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EFFECT OF PHARMACEUTICAL EFFLUENT ON SOIL PHOSPHATE CONCENTRATION

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ABSTRACT :

Soil becomes contaminated when there is presence of xenobiotic chemicals or other alteration in the natural soil environment. In Nigeria, one of the sources of soil pollution is the effluent discharge from industries. Most pharmaceutical effluents are known to contain varying concentrations of organic compound and heavy metals such which, when discharged from pharmaceutical industries affect the concentration of the soil chemical constituents. Phosphate, one of the chemical constituent found in the soil, is a natural source of phosphorous, an element that provides a quarter of all the nutrients that plants need for growth and development. This study is aimed at determining the effect of effluent from JUHEL pharmaceutical company on the concentration of phosphate around Emene industrial area in Enugu. The concentration of phosphate was determined using Spectrophotometric method. The results showed that the concentration of phosphate was lower at the points of discharge, liquid syrup (1.5533 ± 0.027mg/l) than in the other sampling points. The concentration of phosphate decreased when a fresh soil samples were spiked at different concentrations with drugs (Barbiclox and Flu-j syrup) and monitored within 28 days. These results showed that the discharge of this pharmaceutical effluent on the soil decreases the concentration of phosphate. There is need for public awareness of the health impact of pharmaceutical effluents and proper treatment effluents before discharge to the environment to prevent adverse effect of the contaminants on soil, aquatic and human health.

Keywords: Pharmaceutical effluents, Xenobiotics, Soil, Environmental pollution, Phosphate, Spectrophotometer.

1. INTRODUCTION :

Pharmaceutical compounds are being used for several beneficial purposes in modern society but simultaneously pharmaceutical industries are releasing very toxic contaminant in the environment directly or after chemical modifications (Halling-Sorensen *et al*., 2002). Moreover, pharmaceutical compounds may enter the environment by different routes such as discharge from landfills sites, sewer lines, and runoff from animal waste etc (Kumar *et al*., 2010). It has been found that pharmaceutical compounds reach the environment and can be considered as environmental pollutant. Several pharmaceutical production facilities were found to be sources of much higher environmental concentration than those caused by the application of drugs (Larson *et al*., 2007). Pharmaceutical effluents are wastes generated by pharmaceutical industries during the process of drug manufacturing. Their risk to human health and environmental species cannot be overemphasized. Some of the most representative pharmaceutical products found in the environment include antibiotics, lipid regulators, anti-inflammatories, antiepileptics, etc (Lateef, 2004). The increase in demand for pharmaceuticals has resulted in a consequent increase in pharmaceutical manufacturing company and hence increased pharmaceutical waste which most times contain substantial amount of heavy metals. These effluents are usually discharged into the environment and when improperly handled and disposed, they affect both human health and the environment (Osaigbovo and Orhue, 2006; Anetor *et al*., 1999). Because of the detection of pharmaceuticals in soil, concern has been raised over the potential for these substances to be taken up into human food items and to pose a risk to human health (Boxall *et al*., 2006). Active pharmaceutical ingredients (APIs) may be released into the soil environment when contaminated sewage sludge, sewage effluents or animal manure is applied to land (Butler *et al*., 2012).

Phosphorus occurs almost entirely as phosphate and both organic and inorganic forms are of major importance in plant-soil-water, and in the general phosphorus biogeochemical cycling in natural systems. The majority of agricultural soils usually do not meet crop demands for phosphorus and as such, fertilization is required (Radojevic & Bakin, 1999). Plants absorb most of their P as $H_2PO_4^-$ and smaller amount as HPO_4^{2-} depending on the pH. Lower pH values will increase the absorption of $H_2PO_4^-$ ion whereas higher pH values will increase the absorption of HPO_4^{2-} ion.

The availability of phosphorus in soils depends on phosphorus recycling from soil organic matter (Brooks *et al*., 2013). This availability of phosphorus is regulated by adsorption of phosphate ions to aluminium and iron oxide compounds present in soil (Cleveland *et al*., 2002). Soil organic phosphorus

mineralization is enzymatic processes which are carried out by a group of phosphatases that catalyze hydrolytic reaction of phosphate groups and so, provides inorganic phosphate to the soil solution (Criquet & Brand, 2008). The activity of acid phosphatase in particular, plays an important role in the hydrolysis of organic phosphorus to orthophosphate ions in acid soils (Huang & Shindo, 2000). Soil phosphatase is a primary indicator of soil quality in various land use systems (Vinhal-Freitas *et al*., 2013).
This study is aimed at exploring the effect of pharmaceutical effluents on soil phosphate concentration.

2. MATERIALS AND METHOD

2.1 SITE DESCRIPTION

JUHEL Nigeria limited is a pharmaceutical company located in Emene, the industrial hub of Enugu in Enugu state Nigeria. It is located on the latitude 6'48''N and longitude7'58''E. Emene is an industrial area with scattered settlement and a population of more than 200,000 people. The company produces analgesics, antipyretic, antioxidants, antihistamines and so many other drugs. The soil samples were collected from this area at about 15cm depth using the soil auger.

2.2 SOIL COLLECTION

Soil samples containing the pharmaceutical effluents were collected in the month of April from the industrial plant discharge points at JUHEL Nigeria limited, Enugu. Also soil samples were collected 500 metres and 1000 metres away from the pharmaceutical company as well as from the school compound.
Four different soil samples were collected from the pharmaceutical company and within the surroundings of the company. From the point of discharge of dry syrups, the soil was collected in a container to avoid contamination. The soil was collected from 15cm depth pit. From the point of discharge of liquid syrups, the soil was collected in a container to avoid contamination. The soil was also collected from 15cm depth pit. From 500 metres away from the site, the soil was collected after the land has been dug to about 15cm depth and then stored in a container. From 1000metres (1 Km) away from the site, the land was dug up to the depth of 15cm and the soil was collected in a container. The soil samples were collected from the above points and triplicates were made.

2.3 REAGENTS AND EQUIPMENTS

The reagents/chemicals used for this work are as follows: Ammonium sulfate ($(NH_4)_2\ SO_4$), Sulfuric acid (H_2SO_4), Ammonium molybdate ($(NH_4)MO_7O_{24}.4H_2O$), Ascorbic acid, Buffer solution (Na_2HPO_4), Phosphate solution etc. All reagents used are of analytical grade.
The equipments used include: UV/VIS Spectrophotometer UNICO, UV 2100PC, Japan; Centrifuge WEST TUNE, 80-2B, China; Analytical weighing balance OHAUS Corporation, SPU4001, China; Fume cupboard Guangdong Technological, ODM OEM, China; pH meter METTLER TOLEDO, FE20-ATC, Columbia; Refrigerator SAMSUNG DURACOOL, ZR26FARAEWW, China; Beakers Pyrex, United States of America; Pipettes Pyrex, United States of America; Volumetric flasks Pyrex, United States of America; Measuring cylinders Pyrex, United States of America; Spatulas Donguan Co. Ltd, China; Glass rod Pyrex, United States of America; Pyrex, United States of America; Water bath Genlab, WBH6IFL, United Kingdom, etc.

PREPARATION OF REAGENTS

2.4.1 Preparation of ammonium molybdate complex.
To prepare this complex, 5 grams of ammonium molybdate was weighed into 500ml volumetric flask. 160 ml of concentrated sulfuric acid was added slowly into this flask. It was then allowed to cool after which the solution was diluted to 500 ml mark with water. This is an exothermic reaction.
2.4.2 Preparation of ammonium sulfate solution.
This was prepared by weighing out 0.75 grams of ammonium sulfate, mixed with 5 ml of sulfuric acid and then made up to 500 ml with water. This is also an exothermic reaction.
2.4.3 Preparation of phosphate standard solution.
This was prepared by weighing 0.220 gram of mono basic potassium phosphate (KH_2PO_4), dissolving it in 500 ml volumetric flask with water and then make up to the mark with water.

2.5 EXPERIMENTAL DESIGN

The soil samples were grouped into five according to location into;

1. Point of discharge, dry syrup
2. Point of discharge, liquid syrup
3. 1 km away from site
4. 0.5 km away from site
5. School compound (FUNAI)

SPIKING OF SOIL SAMPLES

Soil samples that were collected at 1000 metres away from the indusrial site, 500 metres away from the industrial site and from the school compound (FUNAI) were spiked with the pharmaceutical products obtained from the pharmaceutical company. The pharmaceutical products that were used to spike the soil samples are Barbiclox dry syrup, an antibiotic that has ampicillin trihydrate and cloxacillin sodium as its active pharmaceutical ingredients (API) and Flu-J liquid syrup that contains paracetamol (acetaminophen), chlorpheniramine maleate, and ascorbic acid and is used in the treatmaent of fever, headache and nasal conjestion. The composition of the drugs are shown below:
Barbiclox dry syrup: 100 ml of the suspension (dry syrup mixed with water) contains

1. 125 mg Ampicillin Trihydrate
2. 125 mg Cloxacillin Sodium

Flu- J liquid syrup: Each 5 ml contains

1. 120 mg Paracetamol
2. 1mg Chlorpheniramine Maleate
3. 25mg Ascorbic Acid

The spiking of the soil samples was done at different concentrations of the drugs giving rise the following classes of spike on the soil samples:

1. HIGH DOSE SPIKE (HDS): 1 ml each of the suspension and the syrup were made up to 10 ml with water. Then 1 ml of each of the solutions formed was made up of 25 ml with water and then mixed with 100 grams of the sieved soil samples.
2. NORMAL DOSE SPIKE (NDS): 1 ml of the solution from high dose spike was made up to 10 ml with water. Then 1 ml of the solution was made up to 25ml with water and then mixed with the sieved soil samples. This was done on both drugs.
3. LOW DOSE SPIKE (LDS): 1 ml from the normal dose spike was made up to 10 ml with water. Then 1 ml of the solution was made up to 25ml with water and then mixed with the sieved soil samples. This was carried out on both drugs.
4. CONTROL: The controls are soil samples from the three sampling points but were not spiked with the drugs.

2.6 DETERMINATION OF SOIL pH

Soil pH was determined using the pH meter.
Procedure:
The pH value of the soil samples was determined in distilled water using a pH meter. 10 grams of the soil samples were weighed into a conical flask. Then to the weighed soil samples, 25 ml of distilled water was poured into the flask and allowed to stand for 30 minutes. Within the 30 minutes, the content of the conical flask was stirred using a glass rod at an interval of about three to four times. The pH was then standardized using a pH buffer of 7, before the reading was taken.

2.7 DETERMINATION OF SOIL PHOSPHATE

The determination of phosphate is based on the spectrophotometric method by Murphy & Riley, (1962).
PRINCIPLE:
The principle is based on the use of a complexation reaction to produce a coloured complex of molybdate and phosphorus. This complex is formed when phosphate (from your sample) is heated with ammonium molybdate in the presence of acid and excess ascorbate ions (which are to prevent the colour degrading as the molybdate oxidizes slowly). The coloured complex formed is dependent on the initial phosphate concentration in the sample. The amount of phosphate present is determined by comparison of the blue colour with known standards of phosphate, subjected to the same reaction with molybdate reagent.

PROCEDURES:
1 gram of the soil samples was weighed into test tubes. 20 ml of ammonium sulfate solution was added into the test tubes, shaked and allowed for 30 minutes. The mixture was then centrifuged for 4 minutes at 1000 rpm. After the centrifugation, 5 ml of the filtrate was added into the boiling tubes. 10 ml of distilled water was added into the boiling tubes. 1 ml of ammonium molybdate complex was added into the test tubes followed by half spatula of ascorbic acid crystal and then shaked thoroughly. The samples were slowly heated to boiling. A deep blue green colour develops and then is allowed to cool. The absorbance of the samples was taken at 650 nm.
The standard was prepared by diluting 0.0 ml, 0.1ml, 0.2ml, 0.3ml, 0.4ml, 0.5ml, 0.6ml, 0.7ml, 0.8ml, and 0.9ml of the standard phosphate solution into test tubes and making the volume up to 10ml with water.

3. RESULTS AND DISCUSSIONS

3.1 pH RESULT OF THE SOIL SAMPLES

Table 1. pH result of the soil samples.

SAMPLE	MEAN ± S.D

PODLS	6.6 ± 0.00
PODDS	7.0 ± 0.00
SC	6.8 ± 0.00
I KM	6.7 ± 0.00
0.5 KM	6.5 ± 0.00

Result expressed as Mean ± standard deviation (SD); n=3

NOTE: PODLS: Point of discharge, liquid syrup; **PODDS**: Point of discharge, dry syrup

SC: School Compound (FUNAI): **1KM**: soil sample collected at 1000 meters away from effluent site; **0.5KM**: soil sample collected at 500 meters away from effluent site;

The pH result obtained from the soil samples showed that the soil samples from 1km away from site(6.7), 0.5km away from site (6.5), school compound (6.8) and point of discharge, liquid syrup (6.6) were less acidic while that for the soil sample from point of discharge, dry syrup was neutral(7.0).

3.2 PHOSPHATE CONCENTRATION IN THE SOIL SAMPLES

Table 2. Result of phosphate concentration in the soil sample

	1 km from site		500m from site		School compound		Point of Discharge	
Sample	Week 1	Week 4	Week 1	Week 4	Week 1	Week 4	Week 1	Week 4
	Phosphate concentration (mg/L)							
HDSB	3.320 ± 0.047	0.720 ± 0.010	0.653 ± 0.009	0.586 ± 0.008	0.253 ± 0.003	0.253 ± 0.003		
NDSB	0.753 ± 0.004	0.320 ± 0.005	0.586 ± 0.008	0.320 ± 0.004	0.320 ± 0.005	0.453 ± 0.006		
LDSB	0.653 ± 0.009	0.453 ± 0.006	0.653 ± 0.009	0.386 ± 0.006	2.653 ± 0.038	0.520 ± 0.007		
HDSF	0.320± 0.005	0.253 ± 0.006	0.186 ± 0.003	0.053 ± 0.009	0.186 ± 0.003	0.120 ± 0.001		
NDSF	0.300± 0.002	0.220 ± 0.007	0.320 ± 0.004	0.253 ± 0.003	0.253 ± 0.003	0.320 ± 0.004		
LDSF	0.253 ± 0.012	0.243 ± 0.003	0.120 ± 0.030	0.092 ± 0.007	1.253 ± 0.018	0.253 ± 0.003		
CONTROL	2.053± 0.028	0.320 ± 0.004	1.920 ± 0.027	0.786 ± 0.011	0.120 ± 0.001	0.520 ± 0.007		
Point of Discharge, Dry Syrup							1.853 ± 0.069	0.186 ± 0.003
Point of Discharge, Liquid Syrup							1.553 ± 0.027	0.520 ± 0.007

Results expressed as Mean ± standard deviation (SD); n=3

NOTE: 1km: soil sample collected at 1000 meters away from effluent site; **0.5km**: soil sample collected at 500 meters away from effluent site; **SC**: soil sample collected from school compound (FUNAI); **PODLS**: soil sample at point of discharge, liquid syrup; **PODDS**: soil sample at point of discharge, dry syrup; **HDSB**: High dose spike, Barbiclox syrup. **NDSB**: Normal dose spike, Barbiclox syrup; **LDSB**: Low dose spike, Barbiclox syrup.; **HDSF**: High dose spike, Flu-J syrup ;**NDSF**: Normal dose spike, Flu-J syrup.; **LDSF**: Low dose spike, Flu-J syrup.

The results of phosphate concentration of the soil samples at the different sampling points were shown in Table 2 and the results expressed as mean ± standard deviation. The concentration of phosphate was found to be very low at the point of discharge, liquid syrup (1.553 ± 0.027 mg/L) compared to other points. The concentrations of phosphate were higher in the spiked soil samples than in the controls. Also, the concentration of the phosphate in all the sampling points and the spiked samples decreased rapidly when monitored within 28 days.

3.3 DISCUSSIONS

The pH result of the soil sample from the various sampling points in Table 1 showed that they are within the region of less acidic to alkaline state (6.5-7.0). The optimal pH range for most plant is between 5.5-7.0. Some plant such as blueberries and azaleas however, prefer more strongly acidic soil, while a few such as ferns, asparagus do best in soil that is neutral to pH. The pH result obtained from the soil sample at the point of discharge, antibiotics was higher than the ones obtained at the other sampling point and this could be attributed to the higher Na, Ca, and Mg component in the pharmaceutical effluent (Osaigbovo & Orhue *et al*, 2006). The increase in the concentration of Ca, Mg, Na, K, and N in the soil is due to the high constituent of these elements in the effluents as some of the active pharmaceutical ingredients contain these elements. For example, Barbiclox, an

antibiotic produced in JUHEL, contains Ampicillin trihydrate ($C_{16}H_{25}N_3O_7S$) and Cloxacillin sodium ($C_{19}H_{19}CIN_3NaO_6S$) as its active pharmaceutical ingredients and is therefore, a potential source of N, Na and S to the effluents.

The result of the phosphate concentration in the soil samples in Table 2 showed that the phosphate concentration of the samples obtained at the points of discharge and within the industrial site were higher than the one obtained from soil sample at school compound. However, there was a decrease in the phosphate concentration of the spiked soil samples when monitored in week 1 and 4. The trend of an increase in the concentration of phosphate as the distance away from the discharge points increases was observed. The phosphate concentration at the point of discharge, dry syrup was higher than that at the point of discharge, liquid syrup and their concentrations decreased when monitored in week 1 and 4.

4. CONCLUSION

The discharge of pharmaceutical compounds in the environment in large quantities poses a great risk. This environmental pollutant was found to decrease the concentration of soil phosphate in the soil. The present system of regulations of their release is not able to control the untreated or partially treated pharmaceutical effluents. The impact of drugs are entering into and occurring on ecosystem, biota and humans and their side effect on human, aquatic and terrestrial animal health need to be investigated through thorough safety and toxicological studies.

Efforts should be geared toward public awareness of the health impact of pharmaceutical pollutants and pharmaceutical companies should be made to provide adequate effluents tanks as part of infrastructure before the approval of the company site. Appropriate functional treatment plants should be installed and all wastewaters should be channeled through the plant before the discharge. Also, regulatory bodies such as Federal Environmental Protection Agency (FEPA) should enforce sound environmental policy and all erring companies should be sanctioned.

Further studies should be carried out on the impact of pharmaceutical industries around the state and record of analysis on effluent treatment should be made available to the regulatory bodies.

These approaches are crucial to the attainment of the much desired goal of a sustainable and healthy environment.

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