil		
	1st SL	2nd SL	3rd SL	1st SL	2nd SL	3rd SL
Ex. Ca	-0.50	-0.38	0	-0.74	0.45	0.23
Ex. Mg	-0.30	0.34	0.30	0.21	0.39	0
Ex. K	-0.23	-0.35	0	-0.22	-0.35	0
Ex. Na	0.65	-0.49	-0.76	0.35	-0.68	-0.97**
HFC	-0.15	0.61	-0.31	0.16	-0.48	-0.60
RUFC	-0.83*	0.20	-0.95**	0.25	-0.11	-0.71

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

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

KEY: Ex. Ca = Exchangeable calcium, Ex. Mg = Exchangeable magnesium, Ex. Mg = Exchangeable potassium, Ex. Mg = Exchangeable sodium, cmol/kg = centimoles/kg, HFC = Heterotrophic fungi count, RUFC = Rubber effluent utilising fungi count

One-sample t-test results for study soil and control soil comparisons for the parameters are presented in Table 4. For topsoil, the test revealed significant results for exchangeable magnesium (P < 0.05) and RUFC (P < 0.01), while there were no significant results (P > 0.05) for exchangeable calcium,

potassium, sodium and HFC. For subsoil, the test revealed significant results ($P < 0.01$) for exchangeable calcium, magnesium, sodium and RUFC, while there were no significant results ($P > 0.05$) for exchangeable potassium and HFC. The significant differences recorded between study soil and control (pristine) soil base cation parameters indicate the effect of the effluent on the study soil. Heterotrophic fungi count (HFC) of study soil was not significantly different from that of control (pristine) soil. This means that stimulation of rubber effluent utilising fungi did not lead to an increase in the total number of fungi in the study soil, even when RUFC increased. RUFC of study soil was significantly different from that of control (pristine) soil due to stimulation of metabolically capable fungi by the effluent in the study soil, leading to their increment. This stimulation was near-absent in pristine soil with little or no exposure to rubber effluent, causing smaller RUFC.

Table 4: One-sample t-test comparing exchangeable bases and mycological parameters of study soil with control soil

Parameters	Topsoil/Topsoil (*P*-values)	Subsoil/subsoil (*P*-values)
Exchangeable calcium	0.1212	0.001685**
Exchangeable magnesium	0.02781*	0.00896**
Exchangeable potassium	0.2151	0.6309
Exchangeable sodium	0.2307	0.0009409**
HFC	0.05644	0.09867
RUFC	0.0001241**	0.0002231**

**Significant at 0.05 alpha level (two-sided)*
***Significant at 0.01 alpha level (two-sided)*
KEY: HFC = Heterotrophic fungi count, RUFC = Rubber effluent utilising fungi count

Table 5 shows the results of a two-sample t-test comparing topsoil and subsoil values for each parameter. The test showed significant results for HFC ($P < 0.01$) and RUFC ($P < 0.05$), but no significant results ($P > 0.05$) for the base cations. There was no significant difference between the top and subsoil for exchangeable cations probably due to rubber root uptake. HFC and RUFC decreased with depth in this study soil. This can be attributed to more vegetal cover, better soil structure and more organic matter in the topsoil [25]. The fungi isolated in the study soil were *Aspergillus* spp, *Penicillium* spp, *Rhizopus* spp, *Fusarium* spp, *Mucor* spp, *Cladosporium* spp, *Absidia* spp and *Chrysosporium* spp.

Table 5: Two-sample independent t-test comparing topsoil and subsoil values of each parameter

Parameters	*P*-values
Exchangeable calcium	0.4172
Exchangeable magnesium	0.4059
Exchangeable potassium	0.6993
Exchangeable sodium	0.5802
HFC	2.947×10^{-12}**
RUFC	0.01129*

**Significant at 0.05 level (two-sided)*
***Significant at 0.01 level (two-sided)*
KEY: HFC = Heterotrophic fungi count, RUFC = Rubber effluent utilising fungi count

4. CONCLUSION

The study revealed that the effluent should be treated before discharge into the environment since some parameters recorded values above permissible limits. The mycological investigations added more weight to the body of evidence in support of the impact of the wastewater on the study area since the stimulation of rubber utilising fungi in a receding manner from the flow channel evidently points to an impact decreasing with increasing distance from the flow channel of the wastewater. Hence, correlation analysis performed on data from microbiological investigation involving the use of a selective substrate can be used to augment or properly interpret results obtained from correlation analysis involving base cation parameters, especially in a situation where, like in this study, pollution is not obvious or where factors like root uptake, leaching and erosion can potentially cancel out

significant correlation results of base cation parameters. Also, the significantly different RUFC of study soil from that of control soil reflects the stimulation (and hence increment) of metabolically-capable fungi in the study soil due to continuous exposure to effluent, an exposure that was absent in control soil. Although the soil was impacted by the rubber wastewater, most base cation parameters still recorded low values due to leaching, erosion and rubber root uptake.

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