# 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, sulphate and chloride conformed to standards. Results also revealed that the rubber effluent impacted the soil but parameters still reported 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 appropriate statistical techniques can be applied to the results of microbiological investigation involving the use of a selective substrate 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.

## 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 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

32 and capacity of the rubber plant. A factory that produces 20-30 metric tonnes of rubber generates an
33 average of 45,000 litres of effluent daily [4].

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

39 People living close to rubber-processing factories often complain about the foul-smelling odour from
40 the factories. Soil physicochemical and microbiological characteristics can become altered when
41 exposed to effluent. These alterations can cause toxicity problems and nutrient imbalance in the soil.
42 Pollution of the soil can also be hazardous to man and the environment when toxic chemicals move
43 through the food chain or percolate into groundwater used for drinking purposes [6]. Various
44 researchers have analysed rubber effluent in Nigeria [7, 5, 8]; however, there has been scanty
45 published research work on the peculiar physicochemical and mycological properties of this particular
46 rubber effluent, and its effects on the soil it impacts. The ever-increasing global spotlight on the
47 environment requires that effluent properties and effluent impact be properly monitored.

48 ## 2. MATERIALS AND METHODS

49
50 ### 2.1. Study area

51 The rubber factory (N 5° 6' 80" and 8° 20' 24" E) is located on the outskirts of Calabar, which is the
52 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",
53 geocoordinates for the second impact point) lies just outside the rubber factory. The factory used to
54 produce latex concentrate, however it currently produces mainly crepe rubber. The factory has been
55 releasing untreated effluent indiscriminately into the environment for decades. Over time, a channel
56 (near the factory) of an average depth of about one metre developed through which the wastewater
57 flows, with rainfall sometimes causing flooding of the surrounding soil. The soils sustaining the rubber
58 plantation are classified as Ultisols [9].

59 ### 2.2 Sample collection

60 #### 2.2.1 Water samples

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

66 #### 2.2.2 Soil samples

67 The experimental layout for soil sample collection around the factory is as shown in Figure 1. The
68 larger stars represent the impact points spaced 25 metres from each other and created along the
69 effluent flow channel. Other sample points (smaller stars) were created on both sides of each impact
70 point and spaced five (5) metres from each other. From each impact and sample point, two samples
71 representing topsoil (0-15cm) and subsoil (15-30 cm) were collected and stored in sterile bags. Soil
72 sampling was done using a cylindrical T-shaped probe. A circle of diameter (30 cm) was created at
73 each sampling point and from within each a decontaminated probe was vertically-driven randomly into
74 the soil three (3) times for collection of samples for mycological analysis and randomly again 3 times
75 for base cation samples. Subsoil samples were collected by driving a decontaminated probe into the
76 holes created during collection of topsoil samples. A control (pristine) soil sample was collected from
77 the vertices of an equilateral triangle (length = 5m) created 100 metres away (measured diagonally
78 from the second impact point through the rightmost sample point of the first impact point).

79

80

81



Effluent channel

**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 (HI2400; Hanna Instruments; Rhode Island, USA), dissolved oxygen meter (HI2400; 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].
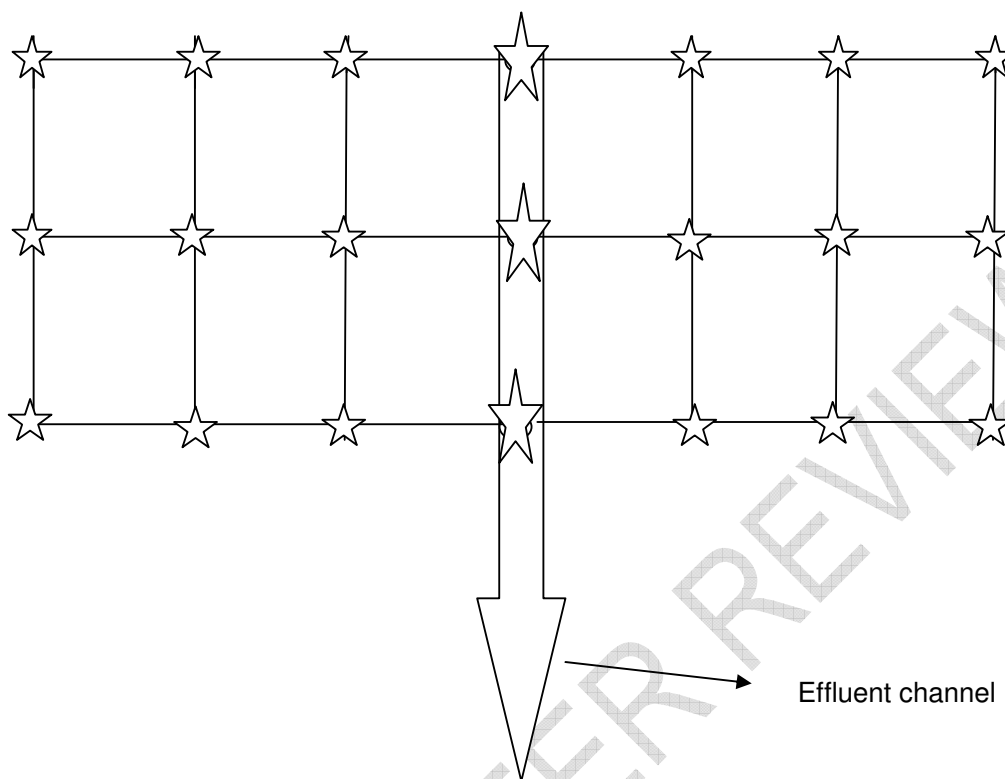
## 2.4 Mycological analysis

### 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 (Zajic and Supplison, 1972) 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 as prepared by [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 (Table 1). The fungi isolated from the effluent were identified as *Aspergillus* spp, *Penicillium* spp, *Rhizopus* spp, *Mucor* spp and *Sporothrix* spp.

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 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 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].

204     **Table 1: Physicochemical and mycological properties of rubber effluent and FEPA standards**

| Parameters | First sample | Second sample | Third sample | Mean ± SEM | FEPA standards |
|---|---|---|---|---|---|
| Temperature ($^{o}$C) | 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 $10^4$ | - |

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

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

215     In this study, a mean nitrate value of 40.13 mg/l was obtained against a limit of 20 mg/l set by FEPA
216     [15]. Iyagba et al. [5] obtained 0.07 mg/l and Asia and Akporhonor [7] recorded 1.36 mg/l. However,
217     Senthil et al. [16] obtained a high value (149 mg/l). A mean phosphate value of 71.98 mg/l, which
218     exceeds the 5 mg/l limit set by FEPA [15] was recorded. This result is consistent with high values (48-
219     94.3 mg/l) recorded by other authors [16,18,21,5,17]. However, Asia and Akporhonor [7] reported a
220     mean of 1.32mg/l. The mean sulphate value was 20.15 mg/l against 500 mg/l set by FEPA [15].  The
221     mean chloride content was 43.87 mg/l against a limit of 600 mg/l set by FEPA [15]; however, Senthil
222     et al. [16] recorded a mean chloride value of 1, 386 mg/l. Differences in the type and quantity of water
223     and chemicals utilised, type of rubber processing or processing conditions are likely responsible for
224     the big variations in physicochemical results obtained by different authors.

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

231     Table 2 presents the overall, topsoil and subsoil means for impact points, sample points and control
232     soil for the parameters. The overall means of exchangeable calcium, potassium and sodium,
233     according to the classification of Landon [22], indicates low contents, except for magnesium. The low
234     base contents can be attributed to erosion, leaching, clay fixation of these base cations. Also, rubber
235     plantations can cause base cations values of soil to decline over time [23,24]. The moderate

236 magnesium content of the study soil indicates that the soil is moderately rich in magnesium minerals
237 like dolomite and serpentine. Rubber effluent utilizing fungi count (RUFC) indicates the presence of
238 fungi that can utilize the rubber effluent. The RUFC was lower than HFC due to the probable toxicity
239 of the effluent to some fungi or lack of suitable substrates or nutrients for others.

240 **Table 2: Means of physicochemical 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 | $5.90\pm1.42 \times 10^3$ | $8.53\pm1.25 \times 10^3$ | $3.27\pm1.58 \times 10^3$ | $1.33\pm2.61 \times 10^4$ | $2.24\pm2.30 \times 10^4$ | $4.19\pm2.92 \times 10^3$ | $1.90\pm1.73 \times 10^4$ | $4.9\pm2.03 \times 10^3$ |
| RUFC | $2.73\pm1.76 \times 10^3$ | $3.57\pm2.16 \times 10^3$ | $1.90\pm1.36 \times 10^3$ | $4.32\pm2.77 \times 10^3$ | $3.10\pm3.28 \times 10^3$ | $1.60\pm2.03 \times 10^3$ | $2.70\pm1.45 \times 10^3$ | $1.30\pm1.20 \times 10^3$ |

241 [+]*Mean±standard error of mean (SEM)*
242 *Units: Ex. Ca, Ex. Mg, Ex. K, Ex. Na = cmol/kg; HFC, RUFC = CFU/g*
243 *KEY: Ex. Ca = Exchangeable calcium, Ex. Mg = Exchangeable magnesium, Ex. Mg = Exchangeable potassium,*
244 *Ex. Mg = Exchangeable sodium, cmol/kg = centimoles/kg, HFC = Heterotrophic fungi count, RUFC = Rubber*
245 *effluent utilising fungi*
246
247 Table 3 presents the results of correlation analysis relating sample lines (distance) to each of the
248 parameters. There were significant negative correlations for sodium ($r$ = -0.97, $P < 0.01$) at the third
249 sample line of subsoil and for RUFC at first ($r$ = -0.83, $P < 0.05$) and third ($r$ = -0.95, $P < 0.01$) sample
250 lines of topsoil; however, there were no significant correlations ($P > 0.05$) for calcium, magnesium,
251 potassium and HFC. The significant negative correlation for sodium implies that other potentially
252 significant correlations were cancelled out by erosion, leaching and rubber root uptake. No significant
253 correlations were observed for HFC (topsoil and subsoil) since the media used was not selective. The
254 significant negative correlations for RUFC highlights the receding effect of the effluent on the study
255 soil. The sample points closer to impact channels were impacted more, leading to stimulation of
256 metabolically capable fungi. The significant correlation for RUFC also indicates that other potentially
257 significant correlations were cancelled out by leaching, erosion and rubber root uptake.

258 **Table 3: Coefficients of correlation ($r$) relating sample lines (distance) to each of the**
259 **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 |

260 *\*Correlation is significant at 0.05 alpha level (two-sided)*
261 *\*\*Correlation is significant at 0.01 alpha level (two-sided)*
262 *KEY: Ex. Ca = Exchangeable calcium, Ex. Mg = Exchangeable magnesium, Ex. Mg = Exchangeable potassium,*
263 *Ex. Mg = Exchangeable sodium, cmol/kg = centimoles/kg, HFC = Heterotrophic fungi count, RUFC = Rubber*
264 *effluent utilising fungi count*
265
266 One-sample t-test results for study soil and control soil comparisons for the parameters are presented
267 in Table 4. For topsoil, the test revealed significant results for exchangeable magnesium ($P < 0.05$)
268 and RUFC ($P < 0.01$), while there were no significant results ($P > 0.05$) for exchangeable calcium,
269 potassium, sodium and HFC. For subsoil, the test revealed significant results ($P < 0.01$) for
270 exchangeable calcium, magnesium, sodium and RUFC, while there were no significant results ($P >
271 0.05$) for exchangeable potassium and HFC. The significant differences recorded between study soil
272 and control (pristine) soil base cation parameters indicate the effect of the effluent on the study soil.
273 Heterotrophic fungi count (HFC) of study soil was not significantly different from that of control
274 (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 physicochemical/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 | $2.947 \times 10^{-12}$** |
| 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 and the mycological investigations added more weight to the body of evidence in support of the impact of the wastewater on the study 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 fungi capable of degrading the rubber effluent in the study soil, an increment 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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