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Effects of Pharmaceutical Effluents on Soil Microbiome and Physicochemical Parameters

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ABSTRACT: Soil contamination from pharmaceuticals is an evolving issue, consequently measurable data on their microbial effects are deficient. Thus, this study investigated the effects of pharmaceutical effluents on soil microbiome and the physicochemical parameters of soil samples obtained from Ugbowo, Benin City, Nigeria using standard procedures. The experiment which lasted for four weeks consists of four treatments of soil samples with pharmaceutical effluents of different percentages and one soil sample without pharmaceutical effluents (control). These include: soil treated with 250 ml of pharmaceutical effluents (25%); soil treated with 500 ml of pharmaceutical effluents (50%); soil treated with 750 ml of pharmaceutical effluents (75%), soil treated with 1000 ml of pharmaceutical effluents (100%) and soil treated without pharmaceutical effluents (0%). There was significant increase in the soil microbial counts in all effluent treatments compared to the control soil. A total of 16 isolates were identified. Ten were isolates belonging to the genera *Bacillus, Arthrobacter, Proteus, Pseudomonas, Staphylococcus, Escherichia, Klebsiella, Enterobacter, Streptococcus,* and *Chromobacterium*, while *Fusarium* sp., *Mucor* sp., *Saccharomyces* sp., *Aspergillus niger*, *Rhizopus* sp. and *Penicillium* sp. were the observed fungal isolates. The mean values of the soil physicochemical properties were all significantly higher in the treated groups compared to the control. This study revealed that pharmaceutical effluents altered the soil microbiological and physicochemical properties. The possibility of these alterations was due to the high nutrient content of the effluent which enriched the soil with additional nutrients needed for microbial growth.

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Pharmaceuticals are bioactive substances utilized in veterinary and human medicine. They are also used in stimulating plant growth and manufacture of food (Gworek *et al*., 2021). It is important to recognise the role that the pharmaceutical industry plays in advancing scientific research and technology that promote both human and animal health. However, the many unsolved problems associated with the residues of active ingredients of pharmaceuticals present in the environment should also be measured (Bartolo *et al*., 2021). In recent times, due to the threats pharmaceuticals pose on biota and their surroundings, they are placed among contaminants of emergent concern (Fernandes *et al*., 2021). This is even more worrisome as population growth, increased wealth and availability of cheap drugs has led to the release of high volume of drugs into our surroundings (Nekui *et al*., 2021, Enagbonma and Babalola, 2019). When pharmaceutical industry, hospitals and veterinary clinic do not handle drug-containing waste or drug-containing wastewater properly, pharmaceuticals and its metabolites are released into the surroundings without treatment (Osayomwanbo *et al*., 2019). The availability, movement, action and fate of pharmaceuticals in the soil and water body is influenced by the biogeochemical properties of the

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soils as well as the properties of the pharmaceuticals (Li *et al*., 2019). The drug active ingredients can be decomposed during abiotic reactions in the soil and this can partly reduce the actual detrimental effects of drugs; though, some breakdown products have related toxicity as their parental constituent. When pharmaceuticals remains go into the soil, the fundamental processes determining their persistence are sorption to organic particles and biodegradation/transformation. Cycoń *et al*. (2019) reviewed that antibiotics affect soil microorganisms by altering their biomass and the relative abundance as well as altering their enzyme activity and ability to metabolize diverse carbon sources. Soil bacterial and fungal community play key role in soil eco-services (Gallego and Martin-Laurent, 2021, Amoo *et al.*, 2021). Hence, this study investigates the effect of pharmaceutical effluents on soil microbiome and physicochemical parameters of soil samples obtained in Ugbowo, Benin City, Nigeria.

MATERIALS AND METHODS

*Study locations and soil collection***:** Fifty (50) g each of soil samples were collected with soil coil from cultivated garden (from 0 – 10 cm depth) (Enagbonma ***et al***., 2020) in Ugbowo, Benin City (6°23'59.3"N 5°36'35.6"E). The sampled soils were well-kept temporarily in ice pack while still in the garden and then transported that same day to the Department of Microbiology Laboratory at the University of Benin, Ugbowo campus, where they were sieved with 2 mm mesh sieve and each equally split into two parts, one for physicochemical analysis (stored at 4℃) and the other for microbial analysis (stored at -2℃) within 7 days (Enagbonma and Babalola, 2020). A measured volume, 2500 ml of the pharmaceutical effluent used in this study were gotten from Nomagbon Pharmaceutical Company, Benin City, Nigeria (6°20'18.4"N 5°37'35.8"E) and these were stored in sterile containers (at room temperature) and properly labelled. The experiment consists of 4 treatments of soil samples with pharmaceutical effluents of different percentages and 1 soil sample without pharmaceutical effluents (control). These include: soil treated with 250 ml of pharmaceutical effluents (25%); soil treated with 500 ml of pharmaceutical effluents (50%); soil treated with 750 ml of pharmaceutical effluents (75%), soil treated with 1000 ml of pharmaceutical effluents (100%) and soil treated without pharmaceutical effluents (0%).

Soil properties analysis: Soil properties were assessed within 7 days of sampling. Soil samples (20 g) that have been pre-processed (i.e. air dried, ground, well mixed, and passed through a 2 mm sieve to remove rubble and solid wooden material) were used for physicochemical analysis. Soil pH in distilled water was measured using a pH-meter in a 1:2.5 soil: water ratio and total nitrogen was determined by the Kjeldhal method. Exchangeable calcium (Ca), magnesium (Mg) and potassium (K) were analyzed after extraction using 1M ammonium acetate method at pH 7.0. Exchangeable Ca and Mg in the extracts were read using an atomic absorption spectrophotometer (AAS) whereas exchangeable K was read by a flame photometer. Available phosphorus (P) was determined spectrophotometrically while organic carbon was determined using method previously described by Enagbonma *et al*. (2021).

Preparation of stock solution: The stock solution of the soil treatment was prepared by weighing 10 g of the soil treatment sample, which was homogenized with 90 ml of sterilized distilled water using sterilized laboratory mortar and pestle. Ten-fold serial dilution was carried out by transferring 1.0 ml of the stock solution into 9.0 ml of sterilized distilled water in a test tube to obtain 10^{-1} dilution after which further dilutions were carried out to obtain 10^{-3} dilution.

Enumeration of microorganisms: Enumeration was done using the pour plate method. An aliquot of 1.0 ml of each dilution was transferred into sterile Petri dishes. About 18-20 ml of molten nutrient agar and potato dextrose agar at 45°C amended with streptomycin were poured into the plates containing sample for the isolation of the total heterotrophic bacteria and fungi. The plates were swirled gently and allowed to solidify at room temperature. Plating was done in triplicates and incubated at 37°C for 24-48 h for nutrient agar while at 25°C for 72 hours for potato dextrose plate. After incubation, the number of discrete colonies were counted and recorded in cfu/g.
Sub-culturing: Pure culture of bacterial isolates were obtained by streaking distinct colonies from countable plates on nutrient agar. They were characterized and identified based on their cultural, morphological, and biochemical characteristics. Fungal isolates were characterized based on their appearance on culture medium, microscopic morphology and type of asexual spores produce.

Characterization and identification of bacterial isolates: All isolates were characterized and identified based on their cultural, morphological, and biochemical characteristics using standard methods.

Statistical analysis: All the assays were done in triplicates. Analysis of variance and descriptive statistics were employed to examine the data gotten from the study using Statistical Package for the Social

Sciences ® version 21, PAST version 2.17c and Microsoft Excel version 2010.

RESULTS AND DISCUSSION

There was increase in the soil microbial counts in all effluent treatment compared to the control soil. The index of the microbial load (10^6 cfu/g) is high and indicates dense population of bacteria and fungi in the pharmaceutical effluent. A total of sixteen isolates were isolated. Ten were isolates belonging to the genera *Bacillus, Arthrobacter, Proteus, Pseudomonas, Staphylococcus, Escherichia, Klebsiella, Enterobacter, Streptococcus,* and *Chromobacterium.* while six of the organisms were fungal isolates. These fungal isolates include: *Fusarium* sp., *Mucor* sp., *Saccharomyces* sp., *Aspergillus niger*, *Rhizopus* sp. and *Penicillium* sp.

Table 1: Microbial count of the soil samples before and after treatment

Treatment	TBC (x 10^4 cfu)/g	TFC (x 10^4 cfu/g)
	Week one	
0%	0.96 ± 0.54	0.42 ± 0.84
25%	1.17 ± 0.25	0.65 ± 0.18
50%	1.29 ± 0.97	0.88 ± 0.99
75%	1.42 ± 1.08	0.96 ± 0.72
100%	1.66 ± 0.84	104 ± 0.84
	Week two	
0%	0.19 ± 0.48	0.51 ± 0.09
25%	1.28 ± 0.87	0.74 ± 0.78
50%	1.51 ± 0.42	0.99 ± 0.08
75%	1.71 ± 0.75	1.06 ± 0.84
100%	1.95 ± 0.84	1.19 ± 0.49
	Week three	
0%	0.17 ± 0.27	0.56 ± 0.08
25%	1.42 ± 0.42	0.85 ± 0.51
50%	1.07 ± 0.54	1.17 ± 0.07
75%	2.08 ± 0.74	1.28 ± 0.81
100%		1.33 ± 0.91
	Week four	
0%	0.16 ± 0.08	0.67 ± 0.17
25%	1.53 ± 0.82	0.95 ± 0.29
50%	1.73 ± 0.42	1.28 ± 0.84
75%	2.04 ± 0.42	1.56 ± 0.48
100%	1.71 ± 0.64	1.65 ± 0.81

Table 2: Bacterial and fungal isolates from soils before and after treatment

Week	Bacterial isolates	Fungal isolates
One	*Bacillus, Arthrobacter, Pseudomonas, Staphylococcus aureus, Escherichia coli, Klebsiella* sp. and *Enterobacter*	*Aspergillus* sp., *Rhizopus* sp, *Yeast* sp. and *Penicillium* sp.
Two	*Bacillus* sp., *Proteus* sp., *Pseudomonas* sp. *Staphylococcus* sp., *Streptococcus* sp. *and Escherichia coli*	*Mucor* sp., *Aspergillus* sp., *Rhizopus* sp., *Yeast* sp. and *Penicillium* sp.
Three	*Bacillus* sp., *Proteus* sp., *Pseudomonas* sp., *Staphylococcus* sp. *and Micrococcus* sp.	*Mucor* sp., *Aspergillus* sp., *Rhizopus* sp, *Fusarium* sp., and *Penicillium* sp.
Four	*Bacillus* sp., *Proteus* sp., *Pseudomonas* sp., *Chromobacterium* sp. and *Micrococcus* sp.	*Mucor* sp., *Aspergillus* sp., *Rhizopus* sp., *Fusarium* sp. and *Penicillium* sp.

Table 3: Physicochemical properties of the soil after pollution

Treatment	pH	C (%)	N %	Ca	P (ppm)	Mg (meg/100g)	K	Na
0%	7.20	1.74	0.07	19.40	1.36	3.96	0.32	0.10
25%	7.90	2.13	0.14	22.50	2.21	4.30	0.35	0.15
50%	8.10	2.20	0.17	22.80	2.26	4.81	0.39	0.18
75%	8.30	2.28	0.18	23.10	2.48	4.96	0.46	0.23
100%	8.40	2.31	0.22	23.40	2.56	5.02	0.47	0.26

The effluent and amended soil contained large number of bacterial loads (9.60 x 10^4 to 2.56 x 10^5 cfu/g) and fungal loads (4.3 x 10^4 cfu/g to 1.63 x 10^5 cfu/g). The index of the microbial load (10^5) is high and it indicates dense population of bacteria and fungi in the effluent. The bacteria isolated from the pharmaceutical effluent and the effluent treated soil samples were *Arthrobacter* sp., *Proteus* sp., *Pseudomonas* sp., *Staphylococcus aureus*, *Escherichia coli*, *Bacillus* sp., *Klebsiella* sp., *Enterobacter* sp., *Streptococcus* sp. and *Chromobacterium* sp. The fungi isolated from the pharmaceutical effluent and the effluent treated soil samples were *Aspergillus niger*, *Mucor* sp., *Rhizopus*

sp., *Fusarium* sp., *Penicillium* sp. and *Saccharomyces cerevisiae*.

Table 4: Physicochemical properties of the pharmaceutical effluent used

Parameters	Values
pH	8.2
Temperature (℃)	28
Total suspended solid (mg/L)	2250
Total dissolved solid (mg/L)	382
BOD (mg/L)	671
COD (mg/L)	1080
Carbon (%)	2.21
Nitrogen (%)	9.68
Phosphorus (ppm)	1.74
Calcium (mg/L)	31.58
Magnesium (mg/L)	22.7
Lead (mg/L)	Not detected
Cadmium (mg/L)	Not detected

All these organisms are potential pathogens of man capable of causing a variety of diseases (Bartolo *et al*., 2021). The isolate of *E. coli* from the pharmaceutical effluent is an indication of faecal contamination of the effluent. *Staphylococcus aureus* causes infections of the skin, deeper tissues and organs, pneumonia and food poisoning, *Proteus* may infect urinary tract and wounds; *E. coli* causes diarrhoea, urinary tract and kidney infections and peritonitis, while *Pseudomonas* causes infections of wounds, burns eyes and ears (Lateef, 2004). The isolation of these pathogens from the effluent is worrisome because the effluents were collected prior to contact with the external environment. In such a case it is not impossible to assume that these pathogens were introduced into the production process by human healthy carrier through handling. The continuous contamination of the process may be enhanced through the processing equipment (Bogomolova and Volobuev, 2020).

The addition of the pharmaceutical effluent increased the soil pH from 7.2 to 8.4 in 100% effluent treatments. However, there was gradual decrease in pH values from week one to four. This decrease in pH values may be attributed to the biodegradation of pharmaceutical effluent influenced by the microbial isolates (Frkova *et al*., 2020). One of the products of the microbial biodegradation of pharmaceutical effluent is organic acid which may have accounted for the decrease in pH (Kudlek *et al*., 2016). The carbon, nitrogen and phosphorus were also raised from 1.74% - 4.20%, 0.07% - 0.51% and 1.36 % - 3.25% in the 100% treatments respectively. The increase in soil pH and decrease in exchangeable acidity is attributed to higher inorganic ions component of the pharmaceutical effluent used (Rastogi and Tiwari, 2022). The increase in carbon, nitrogen and phosphorus in the soil at the end of the analysis is due to the high constituent of these elements in the effluent. The higher component of phosphorus in the soil could be a result of fixation attributed to high pH brought about by the increased component of the effluent in the soil. The decrease in pH may also be due to the occurrence of high microorganism activities, which assisted in the biodegradation of pharmaceutical effluent in the amended soils (Rastogi and Tiwari, 2022, Lateef, 2004, Abioye *et al*., 2015). The increase in the organic carbon is as a result of high total solid present in the effluent, which may have mineralized. In addition, the increase in the microbial population in the amended soils with effluent as the concentration level of treatment may be due to the high nutrient content of the effluent which enriched the soil with additional nutrient needed for microbial growth (García-Santiago *et al*., 2017).

Conclusion: This study revealed that pharmaceutical effluent altered the soil microbiological and physicochemical properties. The possibility of these alterations was linked to the high nutrient content of the effluent which enriched the soil with additional nutrient needed for microbial growth. However, more studies are encouraged to substantiate this claim.

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