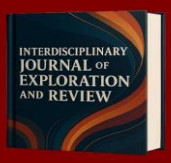

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MICROBIAL DIVERSITY IN PHARMACEUTICAL WASTEWATER AND ITS IMPACT ON SOIL HEALTH

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ABSTRACT

A good agricultural soil is characterized by adequate nutrients. Most agricultural soils in Nigeria have been subjected to different kinds of pollutants resulting from anthropogenic activities, notably untreated pharmaceutical effluents (UPE) which have emerged as the major threatening factor to the quality of Nigerian agricultural soils. This study investigated the impact of untreated pharmaceutical effluents (UPE) on soil microbiological characteristics. UPE samples were collected from five major pharmaceutical companies in Anambra State, Nigeria, and analyzed for microbiological properties, heavy metal level using Atomic Absorption Spectroscpic technique and nutritive values using gravimetric technique. The results showed that the microbial parameters of the UPEs exceeded the World Health Organization (WHO) stipulated limits. Notably, UPE P4 exhibited the highest inhibitory activity against microbes. Furthermore, the study revealed a significant ($P<0.05$) increase in heavy metals (Arsenic, Cadmium, Cobalt, Nickel, Lead) and a decrease in nutritive values (proteins, carbohydrates, fats) of Zea mays seeds harvested from UPE-polluted sites, particularly in 100L/9m² UPE-polluted sites. The findings suggest that UPE pollution significantly reduces soil microbiological properties and nutrient cycling microbes while increasing heavy metal levels. These results have important implications for environmental and human health, highlighting the need for proper treatment and disposal of pharmaceutical effluents.

Keywords: Untreated pharmaceutical effluents (UPE), Heavy- metals, Nutritive -values, Zea mays, Pollution, Public health

INTRODUCTION

Many different active substances of pharmaceuticals, which are present in biosolids produced mainly from municipal sludge, whose agricultural use may cause contamination of soils as well as of surface and groundwater, may often lead to the accumulation of these substances in plants. Bioaccumulation, according to the EPA definition, is the net uptake of a pollutant from the environment. Bioaccumulation factors (BAF) are calculated by considering pollutant tissue concentrations with respect to environmental pollutant concentrations. BAF values >1, indicate that the accumulation in the organism is greater than of the medium (eg. soil or water) from which the pollutant was taken. These factors can be calculated on a total organism basis or normalised to the lipid content of the organism.

Kodesova *et al*. (2016), considering the fact that sewage sludge and manure are potential source of bioactive substances, such as medicines used in human and veterinary treatment, examined the absorption of melformin (an antidiabetic medicine), ciprofloxacin (antibiotic) and narasin (coccidiostat) in carrot (*Daucus carota* ssp. *Sativus* cv. Napoli) and barley (*Hordeum valgare).* It was shown that the bioaccumulation coefficients of ciprofloxacin and narasin in all the analysed plant parts were below one.

Melformin traced in sewage sludge, in a concentration of 0.5 – 1. 6mg/kg dry weight, generally showed a higher coefficient of bioaccumulation in the roots (2 – 10) than in the leaves (0.1 – 1.5). It was observed that the bioaccumulation factor in turnip seeds was above one and was 40 – 60 times higher than that in barley or wheat seeds. The uptake of antibiotics used in veterinary medicine by plants grown on soils fertilized with manure and the consumption of these plants by humans pose potential risk of exposure to residues of active drug substances, in particular chlortetracycline (Xiaolei *et al*., 2020).

The pharmaceuticals introduced into the soil have a tendency to accumulate there for a longer time period. Some pharmaceuticals can be taken up by plants and then they can accumulate in various plant tissues, example, an uptake of chlortetracycline by carrots results in accumulation of this compound in roots while the uptake by lettuce and corn results in the accumulation of the pollutant in leaves. The concentrations of chlortetracycline and sulfamethazine in plant tissues were low (2 - 17µg/kg), however, these concentrations increased in proportion to their concentrations in manure used as fertilizer (Al-Farsi *et al*., 2017). The highest concentrations of these compounds have been described in the tissues of maize, lettuce and potatoes. Diazinon, enrofloxacin, florfenicol and trimethoprim accumulated also in leaves of lettuce, while florfenicol, and levamisol and trimethoprim in carrot roots (Pang *et al*., 2019). The results of research carried out so far showed a low risk

of exposure to these substances through the consumption of vegetables. However, the risk may be important in the case of compounds whose daily acceptable dose is very low, or those that produce subtle effects over a longer period of time, or when their consumption occurs from various sources simultaneously (Tang *et al*., 2018)

The mechanism of detoxification of the green algae in question permits to take advantage of the species for bioindication in the environment risk assessment, whereas from an ecological viewpoint, it showed the potential for sulfathiazole bioaccumulation, which with the role of macroalga as the main producer in the trophic network, poses a risk of biomagnification (Nkoom *et al*., 2019).

MATERIALS AND METHODS

Study Area

The study was carried out in Anambra State. Anambra State is located on latitude 5 0 30" to 6 0 40" North of the equator and on longitude 6 0 40" to 7 0 20" East of the Prime Meridian, with coordinates of 6.2209° N, 6.9370° E with an elevation of 134.83 meters (442.36 feet) above the sea level. Anambra State is a Nigerian State, located in the Southeastern region of the country. Anambra State has total land area of 4,416 sq. km, Anambra State, situated on a generally low elevation on the eastern side of the River Niger and bounded by Delta State to the West, Imo State I metropolis area in Africa. It includes the valley of the lower Anambra River, which is a tributary of the Niger River. It has tropical climate (rainy and dry seasons) with double maximal rainfall. The rainy season is between April and October, and the dry season is between November and March. The annual rainfall ranges from 1800 mm to 2000 mm. The city's yearly temperature is 28.99ºC (84.18ºF) and it is -0.47% lower than Nigeria's averages. Anambra typically receives about 212.36 millimeters (8.36 inches) of precipitation and has 243.38 rainy days (66.68% of the time) annually. The major anthropological activities are farming/agriculture, trading and industries, of which pharmaceutical companies is one of the major industrial practices. In this study, samples were collected from five pharmaceutical companies located in Awka, Ogidi, and Onitsha towns in Anambra State, Nigeria.

Field descriptions of study area

This work considered five different pharmaceutical companies as sources for sampling collection. They are Juhel, Gauze, Kingsize and Rico pharmaceuticals, and Alben health care industries Ltd. The companies are described as follow:

1 Juhel Pharmaceutical Company: It is an indigenous pharmaceutical company located at Plot PD/5, Executive Business Layout, Enugu-Onitsha Express, Awka 420116, Anambra State, Nigeria, with Latitudes 6^{o} 24^{l} 619^{ll} N and Longitudes 7^{o} 09^{l} 553^{ll} E (Fig. 1). It is a pioneer in both oral and

parenteral pharmaceutical manufacturing in west Africa. The company manufactures Small Volume Parenteral (Injectable), Large Volume Parenteral and Ophthalmic solutions.

2.Gauze pharmaceutical and laboratory Nig. Limited: is located off Best Western Meloch Hotel, Enu-Ifite, Near Govt. House, Awka south, Anambra State. The company falls within Latitudes 6^{o} 23^{l} 788^{ll} N and Longitudes 7^{o} 09^{l} 155^{ll} E (Fig. 1). The company is into healthcare and pharmaceutical products. The company deals on production of Oral Solid Dosage (Tablet) and Oral Liquid Dosage (Syrups and Suspension), External Preparation, Oral Dry Powders (ORS) and Beta-Lactam dry powder (Dry Syrup for reconstitution).

3.Kingsize Pharmaceuticals Nig. Limited: is located along Km 15 old Enugu road, at No. 1 Afor Igwe Uru-Orji Village Ogidi, Idemili North, Anambra State, Nigeria, with Latitudes 6^{o} 14^{l} 939^{ll} N and Longitudes 6^{o} 88^{l} 843^{ll} E (Fig. 1). The company's products include Aspirin Tablet – Acetylsalicylic Acid 300 mg; Metronidazole Tablet – 200 mg; Paracetamol Tablet- 500mg. The company's product lines include Oral Solid Dosage (Tablet), Oral Liquid Dosage (Syrups and suspension).

4.Alben Healthcare Industries Limited: is located at Km 15 Old Onitsha – Enugu Road, Ogidi, Idemili south, Anambra State, within Latitudes 6^{o} 14^{l} 528^{ll} N and Longitudes 6^{o} 90^{l} 481^{ll} E (Fig. 1). The company's corporate office is located at plot 102 Maganda Road, Bompai Industrial Area, Kano. In December, Alben Healthcare got approval from Pharmacist Council of Nigeria [PCN], to manufacture three products lines: Tablets, Liquid and ORS. Ref. No: PCN/C/4459/XVI/2128.. The company's product lines are Oral Solid Dosage (Tablet and Capsule), and Oral Liquid (Syrups).

5.Rico Pharmaceuticals Industries Limited: is located at No. 26. Nawfia street, Omagba Phase ll, Onitsha, Anambra State, Nigeria, and situates within Latitudes 6^{o} 13^{l} 204^{ll} N and Longitudes 6^{o} 79^{l} 669^{ll} E (Fig. 1). The company's product lines include; Oral Solid Dosage (Tablet and Capsule), Oral Liquid Dosage (Syrups), Oral Powder (Oral Rehydration Salt)

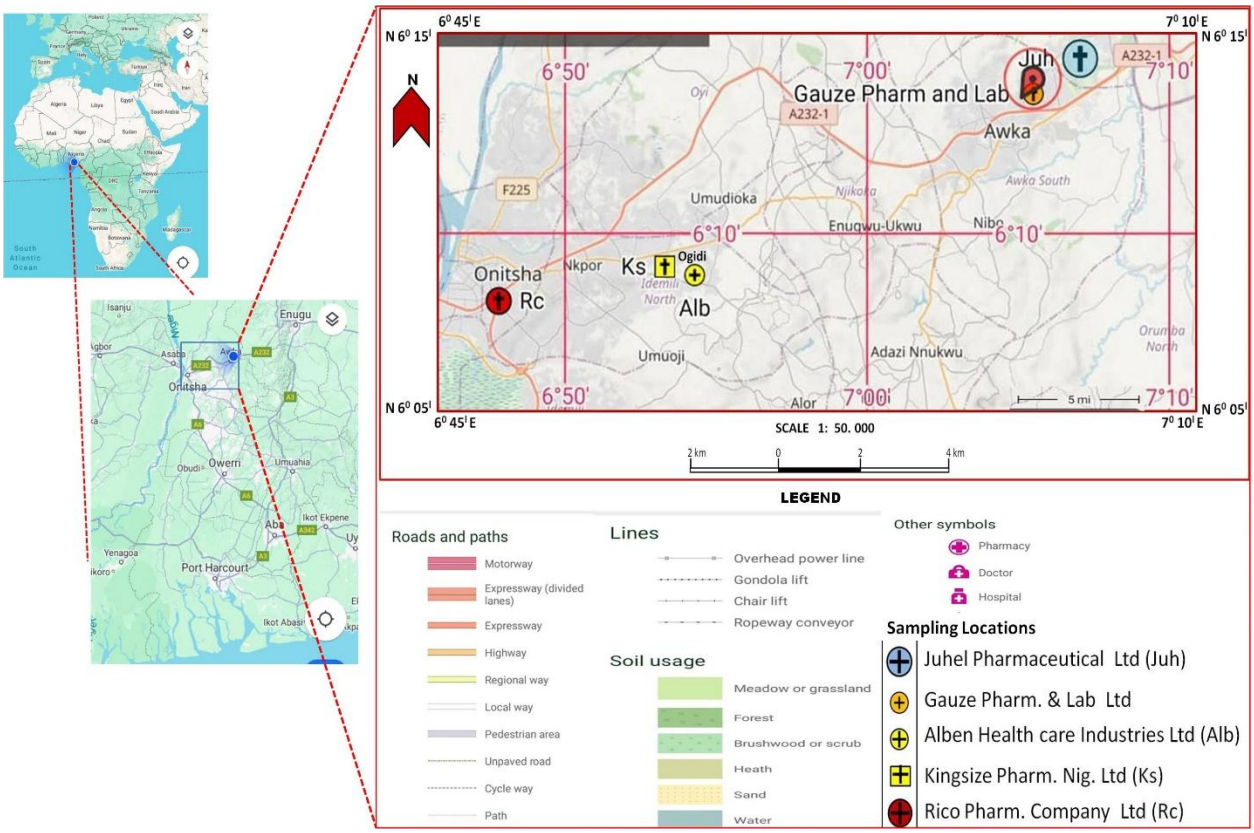

Figure1: Map of Africa showing part of the regional topographical map of southeastern Nigeria, locations and access routes of the study area, and sampling point

Sample Collection

Collection, Handling and transportation of pharmaceutical effluents, soil samples: The samples used for this study were untreated pharmaceutical effluent, collected from five notable pharmaceutical companies in Anambra State. The pharmaceutical companies were Juhel pharmaceutical company at Awka, AL-Ben Healthy Care at Ogidi, Gauze pharmaceutical company at Awka, King Size pharmaceutical company at Ogidi and Rico pharmaceutical company at Onitsha. Samples were taken from the sampling sites in triplicates. The effluent samples were collected with sterile containers. The containers were thoroughly washed with detergent, rinsed with water, and then rinsed with 70% ethanol and final rinsed three times with distilled water. During collection, the containers were placed inverted in order to drain the water inside them. The container was placed on the discharged point, then, placed vertically for the effluent sample to refill the sample container. This sample was covered immediately and kept in a cooler containing ice block. Similarly, soil samples were collected at the site of discharge of the effluent using a soil auger at the depth of 10 cm. Samples were collected at 5 cm distance apart and the samples were mixed together to formulate a composite sample and were put in a clean polyethene material. Also, cobs of maize were purchased at Eke Awka Market and put in a clean polyethene material. All the samples were transported to the laboratory for immediate analysis.

Microbiological analysis

Determination of Total Bacterial Counts (TBC): One milliliter (1.0 ml) sample was aseptically transferred into a sterile test tube (Pyrex) containing 9.0 ml of the diluent (sterile normal saline) and from this; ten-fold serial dilutions were made up to 10^{-3}. One milliliter of the diluted sample (10^{-3}) was plated on Petri dishes (60 mm OD × 55 mm ID × 13mm high) containing Nutrient agar medium (NA/Biotech) using pour plate method. All the plates in triplicates were incubated inverted at 37±2˚C for 24 h. The total bacterial counts were determined after incubation using an electric colony counter and colonies counted were expressed at CFU/ml as described by APHA (2012). The procedure was repeated for other samples.

Determination of Total Coliform Counts (TCC): One milliliter (1.0 ml) sample was aseptically transferred into a sterile test tube (Pyrex) containing 9.0 ml of the diluent (sterile normal saline) and from this; ten-fold serial dilutions were made up to 10^{-3}. One milliliter of the diluted sample (10^{-3}) was plated on Petri dishes (60 mm OD × 55 mm ID × 13mm high) containing MacConkey agar medium (MA/Biotech) using pour plate method. All the plates in triplicates were incubated inverted at 37±2˚C for 24 h. The total coliform counts were determined after incubation using an electric colony counter and colonies counted

were expressed at CFU/ml as described by APHA (2012). The procedure was repeated for other samples.

Determination of Total Faecal Coliform Counts (TFC): One milliliter (1.0 ml) sample was aseptically transferred into a sterile test tube (Pyrex) containing 9.0 ml of the diluent (sterile normal saline) and from this; ten-fold serial dilutions were made up to 10^{-3}. One milliliter of the diluted sample (10^{-3}) was plated on Petri dishes (60 mm OD × 55 mm ID × 13mm high) containing Eosin Methylene Blue agar medium (EMB/Biotech) using pour plate method. All the plates in triplicates were incubated inverted at 44.5˚C for 24-48 h. The total coliform counts were determined after incubation using an electric colony counter and colonies counted were expressed at CFU/ml as described by APHA (2012).

Total Mold Counts (TMC): One milliliter (1.0 ml) sample was aseptically transferred into a sterile test tube (Pyrex) containing 9.0 ml of the diluent (sterile normal saline) and from this; ten-fold serial dilutions were made up to 10^{-3}. One -tenth milliliter of the diluted sample (10^{-3}) was plated on Petri dishes (60 mm OD × 55 mm ID × 13mm high) containing Sabouraud Dextrose agar medium (SDA/Biotech) using pour plate method. All the plates in triplicates were incubated at inverted position at 30±2˚C for 5-7 days. The total mold counts were determined after incubation using an electric colony counter and colonies counted were expressed at CFU/ml as described by APHA (2012).

Total Yeast Counts (TYC): One milliliter (1.0 ml) sample was aseptically transferred into a sterile test tube (Pyrex) containing 9.0 ml of the diluent (sterile normal saline) and from this; ten-fold serial dilutions were made up to 10^{-3}. One-tenth milliliter of the diluted sample (10^{-3}) was plated on Petri dishes (60 mm OD × 55 mm ID × 13mm high) containing Sabouraud Dextrose agar medium (MA/Biotech) using pour plate method. All the plates in triplicates were incubated inverted at 37±2˚C for 24 h. The total yeast counts were determined after incubation using an electric colony counter and colonies counted were expressed at CFU/ml as described by APHA (2012). The procedure was repeated for other samples. The CFU/ml was further converted log CFU/ml.

Estimation of Total Heterotrophic Bacterial Counts (THBC): The prepared samples were aseptically introduced (1.0 mL) into Petri dishes (90 mm X 15 mm) containing sterile prepared nutrient agar (BIOTECH) as described in the study published by Frank and Robert (2015). These were placed in electric incubator in vertical positions at 35±˚C for 24 h. THBC were enumerated by counting the number of colonies in each plate after 24 h, and the mean counts were calculated and presented in form of mean ± standard deviation.

Estimation of Lipolytic Bacterial Counts (LBC) : The prepared samples were aseptically cultured on sterile poured plates (90 mm x 15 mm) containing Tributyrin agar (TA) as described in the study published by Agboli *et al*. (2017). The plates were incubated in inverted position in electric incubator (STXB128) at 30±2°C for 24 – 48 h. LBC was enumerated by counting the number of colonies surrounded by the clear zones.

Estimation of Phosphate Solubilizing Bacterial Count (PSBC): The prepared samples were aseptically cultured on sterile poured plates (90 mm X 15 mm) containing National Botanical Research Institute's Phosphate Growth Medium (NBRIP) which comprises 10 g glucose, 5 g $Ca(PO_4)2$, 5 g $MgCl_2.6H2O$, 0.25 g $MgSO_4.7H_20$, 0.2 g KCl and 0.1 g $(NH_4)_2SO_4$ in 1000 mL of distilled water as described in the study published by Agboli *et al.* (2017). These were placed in electric incubator (STXB128) in vertical positions at 30±2°C for 24 – 48 h. PSBC were enumerated by counting the number of colonies in each plate after 24 – 48 h, and the mean counts were calculated and presented in form of mean ± standard deviation.

Estimation of Nitrifying Bacteria Counts (NBC) : The prepared samples were aseptically cultured on sterile poured plates (90 mm X 15 mm) containing Glucose Nitrogen Free Mineral Medium (GNFMM) which comprises 1.0 g K_2HPO_4, 1.0 g $CaCl_2$, 0.5 g NaCl. 0.25 g $MgSO_4.7H2O$, 0.01 g $FeSO_4.7H2O$, 0.1 g $Na_2MoO4.2H_2O$, 0.01 g $MnSO_4.5H_2O$ and 7.0 g glucose in 1000 mL distilled water as described in the study published by Zaw *et al*. (2020). These were incubated in vertical positions at room temperature (30±2°C). The NBC were enumerated after 48 h.

RESULTS

Microbiological qualities of the pharmaceutical effluents.

The microbiological qualities of the pharmaceutical effluents (P1, P2, P3, P4 and P5) are shown in Table 1. The physicochemical qualities of the pharmaceutical effluents collected from discharge points of five pharmaceutical companies (P1, P2, P3, P4 and P5) in Anambra State are presented in Table 2. The study revealed that the total bacterial counts (TBC), total yeast counts (TYC) and total mold counts were within the WHO stipulated limited. The total coliform counts (TCC) and faecal coliform counts (FCC) deviated from the WHO stipulated limits. The study further revealed that TBC of the pharmaceutical effluents were significantly ($P<0.05$) higher than the TCC, FCC, TYC and TMC as shown in Table 1 and P4 recorded the highest microbial counts.

Effects of the untreated pharmaceutical effluents on the soil microbes

Tables 3-7 showed the effects of the untreated pharmaceutical effluents on the soil microbes. The effects of the untreated

pharmaceutical effluents on the soil microbes are presented in Tables 3-7. There was significant ($P<0.05$) decrease in the microbial counts of soil microbes from month 1 to month 4, and the decrease was mostly pronounced after the third month mostly for Total Bacterial Counts (TBC), Lipolytic Bacterial Counts (LBC), Phosphate Solubilizing Bacterial Counts (PSBC) and Nitrifying Bcaterial Counts (NBC). A decrease in Total Yeast Counts (TYC) and Total Mold Counts (TMC) were also observed from month 1 to month 4, but the decrease were not pronounced and were statistically not significant ($P>0.05$) more especially for the TMC.

The study revealed that there was deviation in the Physicochemical properties of the pharmaceutical effluent-polluted soil samples as shown in Tables 10-14. There was significant ($P<0.05$) decrease in the pH of the soil samples from month 1 to month 4, and the decreased became severe after the third month. There was increase in the electrical conductivity from month 1 to month 3, and the increase became significant ($P<0.05$) after the third month. The sulphate contents showed slight increase from month 1 to month 3, but later decreased after the third month. Also deviation occurred in the appearance of the soil samples.

Characteristics and Identities of the Bacterial and Fungal Isolates from the Samples

The cultural and morphological characteristics of the bacterial isolates from the effluent and soil samples are shown in Table 8. The isolates; A, B, C, D, E and F exhibited varying characteristics culturally and microscopically. Isolates B, C, D and F were Gram negative rods, circular colonies with varied appearance on nutrient agar plates. Isolates D was yellow in color, with entire margin, non-capsulated and motile. Isolate C was colorless with smooth margin, raised elevation, non-capsulated and motile. Isolate D was colorless and mucoid on nutrient agar plate, raised colonies with smooth margin, capsulated and non-motile. Isolate F exhibited pale yellow colonies initially and later turned white, which also fluorescenced when exposed to direct sunlight. The colonies were convex with entire margin, non –capsulated and motile. Isolate A was Gram positive rod, endospore positive, motile with flat or concave colonies. The colonies were milkish white, irregular shaped with filamentous margin. Isolate E was yellow on nutrient agar plates, circular colonies, non-motile Gram positive cocci bacterium. The isolates were catalase positive and utilized glucose. Isolate B, E and F were oxidase positive. They exhibited varied degree of utilizing sugar molecules as shown in Table 9.

Table 1: Microbial qualities of the pharmaceutical effluents

Effluents Source	TBC ($x10^2$ CFU/ml)	TCC ($x10^2$ CFU/ml)	FCC ($x10^2$ CFU/ml)	TYC ($x10^2$ CFU/ml)	TMC ($x10^2$ CFU/ml)
P1	61.33±0.11	12.62±0.11	4.21±0.01	7.48±0.03	4.39±0.07
P2	87.11±0.33	18.27±0.14	9.33±0.11	11.21±0.07	7.26±0.03
P3	79.21±0.67	14.72±0.11	7.23±0.01	7.18±0.33	5.19±0.03
P4	91.31±0.14	21.33±0.12	9.47±0.14	16.27±0.03	9.11±0.03
P5	68.42±0.11	11.89±0.07	4.14±0.01	6.16±0.03	4.52±0.03
WHO	10 -	0	0	-	-

TBC – Total Bacterial Counts; ; TCC – Total Coliform Counts; FCC – Faecal Coliform Counts; TYC – Total Yeast Counts; TMC – Total Mold Counts; WHO – World Health Organization.

Table 2: Microbial qualities of the unpolluted soil samples

Parameter ($X10^6$ CFU/g)	Value
TBC	12.10±0.10
LBC	1.20±0.10
PSBC	1.80±0.03
NBC	2.40±0.10
TYC	1.30±0.07

TMC	0.70±0.03

TBC – Total Bacterial Counts , LBC – Lipolytic Bacterial Counts, PSBC – Phosphate Solubilizing Bacterial Counts, NBC – Nitrifying Bacterial Counts, TYC – Total Yeast Counts, TMC – Total Mold Counts, CFU/g – Colony Forming Unit per gram

Table 3: Microbiological qualities of the pharmaceutical effluent-polluted soil from Juhel Pharmaceuticals Ltd

Parameter ($x10^6$ CFU/g)	Month 1	Month 2	Month 3	Month 4
TBC	9.88±0.07	6.79±0.02	2.94±0.03	1.08±0.01
LBC	9.88±0.07	6.79±0.02	2.94±0.03	0.21±0.01
PSBC	1.51±0.01	0.94±0.01	0.67±0.03	0.33±0.01
NBC	1.92±0.03	1.36±0.03	1.02±0.01	0.71±0.01
TYC	1.22±0.01	1.08±0.01	0.94±0.01	0.72±0.01
TMC	0.68±0.01	0.64±0.01	0.61±0.01	0.57±0.01

TBC – Total Bacterial Count; LBC – Lipolytic Bacterial Count; NBC – Nitrifying Bacterial Count; PSBC – Phosphate Solubilizing Bacterial Count; TYC – Total Yeast Count; TMC – Total Mold Count.

Table 4: Microbiological qualities of the pharmaceutical effluent-polluted soil samples from King size pharmaceuticals Ltd

Parameter ($x10^6$ CFU/g)	Control	Month 1	Month 2	Month 3	Month 4
TBC	12.10±0.10	8.64±0.01	4.29±0.07	1.02±0.01	0.72±0.01
LBC	1.20±0.10	1.00±0.01	0.57±0.01	0.32±0.03	0.11±0.01
PSBC	1.80±0.03	1.42±0.07	0.83±0.01	0.47±0.01	0.16±0.01
NBC	2.40±0.10	1.16±0.03	0.92±0.01	0.67±0.01	0.53±0.01

TYC	1.30±0.07	1.22±0.01	1.08±0.01	0.94±0.01	0.72±0.01
TMC	0.70±0.03	0.64±0.01	0.61±0.01	0.57±0.01	0.47±0.01

TBC – Total Bacterial Count; LBC – Lipolytic Bacterial Count; NBC – Nitrifying Bacterial Count; PSBC – Phosphate Solubilizing Bacterial Count; TYC – Total Yeast Count; TMC – Total Mold Count.

Table 5: Microbiological qualities of the Pharmaceutical effluent-polluted soil from Rico pharmaceuticals Ltd

Parameter	Month 1	Month 2	Month 3	Month 4
TBC ($x10^6$ CFU/g)	7.86±0.03	2.14±0.01	0.64±0.01	0.22±0.00
LBC ($x10^6$ CFU/g)	0.80±0.01	0.41±0.01	0.12±0.01	0.02±0.00
PSBC ($x10^6$ CFU/g)	1.34±0.01	0.70±0.03	0.18±0.01	0.06±0.00
NBC ($x10^6$ CFU/g)	1.60±0.033	0.74±0.01	0.14±0.01	0.03±0.00
TYC ($x10^6$ CFU/g)	0.90±0.01	0.60±0.01	0.47±0.01	0.41±0.01
TMC ($x10^6$ CFU/g)	0.70±0.01	0.50±0.01	0.43±0.01	0.37±0.01

TBC – Total Bacterial Counts; LBC – Lipolytic Bacterial Counts; NBC – Nitrifying Bacterial Counts; PSBC – Phosphate Solubilizing Bacterial Counts; TYC – Total Yeast Counts; TMC – Total Mold Counts.

Table 6: Microbial qualities of untreated pharmaceutical effluent-polluted soil samples from Gauze pharmaceuticals Ltd

Parameter ($x10^6$ CFU/g)	Control	Month 1	Month 2	Month 3	Month 4
TBC	12.10±0.10	9.88±0.07	6.79±0.02	2.94±0.03	1.08±0.01
LBC	1.20±0.10	9.88±0.7	6.79±0.02	2.94±0.03	0.21±0.01
PSBC	1.80±0.03	1.51±0.01	0.94±0.01	0.67±0.03	0.33±0.01
NBC	2.40±0.10	1.92±0.03	1.36±0.03	1.02±0.01	0.71±0.01
TYC	1.30±0.07	1.22±0.01	1.08±0.01	0.94±0.01	0.72±0.01
TMC	0.70±0.03	0.68±0.01	0.64±0.01	0.61±0.01	0.57±0.01

TBC – Total Bacteria Count; LBC – Lipolytic Bacterial Count; NBC – Nitrifying Bacterial Count; PSBC – Phosphate Solubilizing Bacterial Count; TYC – Total Yeast Count; TMC – Total Mold Count.

Table 7: Microbial qualities of untreated pharmaceutical effluent-polluted soil samples from Alben pharmaceutical Industries Ltd.

Parameter ($x10^6$ CFU/g)	Control	Month 1	Month 2	Month 3	Month 4
TBC	12.10±0.10	8.64±0.01	4.29±0.07	1.02±0.03	0.72±0.01
LBC	1.20±0.10	1.00±0.7	0.57±0.02	0.32±0.03	0.11±0.01
PSBC	1.80±0.03	1.42±0.07	0.83±0.01	0.47±0.01	0.16±0.01
NBC	2.40±0.10	1.16±0.03	0.92±0.03	0.67±0.01	0.53±0.01
TYC	1.30±0.07	1.22±0.01	1.08±0.01	0.94±0.01	0.72±0.01
TMC	0.70±0.03	0.64±0.01	0.61±0.01	0.57±0.01	0.47±0.01

TBC – Total Bacteria Count; LBC – Lipolytic Bacterial Count; NBC – Nitrifying Bacterial Count; PSBC – Phosphate Solubilizing Bacterial Count; TYC – Total Yeast Count; TMC – Total Mold Count.

Table 8: Cultural and morphological characteristics of the bacterial isolates from the effluent and soil samples

Parameter	A	B	C	D	E	F
Appearance on NA	Milkish white	Yellow	Colorless	Colorless	Yellow	Pale yellow, later white
Shape of colony	Irregular	Circular	Circular	Circular	Circular	Circular
Elevation	Flat/Concave	Convex	Raised	Raised	Raised	Convex
Margin	Filamentous	Entire	Smooth	Smooth	Smooth	Entire
Gram Reaction	Positive	Negative	Negative	Negative	Positive	Negative
Cell Morphology	Rods	Rods	Rods	Rods	Cocci	Rods
Endospore	Positive	Negative	Negative	Negative	Negative	Negative
Capsule	Negative	Negative	Negative	Positive	Negative	Negative
Motility	motile	motile	Motile	Non-motile	Non-motile	motile
Possible Bacterium	*Bacillus*	*Burkholderia*	*Enterobacter*	*Klebsiella*	*Micrococcus*	*Pseudomonas*

Table 9: Biochemical characteristics of the bacterial isolates

Parameter	B	D	E	M	N	P
Catalase	+	+	+	+	+	+
Oxidase	_	+	_	_	+	+
Citrate	+	+	+	+	_	+/_
Indole	_	_	_	_	_	_
Methyl Red	+	_	_	_	_	_
Voges Proskauer	+	_	+	+	+/_	_
Urease	+	_	_	+	+/_	_
Hydrogen sulphide	_	_	_	_	_	_

Glucose	+	+	+	+	+	+
Maltose	+	_	+	+	_	_
Lactose	+	_	+	+	_	_
Mannitol	+	+	+	+	_	_
Mannose	+/_	+	+	+	_	_
Sorbitol	+/_	_	+	+	_	_
Bacteriu m	*Bacillus*	*Burkholderia*	*Enterobacter*	*Klebsiella*	*Micrococcus*	*Pseudomonas*

DISCUSSION

The microbiological and physicochemical qualities of the unpolluted soil samples were within the stipulated limits of WHO and Environmental Protection Agency (EPA). The total bacterial counts (TBC), nitrifying bacterial counts (NBC), lipolytic bacterial count (LBC), phosphate solubilizing bacterial counts (PSBC) were within the stipulated limits of soil enriched with nutrients that is designed for agricultural purposes as reported by many researchers (Less *et al.,* 2016). The deviation of the physicochemical properties of the UPE-polluted soil recorded in the present study agreed with the findings of Lalwani *et al.* (2020) but deviated from the reports of Kumar *et al*. (2019) who only focused on the treatment strategies, human usage and heavy metal constituents of pharmaceutical effluents-polluted soil. There were reductions in the number and loads of nutrient enriched soil microbes such as NBC, PSBC, and LBC in UPE-polluted soil. Similar observations were recorded by many researchers (Gworek *et al*., 2021). Ezeogo *et al.* (2021) also reported that the presence of contaminants in form of heavy metals led to loss of valuable microbes in the soil. Consequently, this significant reduction observed in the present study could be attributed to the reduction and disaggregation of soil nutrients and soil structure due to reduction or loss of valuable nutrient cycling microbes as of constant exposure to high concentration of UPE. The presence of heavy metals such as arsenic, cadmium, cobalt, chromium, copper, nickel, lead, and zinc in the edible part of the *Zea mays* supported the reports of Zhi *et al*. (2019). Some researchers reported the occurrences of heavy metals in UPE-polluted sites, and these were detected in the crops planted in those sites. Studies have shown that nickel (Tang *et al*., 2018), copper (Kumar *et al*., 2019; Pobi *et al.*, 2019), lead (Sousa *et al*., 2018), cadmium (Shahmahidi *et al*., 2020) and chromium (Zhu *et al.,* 2018), cobalt and zinc were mostly detected in UPE-polluted soil and the crops planted in the sites. The decrease in the nutritive values of both fresh and dried maize samples harvested from the UPE-polluted soil could be attributed to a variety of essential nutrients in the polluted soil.

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