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EFFECT OF PHARMACEUTICAL EFFLUENT ON SOIL NITRITE 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. Nitrite is widely used in pharmaceutical industries and it is an intermediary compound formed during nitrification and denitrification. This study is aimed at determining the effect of effluent from JUHEL pharmaceutical company on the concentration of nitrite around Emene industrial area in Enugu. The concentration of phosphate was determined using Spectrophotometric method. The results showed that the concentration of nitrite was lower at the points of discharge, liquid syrup (0.6667 ± 0.092 mg/l) than in the other sampling points. The concentration of nitrite increased 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 nitrite. 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, Nitrite, Spectrophotometer.

1. INTRODUCTION:

Pharmaceutical drugs, classified as analgesics, antibiotics, antiepileptic, hormones contraceptives, etc which are being used for human and veterinary medicines are emerging as environmental pollutants (Halling-Sorensen *et al*., 2002). The environmental exposure routes of pharmaceuticals into the environment are manufacturing units and hospital effluents, land applications such as biosolids and water reuse, etc (Daughton & Ternes,1999). Sewage treatment services are not always successful in removing the active chemicals from wastewater especially wastewater that are directly released to the soil (Chanti & Durga, 2015). Studies on antibiotics have shown that up to 95% of antibiotics compound can be released unaltered into the sewage system and higher concentration of the antibiotics can lead to change in microbial community structure and ultimately affect food chain. Such actions of antibiotics are also applicable to non-steroidal anti-inflammatory drugs (NSAIDs) like ibuprofen, naproxen, acetylsalicylic acid and diclofenac (Kreissberg, 2005). Globally, the detection of pharmaceuticals in the environment creates the risks which are associated with their introduction into human, aquatic life and wildlife and has become a serious problem equally for both regulators and the pharmaceutical industry (Daughton &Ternes, 1999). The long term exposure of lower concentration of complex pharmaceutical mixtures on living organism of land and water may result in acute and chronic damages, behavioural changes, accumulation on tissues, reproduction damage and inhibition of cell proliferation (Pomati *et al*., 2006).

Nitrite is an intermediary compound formed during nitrification (biological oxidation of ammonia to nitrate) as well as denitrification. Nitrate (NO_3^-) is a form of inorganic nitrogen (N) that naturally occurs in soils. Sources of soil nitrate include decomposing plant residues and animal manure, chemical fertilizers, exudates from living plant, rainfall as well as lightening (Dick *et al*., 2000). Nitrate taken up by microorganisms are converted into organic forms and released back to the soil in plant-available form when dead organisms are fed upon or decompose. In well drained soils, ammonium and ammonia are converted into nitrate by specific population of aerobic bacteria, a process known as nitrification (Angle *et al*., 1993). The determination of inorganic nitrogen, mainly nitrate and ammonium in soil is often useful, because, despite their usually low level, these inorganic forms are readily available for plant uptake (Tisdale & Nelson, 1970).

Nitrite (NO_2^-) is a versatile chemical agent that is applied in the manufacture of drug and in food preservation among others. Its harmful effect is that it produces carcinogenic nitrosamines in the human body through its reaction with amines or amides (Ensafi *et al.,* 2004). One of the pollutants found in the atmosphere and natural water is nitrite (Manzoori *et al*., 1998). It is formed from the reduction of nitrate and it is an important intermediate in biological nitrogen cycle. Soil nitrate and nitrite along the soil profile can be used as an indicator of nitrogen loss by leaching (Pabalinga *et al*., 2014). Excessive nitrogen fertilizer use accompanied by over irrigation can result in nitrogen leaching in the soil profile and contaminating ground water.

Excess nitrogen accompanied by low oxygen diffusion down the soil alters microbial diversity as it tends to promote denitrifiers thereby changing microbial diversity which at the same time affect nutrient cycling (Pabalinga *et al*., 2014).
This study is aimed at exploring the effect of pharmaceutical effluents on soil nitrite 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: Disodium trioxocarbonate (Na_2CO_3), Sulfanilic acid, Hydrochloric acid (HCl), Methyl Anthranilate, Sodium hydroxide (NaOH), etc. All reagents used are of analytical grade.
The equipment 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 disodium carbonate solution (Na_2CO_3)

To prepare this, 5 grams of disodium carbonate was weighed and dissolved in 1000 ml volumetric flask with water. The volume was made up to 1000ml with water.

2.4.2 Preparation of 0.5% sulphanilic acid

This was prepared by weighing out 0.5 grams of sulfanilic acid, dissolving it with water and the making it up to 100ml on the volumetric flask with water.

2.4.3 Preparation of 2M hydrochloric acid (HCl).

To prepare 2M HCl, 0.689 ml of the acid was pipetted into a beaker containing 500 ml of distilled water. It was then transferred to a 1000ml volumetric flask and the volume was made up to the mark with distilled water.

2.4.4 Preparation of nitrite solution

Nitrite solution was prepared by dissolving 0.1500 g sodium nitrite in water and diluting to 100 ml.

2.4.5 Preparation of 0.5% methyl anthranilate

To prepare this, 0.5 ml of methyl anthranilate was pipetted into a 100 ml volumetric flask and made up to the mark with ethanol.

2.4.6 Preparation of 2M sodium hydroxide (NaOH)

To prepare 2M NaOH, 80 grams of NaOH was dissolved in distilled water and was made up to the 1000 ml mark on the volumetric flask.

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 NITRITE

The determination of nitrite is based on the spectrophotometric method by Badiadka & Kenchaiah (2009).

PRINCIPLE:

Sulfanilic acid was diazotized in acidic medium and coupled with methyl anthranilate to give a colored dye having absorption maximum at 493 nm. Determination of nitrjte is based on the reduction of nitrate to nitrite in the presence of Zn/NaCl. The produced nitrite is subsequently diazotized with sulfanilic acid and then coupled with methylanthranilate to form an azo dye and was measured at 493 nm.

PROCEDURE:

1 gram of the soil samples was weighed into different test tubes and 10 ml of 0.5M Na_2CO_3 was added into the test tubes and shaked for about 2 minutes and allowed to settle. About 5ml of the upper layer was measured into the centrifuge tubes and centrifuged at 1000 rpm for 5 minutes. 1 ml of the aliquot (the supernatant) was pipetted into test tubes and 1 ml of 0.5% sulfanilic acid was added to the test tubes. Then 1 ml of 2M hydrochloric acid was added to it and was shaked very well for about 5 minutes to allow for diazotization reaction to complete. After that, 1 ml of 0.5% methyl anthranilate and 2 ml of 2M sodium hydroxide solution were added to form an azo dye and the contents were diluted to 10 ml using water. After dilution to 10 ml with water, absorbance of the red colored dye was measured at 493 nm.

The standard (blank) was prepared by pipetting 0ml, 1ml, 2ml, 3ml, 4ml, 5ml, 6ml, 7ml, 8ml and 9ml of nitrite solution into test tubes and diluting each to 10 ml using distilled 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 NITRITE CONCENTRATION IN THE SOIL SAMPLES

Table 2. Result of nitrite concentration in the soil samples.

	1 km from site		500m from site		School compound			
Sample	Week 1	Week 4	Week 1	Week 4	Week 1	Week 4	Week 1	Week 4
	Nitrite concentration (mg/L)							
HDSB	5.921 ± 0.817	8.980 ± 1.239	2.392 ± 0.330	6.078 ± 0.839	1.294 ± 0.179	2.235 ± 0.308		
NDSB	5.725 ± 0.790	10.549 ± 1.456	1.803 ± 0.249	4.666 ± 0.644	1.843 ± 0.254	2.902 ± 0.400		
LDSB	3.215 ± 0.443	7.607 ± 1.050	2.156 ± 0.298	5.451 ± 0.752	0.902 ± 0.124	3.294 ± 0.455		
HDSF	4.470± 0.617	9.176 ± 1.266	4.352 ± 0.601	5.764 ± 0.795	0.823 ± 0.114	2.000 ± 0.276		
NDSF	2.392 ± 0.330	8.196 ± 1.131	2.235 ± 0.308	2.529 ± 0.211	0.901 ± 0.124	2.588 ± 0.357		
LDSF	2.196± 0.303	10.941 ± 1.510	1.803 ± 0.249	5.098 ± 0.704	1.294 ± 0.179	2.509 ± 0.346		
CONTROL	2.313 ± 0.319	7.921 ± 1.093	1.798 ± 0.152	2.352 ± 0.325	1.176 ± 0.162	2.627 ± 0.362		
Point of Discharge, Dry Syrup							1.647 ± 0.227	1.545 ± 0.379
Point of Discharge, Liquid Syrup							0.666 ± 0.092	0.545 ± 0.175

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 concentration of nitrite in the soil samples were shown in Table 2. The results were expressed as mean ± standard deviation. The concentration of nitrite was lower at the point of discharge, liquid syrup (0.666 ± 0.092 mg/L) than in other sampling points. There was an increase an increase in the concentrations of nitrite in the spiked soil samples and control (non-spiked soil samples) as well as in the sampling points when monitored within 28 days.

3.3 DISCUSSIONS

The pH result of the soil sample from the various sampling points 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 nitrite concentration in the soil samples showed that the concentration of nitrite in the soil sample obtained at the points of discharge and within the industrial site (1km and 0.5km away) were higher than the one obtained from the school compound. This may be attributed to the differences in the nature of the soils found Emene and FUNAI. There was a significant reduction in the concentration of nitrite between week 1 and week 4 for the soil samples obtained at the two points of discharge. In contrast to the above statement, the concentration of nitrite in the soil samples spiked with Barbiclox and Flu-J at different doses in 1km away from site, 0.5km away from site and from school compound had a significant increase. The concentration of nitrite in the soil sample at the point of discharge, liquid syrup was very low compared to the samples at the other points. The increase in the concentration of nitrite in the soil sample spiked with Barbiclox and Flu-J when monitored between week 1 and 4 may be as a result of the presence of nitrite component in the active pharmaceutical ingredient. It was observed that the farther the distance away from the discharge points, the higher the concentration of the soil samples. The concentration of nitrite was found to be higher at the point of discharge, dry syrup than at the point of discharge, liquid syrup. This may be because, as earlier stated, the active pharmaceutical ingredients used in the production of antibiotics are a good source of nitrite components.

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 nitrite 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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