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Effects of Effluents' Discharge from Some Paint Industries on Soil's Physicochemical Properties and Bioattenuation of Polluted Soil

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ABSTRACT: Rapid population growth resulting in industrial proliferation and urbanization has led to the rapid increase in pollution of the environment. Paint industries in urban areas mostly channel their wastewater into streams and on land, which results in the pollution of the receiving environment. This study aims to determine the impact of effluent discharges from paint industries on the soils' physicochemical properties and the clean-up of the polluted soil through monitored natural attenuation. Composite samples of paint-effluents and soils were collected from paint industries. Their bioattenuation levels and changes in their physicochemical properties were monitored over a six-month period. Fungal isolates from the effluents include *Saccharomyces cerevisiae* (20%), Rhodotorula species (15%), *Aspergillus niger* (25%), *Aspergillus flavus* (15%), and *Penicillum notatum* (25%), while the bacterial isolates include *Staphylococcus aureus* (30%), *Bacillus* sp. (20%), *Klebsiella* sp.(15%), *Escherichia coli* (15%), *Salmonella* sp. (10%), and Staphylococcus species (10%). The effluents showed slightly alkaline pH values while the soils showed slightly acidic pH values. There were significant reductions in the heavy metal contents of the effluent polluted soils as remediation time increased, thus reducing the toxicity of such soil environments. Monitored natural-attenuation methods should be employed and improved as a means of reducing the toxicity of effluents on the environment since they are cheap and effective compared to other methods.

KEYWORDS: Autochthonous microorganisms; heavy metals; monitored natural attenuation; soils; paint effluents

1. Introduction

Paint industries pollute the environment through effluent discharge, gas emissions, and waste disposal in the form of organic and inorganic substances. It is recorded that of all the wastes discharged by paint industries, effluents are by far the most significant due to their heavy metal compositions [1]. It is recorded that of all the wastes discharged by paint industries, effluents are by far the most significant due to their heavy metal compositions [2]. The heavy metals

have cumulative effects over years; the presence of these heavy metals in the ecosystems has increased as a result of an increase in the industrialization process. Health problems such as genetic mutation, deformation, cancer, kidney problems, etc., have been attributed to pollution by heavy metals. Paint effluent often contains all the components of the precursor paints with insignificant dilution [3]. They may be released as contaminated water containing residual acids, plating metals, and toxic chemicals [4].

The numerous chemicals used for the production of paints are responsible for the high concentrations of organic acid compounds, suspended solids, colored materials, and hazardous pollutants like heavy metals in the generated waste [6]. Generally, industrial effluents can potentially affect hydrological and environmental parameters of a catchment as well as pose a significant threat to man and natural ecology. In Nigeria, paint production uses a large volume of water without adequate water treatment plants. Hence, large quantities of both hazardous and non-hazardous wastes are inherently released into the environment, thus causing health-related problems, ecological imbalances, and bioaccumulation in aquatic organisms [7].

Biological treatment most commonly involves the breakdown of contaminants into non-toxic forms by microbiological processes. Microorganisms exist naturally or are cultured especially and used for microorganism remediation under controlled conditions to transform toxic contaminants into non-toxic substances [8]. Microorganisms that naturally occur in paint effluent are adapted to the conditions within the wastewater and should therefore be useful in the biological treatment thereof [9]. A number of yeasts, molds, and other fungi associated with paints will most likely also be present in paint wastewater [2].

Intrinsic bioremediation, also known as natural attenuation, is an in-situ bioremediation technique that involves passive remediation of polluted sites without any external force (human intervention). The process relies on microbial (aerobic and anaerobic) processes to biodegrade polluting substances, including those that are recalcitrant. The absence of external force implies that the technique is less expensive compared to other in situ techniques. Nevertheless, the process must be monitored in order to establish that bioremediation is ongoing and sustainable. Moreover, it was reported that intrinsic bioremediation does not result in adequate polyaromatic hydrocarbon (PAH) removal and a corresponding reduction in polluted soil eco-toxicity [10].

The aim of this study, therefore, is to determine the effect of discharges from paint industries on the soil's physicochemical characteristics and to remediate the polluted soil using the indigenous microbes from the effluents and the soil. The goals of this research were to find out the microbiological and physicochemical properties of both unpolluted and polluted soils as well as the effluents from the paint industry. They also wanted to find out how the effluents from the paint industry affected the physicochemical properties of the soil and how natural attenuation could be used to clean up polluted soil.

2. Materials and Methods

2.1. Study area.

Six (6) paint producing companies in Aba metropolis were chosen for the study. Aba is in Abia State, whose capital is Umuahia. Aba is located approximately between Latitudes 05^{o}01’30” and 05^{o}07’00” North of the Equator and Longitudes 07^{o}22’00” 07^{o}26’00” East of the Greenwich meridian [11]. The paint industries used for this study were those that have operated

for more than six years and do not treat their effluents before discharge, and those whose effluents are discharged directly into the receiving lithospheric environment.

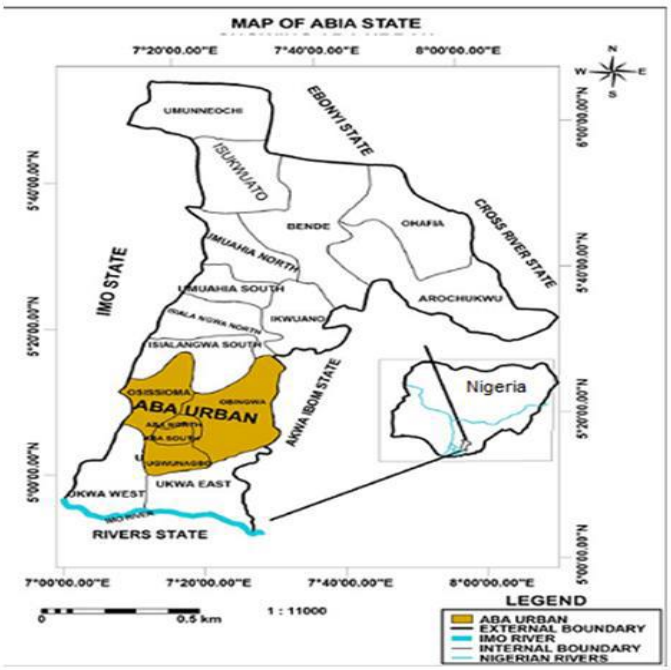

Figure 1. Study area at Abia State [12].

2.2. Sample collection.

One thousand two hundred milliliters of effluent (1200 ml from each paint industry) were collected from six paint industries involved in emulsion and oil paint production in triplicates at different seasons (dry and rainy) and different times using sterile bottles. All the samples were labelled A, B, C, D, E, and F. The effluents were collected according to the previous method [2]. For each of the industries, three representative samples were collected from different points on their effluent discharge unit. The samples were first collected in the month of February 2018 for the dry season and then the same process was repeated in October of the same year for the wet season. The sampling was done in three shifts, i.e., the morning shift between 07:00 a.m. and 09:00 a.m., the afternoon shift between 02:00 p.m. and 04:00 p.m., and the evening shift between 07:00 p.m. and 09:00 p.m. Polythene bottles of 2.5 litres and 2.0 litres were used to collect the grab effluent samples. The bottles were thoroughly cleaned with hydrochloric acid, washed with tap water to render them free of acid, washed with distilled water twice, again rinsed with the effluent sample to be collected, and then filled up with the sample, leaving only a small air gap at the top. The sample bottles were stopped and sealed with paraffin wax. The soil samples were taken from the places where the effluents from the paint factories are dumped.

2.3. Incorporation of the soil sample and the paint effluents.

The soil samples with the paint effluents was done using the previous method [13]. Five hundred grams (500 g) of unpolluted soils from each of the paint industries were moistened with sterile water and kept at room temperature in the Microbiology laboratory for one week to make for proper homogenization. The unpolluted soil samples from each of the paint

industries were polluted with their respective paint effluents in the ratio of 5:2 i.e. 500 g of soil was mixed with 200 ml of the respective paint-industry effluent from each of the paint industries and kept for 2 weeks for proper homogenization.

2.4. Isolation of indigenous microorganisms from the effluents and the virgin soil.

Isolation of indigenous microorganisms from the effluents and the virgin soil was done using the previous method [14]. The containers containing the effluents were shaken well to homogenize their contents before use. One milliliter of the wastewater effluents from each sample was aseptically transferred to 9 ml of sterile distilled water and serial dilution was performed to obtain a suspension up to 10-6. Six sets of test tubes in racks were used for the serial dilution of the samples. A set of five test tubes was used for each sample. Ten grams of the soil sample were added to a 100ml sterile bottle containing 50ml of sterile water, shaken and allowed to stand for 10 minutes. This was to enable the soil to detach any particles or microorganisms from the soil before a second shaking. From each sample, 1 ml of liquid was aseptically pipetted out and serially diluted through five tubes. The second and fourth test tubes in each set were selected for inoculation on the media plates, NA and SDA, the former for bacteria and the latter for fungi. Using the spread plate method, 0.1ml of each dilution (10-2 and 10-4) of both the effluent and the supernatant from the virgin soil were inoculated on Nutrient agar medium and Sabouraud dextrose agar plates in duplicates. 0.1 ml of the diluted samples was introduced onto the agar media surface and spread using a sterile bent glass spreader. The NA plates were incubated at 37°C for 24 hours, whereas the SDA plates were incubated at room temperature (28°C) for 72 hours. The heterotrophic bacterial and fungal counts were determined after which sub-culturing was done using the streaking method for bacteria and the stab inoculation method for fungi to obtain discrete colonies (pure cultures). The discrete colonies were re-inoculated into appropriate media slants and kept at 4°C for further identification.

2.5. Physicochemical examination of the effluents

Effluent digestion and other physicochemical analyses were conducted on the effluents using a Varian AA240 Atomic Absorption Spectrophotometer.

2.5.1. Phosphorus determination

Phosphate was measured using Standard Method 4500-P B-5 and 4500-PE [15]. Exactly 100ml of the homogenized and filtered sample was pipetted into a conical flask. The same volume of the distilled water (serving as a control) was also pipetted into another conical flask. 1ml of 18M H_2SO_4 and 0.89g of ammonium phosphate were added to both conical flasks and gently boiled for 1 1/2 hours with distilled water to maintain a volume of 25-50 cm3. It was then cooled, one drop of phenolpthelein indicator was added and neutralized to a faint pink color with 2M HCl, and the solution made up to 100ml with distilled water. For the colorimetric analysis, 20ml of the sample was pipetted into test tubes, 10ml of the combined reagent added, shaken and left to stand for 10 minutes before reading the absorbance at 690nm on a spectrophotometer, using 20ml of distilled water plus 1ml of the reagent as reference.

2.5.2. Nitrogen determination

Total Nitrogen content was determined using the method of AOAC [16]. Exactly 1 ml of the effluent sample was weighed into a 300 ml Kjehdal flask (gently to prevent the sample from touching the walls of the flask) and then the flask was stopped and shaken. Then 1g of the Kjedahl catalyst mixture was added. The mixture was heated cautiously in a digestion rack under fire until a clear solution appeared. The clear solution was then allowed to stand for 30 minutes to cool. After cooling, about 100ml of distilled water was added to avoid caking and then transferred to the Kjedahl digestion apparatus. A 500 ml receiver flask containing 5 ml of boric acid indicator was placed under the condenser of the distillation apparatus so that the tap was about 20 cm inside the solution. The 10 ml of 40% sodium hydroxide was added to the digested sample in the apparatus and distillation commenced immediately until the 35 ml mark of the receiver flask was reached, after which it was titrated to punk color using 0.01N hydrochloric acid.

2.5.3. Determination of Total Organic Carbon and Ferroin titration

The moisture content of the air-dry soil that has been ground to pass a 0.42 sieve was determined. Enough effluent was accurately weighed to contain between 10 g and 20 mg of carbon into a dry-rated 20ml conical flask (between 0.5 g and 1 g for topsoil, and 2 g and 4 g for subsoil). 10ml of 1 N $K_2Cr_2O_7$ was accurately added, and the flask was swirled gently to disperse the soil in the solution. A 20 ml concentration of H_2SO_4 was added while directing the steam into the suspension. The flask was immediately swirled until the soil and the reagent were mixed. A 200 °C thermometer was inserted, and the flask and contents were heated by swirling them on a hot plate or over a gas burner and gazing until the temperature reached 139 °C. The flask was set aside to cool slowly on an asbestos sheet in a fume cupboard. Two blanks (without soil) were run in the same way as the standardized $FeSO_4$ solution. When cool (20–30 mins), 200ml of deionized water was used to dilute the solution, and the titration of the $FeSO_4$ proceeded, using the ferroin indicator or potentiometrically with an expending scale PH/MV meter or auto titrator [15]. Exactly 3 drops of ferroin indicator were added to the effluent samples, and 0.4N $FeSO_4$ was used to titrate the solution. As the end point was approached, the solution took on a greenish color and then changed to a dark green. At this point, the $FeSO_4$ was added drop-by-drop until the color changed from blue green to reddish grey. If the end point was overshot, 0.5ml or 1.0ml of 1N $K_2Cr_2O_7$ would be added, and the end point would be re-approached drop by drop. A correction for the extra volume was added if over 8ml of the 10ml dichromate had been consumed by the determination was repeated with a smaller soil sample [15].

2.5.4. Determination of electrical conductivity

A 10 % w/v suspension of the effluent sample was prepared in distilled water. The conductivity cell was rinsed with at least three portions of the sample, and the temperature of the sample was then adjusted to 20°C ± 0.1°C. The conductivity cell containing the electrodes was immersed in a sufficient volume of the sample. The conductivity meter was turned on and the conductivity of the sample was recorded [15].

2.5.5. Determination of pH and heavy metals in effluent samples

The pH was determined by placing a pH probe (Hanna instrument C-99-USA) into the respective effluent sample in a 250 ml conical flask and allowing it to equilibrate for 3 minutes, and the pH meter was read and recorded accordingly [15]. The determination of Cu, Zn, Mn, Fe, Cr, Cd, Ni, and Pb was carried out directly in each final solution using Standard [17]. An Atomic Adsorption Spectrophotometer (AAS-model-GBC-932 plus Chem Tech - USA) was used to analyze each metal. The wastewater samples were digested as follows: 100 millilitres of the sample were transferred into a beaker and 5 mL of concentrated HNO_3 was added. The beaker with the content was placed on a hot plate and evaporated down to about 20 ml. The beaker was then cooled and another 5 mL of concentrated HNO_3 was added. The beaker was covered with watch glass and returned to the hot plate. The heating was continued, and then a small portion of HNO_3 was added until the solution appeared light-colored and clear. The beaker wall and watch glass were washed with distilled water, and the sample was filtered to remove any insoluble materials that could clog the atomizer. The volume was adjusted to 100 cm^3 with distilled water, and the result was read in mg/l.

2.6. Microbiological and physicochemical analyses of polluted soil

Microbiological examination of the effluents mixed with the virgin soil was done by using the previous method [18].

2.7. Biochemical identification of isolates

Microscopic and biochemical tests done using standard methods [19] include gram staining, motility, catalase, urease, citrate, sugar tests, and indole. In identifying fungi, microscopic and macroscopic examinations, including Gram staining for yeast cells and the lactophenol cotton blue stain test for molds for morphological characteristics, were carried out on fungal isolates.

2.8. Statistical analyses

One-way ANOVA with meanstandard deviation was used for statistical analysis. The standard deviations (error bars) and statistical differences (5% level of significance) were analyzed by observing the changes in the physicochemical properties of the polluted soil with time by using Statistical Package for Social Science (SPSS) version 21.0 and GraphPad Prism 6® software (trial version) (GraphPad Software, CA, USA).

3. Results and Discussion

3.1 Microbial and biochemical properties of the paint-industry effluents and soil

The Mean Heterotrophic Count (THC) of the Bacterial and fungal Isolates from the Paint effluents is shown in Table 1. The effluent samples from sample F showed the highest bacterial count of 6.56 ± 2.51 x10^4cfu/ml, while sample B exhibited the least bacterial count of 1.6 ± 2.00x10^4 cfu/ml. For the fungal count, sample B showed the highest colony count of 7.4 ± 3.21 x10^4 cfu/ml while sample E showed the least count of 5.6 ± 12.10 x10^4 cfu/ml.

Table 1. Mean Heterotrophic Count (THC) of the bacterial and fungal isolates from the paint effluents.

Effluent Sample	Bacteria (10^4cfu/ml)	Fungi (10^4cfu/l)
A	2.8 ± 1.00	7.2 ± 2.63
B	1.6 ± 2.00	7.4 ± 3.21
C	2.6 ± 2.64	6.57 ± 3.78
D	2.2 ± 1.52	6.23 ± 1.15
E	4.23 ± 3.05	5.6 ± 12.10
F	6.56 ± 2.51	6.7 ± 1.73

Results are expressed in Mean ± Standard deviation of triplicate determinations

Table 2. Mean Heterotrophic Count (THC) of the bacterial and fungal isolates from the virgin soil.

Soil sample	Bacteria (10^4cfu/g)	Fungi (10^4cfu/g)
A	1.30±0.20	1.10±0.30
B	1.20±0.20	1.30±0.44
C	1.70±0.01	2.30±0.28
D	1.10±0.31	2.20±0.24
E	1.50±0.20	1.8±0.31
F	6.11±0.20	1.70±0.01

Results are expressed in Mean ± Standard deviation of triplicate determinations

The plate counts of the virgin soil samples from the various paint industries are shown in Table 2. Virgin Soils from sample F showed the highest bacterial plate counts of $6.11\pm0.20x10^4$cfu/g while sample D had the least bacterial count of $1.10\pm0.31x10^4$cfu/g. The fungal counts of the virgin soils indicate sample C as having the highest count of $2.30\pm0.28x10^4$cfu/g while sample A had the least count of $1.10\pm0.30x10^4$cfu/g.

Table 3. Microscopic and biochemical tests for the identification of the fungal isolates from paint effluents.

Isolate	Gram staining	Lactophenol observations	Probable organisms
I	+	Circular buds	*Saccharomyces cerevisiae*
II	+	Oval buds	*Rhodotorula* species
III		Aseptate hyphae, blastospores	*Rhizopus* species
IV		Aseptate hyphae, sporangium, sporangiospores, sporangiophores	*Penicillum notatum*
V		Aseptate hyphae, clustered conidiospores, conidiosphores	*Aspergillus flavus*
VI		Aseptate hyphae, sporangiospores, sporangium, sporangiosphores	*Aspergillus niger*

Table 3 shows the microscopic and biochemical tests for the identification of the fungal isolates from the paint industry effluents. For the yeast, Gram stain was used, and lactophenol cotton-blue stain was used for the mold. Their various reactions to these reagents are shown in this table. The fungal isolates include *Saccharomyces cerevisiae*, *Rhodotorula* species, *Rhizopus* species, *Aspergillus niger*, *Aspergillus flavus*, and *Penicillum notatum*. They were characterized based on shape, color, texture, etc. The fungi isolates include yeasts and molds. The growth rate varied, as some was rapid and some was slow. The surface color and underside color varied.

Table 4. Biochemical Identification of bacterial Isolates from the effluents.

Sample	IN	CL	CT	CG	VP	MR	Gram Stain	Shape	Sugar Fermentation Test					Probable Organism
									Glu	Fru	Suc	Lac	Mal	
A	-	+	+	-	+	-	+	Rod in chains	AG+	AG+	AG-	AG-	AG+	*Bacillus spp*
B	-	+	+	-	+	-	+	Short rods in chains	AG+	AG+	AG-	AG-	AG+	*Bacillus spp*
C	+	+	-	-	-	+	+	Rods in chains	AG+	AG+	AG+	AG-	AG+	*Bacillus spp*
D	-	+	+	-	-	-	-	Rods in chains	AG+	AG+	AG+	AG-	A+G-	*Rhizobium spp*
E	-	+	+	-	+	-	-	Rods in chains	AG+	AG+	AG-	AG-	AG+	*Pseudomonas spp*
F	-	+	+	-	+	-	+	Rods in chains	AG+	AG+	AG-	AG-	AG+	*Serratia spp*

Key: IN: Indole test, CL: Catalase test, CT: Citrate test, CG: Coagulase test, VP: Vogues Proskauer test, MR: Methyl Red Test

Table 4 and Table 5 shows the microscopic and biochemical tests for the identification of the bacterial isolates from the paint effluents. The isolates displayed different reactions to gram stain as some were gram positive and some were gram negative. The bacterial isolates include species of Rhizobium*, Pseudomonas, Bacillus, and Serratia.*

Table 5. Morphological characteristics of indigenous molds from the paint effluents

Sample	Nature of hyphae	Type Of Spores	Growth	Front view	Back view	Probable Organism
A	Ellipsoidal	Conidiospore	Rapid	White	Whitish	*Mucor sp.*
B	Non-Septate	Conidiospores	Rapid	Grey black	Dark brown	*Aspergillus niger*
C	Septate	Conidiospore	Abundant	Pale brown	dark zonation	*Fusarium* sp.
D	Non-Septate	Conidiospores	Rapid	Grey black	Dark brown	*Aspergillus niger*
E	Arial	Conidiospores	Rapid	White with brown	Creamyellow /red	*Penicillium funiculosum*
F	Septate	Conidiospores	Rapid	White	White	*Geotrichum* sp.

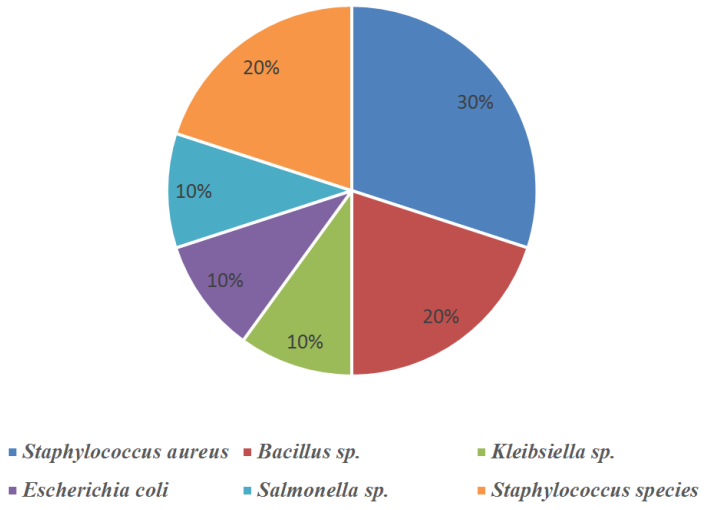

Figure 2. Occurrence of the bacterial isolates paint industry effluents

Figure 2 shows the pie chart for the percentage occurrence of the bacterial isolates. *Staphylococcus aureus* had the highest percentage occurrence of 30%, *Bacillus* sp. had a 20% occurrence, and other bacterial isolates had a 10% occurrence. Figure 3 shows the pie chart for the percentage occurrence of the fungal isolates. *Penicillium notatum* and *Aspergillus niger* both have the same percentage occurrence of 25%. Other fungal isolates have a percentage occurrence of 12.5%.

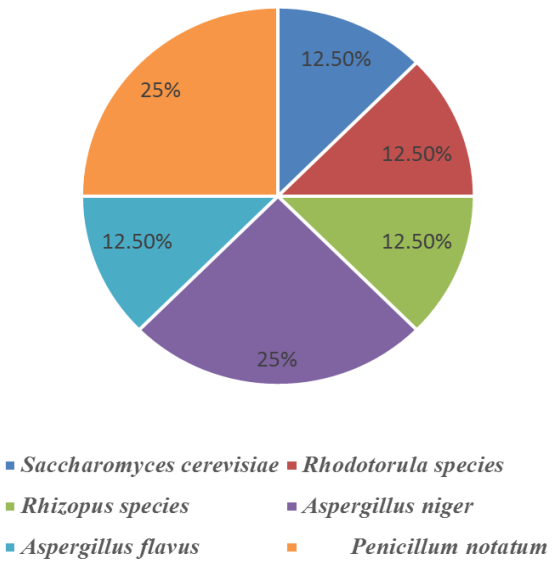

Figure 3. Percentage occurrence of the fungal isolates from paint industry effluents.

Figure 4 shows the changes in the Mean Microbial Counts of the Paint Effluent Micro-Deteriorated Soil over a 6-Month Remediation Period using the autochthonous microorganisms from the effluents and the soil. Sample F showed the highest mean bacterial counts while sample C showed the least.

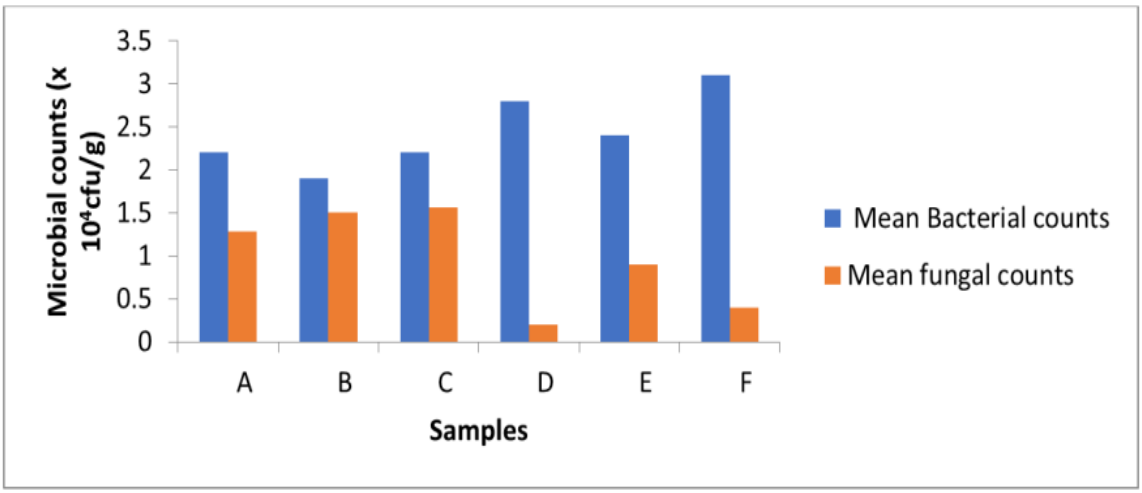

Figure 4. Changes in the mean microbial counts of the paint effluent micro-deteriorated soil over a 6-Month remediation period.

3.2. Physicochemical properties of the effluents and soils

Table 6. Initial physicochemical properties of the effluents from paint-industry effluents contaminated sites.

Parameters	A	B	C	D	E	F
pH	7.55 ± 0.11	7.86 ± 0.21	7.44 ± 0.07	7.98 ± 0.24	7.04 ± 0.33	8.02 ±0.08
Lead	0.34± 0.01	0.07± 0.00	0.72 ± 0.06	0.25 ± 0.03	0.48 ± 0.04	0.25 ± 0.04
Copper (ppm)	0.05 ± 0.04	0.00 ± 0.00	0.03 ± 0.00	0.74 ± 0.03	0.18 ± 0.00	0.34 ± 0.03
Zinc (ppm)	1.20 ± 0.04	6.76 ± 0.61	14.18 ± 0.43	1.11 ± 0.01	19.43 ± 0.50	4.10 ± 0.52
Cobalt (ppm)	0.00 ± 0.00	0.06± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.31 ± 0.05	2.19 ± 0.04
Conductivity (us/cm)	93.13 ± 0.55	107.25 ± 1.31	81.65 ± 0.59	117.17± 1.09	186.16 ± 1.44	86.10 ± 1.07
Phosphorus(mg/l)	5.74 ± 0.09	4.96± 0.01	5.02 ± 0.21	3.96± 0.05	5.38 ± 0.58	4.51± 0.44
Total nitrogen %	7.00 ± 0.17	5.17 ± 0.41	5.01 ± 0.00	4.35 ± 0.51	7.83 ± 0.09	5.17 ± 0.35
Total organic carbon	0.91 ± 0.05	0.73 ± 0.04	0.85 ± 0.02	0.64 ± 0.08	0.54 ± 0.02	0.63± 0.00

Results are expressed in mean ± standard deviation of triplicate determinations

The physicochemical tests conducted on the effluent samples indicated that the effluent samples contained some metals which are detrimental to health and could lead to pollution problems in excess amounts. Table 6 shows the Initial Physicochemical Properties of the Effluents from paint-industry effluents contaminated sites. There was presence of some heavy metals in the effluents and pH values were mostly alkaline. Table 7 shows the Initial Physicochemical Properties of soil from paint-industry effluents contaminated sites. The pH of the soils from the contaminated sites showed slightly acidic pH values.

Table 7. Initial physicochemical properties of soil from paint-industry effluents contaminated sites.

Parameters	A	B	C	D	E	F
pH	6.30 ± 0.08	6.07 ± 0.14	5.73 ± 0.02	5.34 ± 0.42	5.95± 0.47	6.15±0.05
Lead	0.44 ± 0.01	0.38 ± 0.01	0.88 ± 0.00	0.34 ± 0.02	0.62± 0.07	0.96± 0.00
Copper (ppm)	0.00 ± 0.00	0.00 ± 0.00	0.06 ± 0.01	0.39 ± 0.02	0.17± 0.21	0.87± 0.17
Zinc (ppm)	18.36 ± 0.36	7.00 ± 0.00	14.25 ± 0.88	1.90 ± 0.02	15.41± 0.58	7.57± 0.46
Cobalt (ppm)	0.00 ± 0.00	0.58 ± 0.05	0.03 ± 0.02	0.00 ± 0.00	1.28± 0.07	0.38± 0.01
Conductivity (us/cm)	93.98 ± 0.29	108.14 ± 0.69	80.79 ± 0.67	117.62 ± 0.42	185.90± 1.47	87.79 ± 0.99
Phosphorus (mg/l)	4.92 ± 0.09	5.53 ± 0.23	4.57 ± 0.13	3.49 ± 0.06	5.13± 0.14	4.72± 0.35
Total nitrogen %	4.77 ± 0.32	4.71 ± 0.42	3.54 ± 0.01	4.56 ± 0.03	6.81± 0.12	4.87± 0.04
Total organic carbon	5.00 ± 0.08	2.70 ± 0.05	4.90 ± 0.05	3.58 ± 0.02	4.71± 0.08	2.79 ± 0.19

Results are expressed in Mean ± Standard deviation of triplicate determinations

Table 8. Mean Physicochemical properties of the paint effluent polluted micro-deteriorated soil at the end of the 6-month remediation period (Natural Attenuation).

Parameters	A	B	C	D	E	F
pH	7.54± 0.08	7.84± 0.06	7.47± 0.04	7.53± 0.36	7.06± 0.05	7.99± 0.04
Lead	0.38± 0.03	0.08± 0.01	0.82± 0.05	0.25± 0.01	0.50± 0.04	0.34± 0.00
Copper (ppm)	0.04± 0.03	0.00± 0.00	0.04± 0.00	0.77± 0.04	0.08± 0.00	0.41 ± 0.00
Zinc (ppm)	1.20± 0.05	6.09± 0.01	14.85± 0.57	1.17± 0.12	19.92 ± 0.77	4.60± 0.10
Cobalt (ppm)	0.00± 0.00	0.07± 0.00	0.00± 0.00	0.00± 0.00	1.28± 0.03	0.26± 0.00
Conductivity (us/cm)	204.32± 1.51	190.79± 0.83	81.16± 1.02	110.96± 0.90	100.99± 1.16	187.30± 1.97
Phosphorus (mg/l)	5.81± 0.03	4.98 ±0.25	4.87± 0.04	4.00± 0.01	5.39± 0.54	4.90± 0.13
Total nitrogen %	6.92± 0.05	5.71± 0.03	5.04± 0.06	4.94± 0.02	7.84± 0.07	4.83± 0.04
Total organic carbon	0.94± 0.05	0.87± 0.09	0.91± 0.01	0.56± 0.03	0.63± 0.01	0.63± 0.00

Results are expressed in Mean ± Standard deviation of triplicate determination

Table 8 shows the Mean Physicochemical properties of the paint effluent polluted micro-deteriorated soil at the end of the 6-month remediation period (Natural Attenuation). Figure 4 shows the Changes in Ph, Lead, Copper, Zinc and Cobalt concentrations of the paint effluent polluted soil respectively over the 6-month remediation period while Figure 5 shows the Changes in Electrical Conductivity, Total Phosphorus, Total Nitrogen and Total Organic Carbon concentrations of the paint effluent polluted soil respectively over the 6-month remediation period. The pH of the effluents from the contaminated sites showed slightly alkaline values while that of the soils from the contaminated sites showed slightly acidic pH values. Lead (Pb), Copper (Cu), Cobalt (Co), and Zinc (Zn) were all detected, with Zinc (Zn) having the highest concentration, an average of 8.226 ppm. Cobalt (Co) had the least concentration, an average of 0.053 ppm.

The microscopic and biochemical characteristics of the fungal isolates from the effluents revealed the presence of *Saccharomyces cerevisiae*, *Rhodotorula* species, *Aspergillus niger*, *Aspergillus flavus,* and *Penicillum notatum.* This was similar to previous findings that identified *Aspergillus* sp. and *Penicillium* sp. when effluents from the paint industry were analyzed [14]. Previous studies obtained fungal isolates, particularly *Aspergillus spp*. and *Fusarium spp*., where *Rhizopus spp*., which were absent in this study, were the dominant species [23-27].

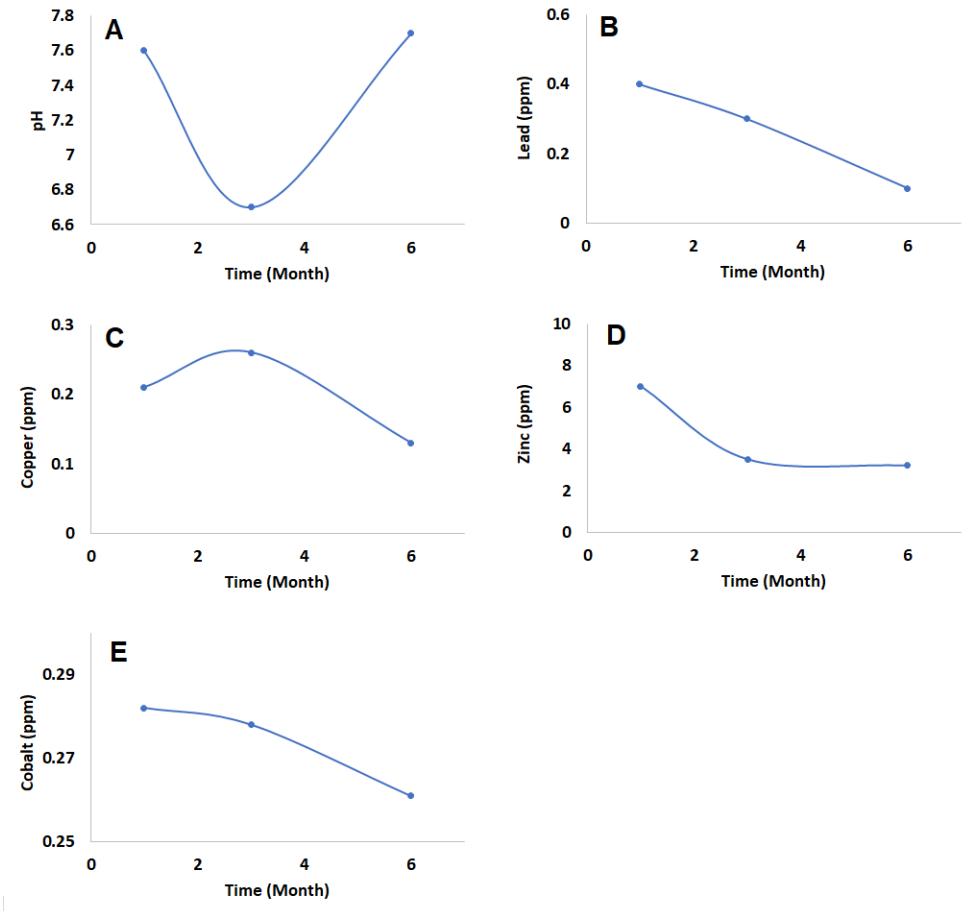

Figure 4. Changes in pH (A), lead (B), copper (C), zinc (D), cobalt (E) of the paint effluent polluted soil over the 6-month remediation period.

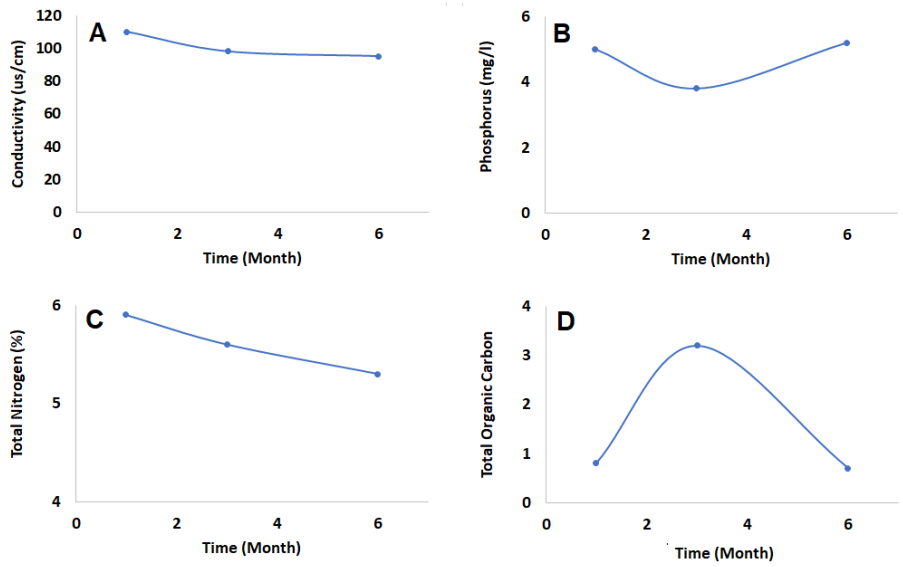

Figure 5. Changes in conductivity (A), phosphorus (B), Total Nitrogen (C), and Total organic Carbon (D) of the paint effluent polluted soil over the 6-month remediation period.

The highest percentage occurrence of bacterial isolates from the effluents was seen in *Bacillus* sp. (30%), *Staphylococcus aureus* had a 20% occurrence, and other bacterial isolates had 10% occurrences each. Bacteria act as bioindicators of highly polluted effluents, which prompted them to analyse the native bacterial population in paint effluent and to use it for

biodegradation [28]. *Aspergillus spp*. and *Fusarium spp*. were the fungi that were found most often in the clean soil samples used in this study [29,30].

Some of the heavy metals obtained in this study from the effluents include lead (Pb), copper (Cu), zinc (Zn), and cobalt (Co), and these are known to be toxic metals at high concentrations, found at varying concentrations in the samples. Heavy metals are potentially toxic trace elements, and their impacts may be felt in organisms at low concentrations. It is a known fact that heavy metals are not biodegradable and so tend to bioaccumulate in organisms' Heavy metals cause health problems when ingested by humans and other organisms [31]. The mean physical properties obtained from the paint-effluent polluted soil during the remediation period were similar to the previous findings [32]. The concentrations of zinc, total nitrogen, total organic carbon, and electrical conductivity levels had a significant statistical difference across the samples throughout the period of remediation at $p < 0.05$. This means that there were significant reductions in the heavy metal contents of the effluent polluted soils as remediation time increased. Heavy metals decreased significantly with an increased remediation period after six months of amendment with different amendments [2,13,14].

The bacterial and fungal counts of the paint-effluent polluted soil over a 6-month remediation period revealed that the bacterial counts for all the samples at the start of remediation were low, but increased between the 8^{th} and 12^{th} weeks, then started to decline up to the 24^{th} week. This reduction in microbial count may be due to the action of biocides in paints, which eliminate microorganisms and so decrease the microbial population [19]. The mean values of the bacterial and fungal counts for all the samples were statistically significant across the weeks up to the 24^{th} week ($p < 0.05$). The *Pseudomonas spp.*, found almost in every case of degradation of materials, is also the most tenacious bacterial isolate in the screening test conducted with mineral salt media. Aspergillus spp. and Pseudomonas spp. have been found in paints more than once during different studies, which shows that they can be used to remove pollutants from paints and paint effluents [29,33].

Microbes degrade contaminants because in the process they gain energy that allows them to grow and reproduce. Microbes get energy from the contaminants by breaking chemical bonds and transferring electrons from the contaminants to an electron acceptor, such as oxygen. The physicochemical assessment showed that as remediation time went on, the heavy metal levels in effluent-polluted soils went down by a lot, making these soil environments less dangerous [2,13,14].

4. Conclusion

The microbial assessment of paint industry effluents and soils from the study revealed the presence of numerous microbes, some of which are pathogenic. Some of these authochthonous microorganisms isolated from paint industry effluents were helpful in the remediation of such soils polluted with effluents over a specific period of time. The survival of these microorganisms in these paint effluents shows that they possess certain factors that promote adaptability to some heavy metal conditions. The physicochemical assessment revealed the presence of some heavy metals, which are toxic to both plants and animals. At the end of the remediation period, there were significant reductions in the heavy metal contents of the effluent-polluted soils as the remediation time increased, thus reducing the toxicity of such soil environments. Monitoring natural attenuation methods (bio attenuation) should be looked into and improved as a way to clean up sites polluted by paint industry effluent because they are

cheaper and better for the environment than other processes and methods.

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Competing Interests

Authors have declared that no competing interests exist.

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