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EFFECT OF PHARMACEUTICAL EFFLUENT ON SOIL DEHYDROGENASE ACTIVITY

Ngwoke Stephen Ifeanyichukwu[1], Ichu Bright Chigozie[2], Hajara Musa[3] and Ozor Gerald Ogochukwu[4]

[1] Department of Material and Energy Technology, Project Development Institute (PRODA), Enugu, Nigeria. stevengwoke93@gmail.com[1]
[2] Department of Material and Energy Technology, Project Development Institute (PRODA) Enugu, Nigeria. brizeditor@gmail.com[2]
[3] Department of Material and Energy Technology, Project Development Institute (PRODA), Enugu, Nigeria. hajjomusa.hm@gmail.com[3]
[4] Department of Industrial and Medicinal Chemistry, David Umahi Federal University of Health Sciences, Uburu, Nigeria.
ozorgeraldogochukwu33@gmail.com[4]

ABSTRACT :

Soil is an important natural resource like water and air and most of the metabolic processes that exist in the soil are catalyzed by enzymes. Soil enzymes play a major role in the maintenance of soil ecology, chemical and physical properties, soil health and key biochemical function in the overall process of organic matter decomposition in the soil system. Enzymes in polluted soils are usually less active due to their exposure to environmental pollutants such as pharmaceutical effluents. This study is aimed at determining the effect of effluent from JUHEL pharmaceutical company on the activity of soil dehydrogenase around Emene industrial area in Enugu. The activity of soil dehydrogenase was determined using Spectrophotometric method. The results showed that the dehydrogenase activity was low at the point of liquid syrup effluent discharge (1.800 ± 1.040 x10-5 mol/L/hr) when compared with other points. The dehydrogenase activity decreased when a fresh soil samples were spiked at different concentrations with drugs (Barbiclox and Flu-j syrup) and monitored within 14 days. These results showed that the discharge of this pharmaceutical effluent on the soil decreases the activity of soil dehydrogenase. 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, Antibiotics resistance strain, Soil, Environmental pollution, Dehydrogenase, Spectrophotometer.

1. INTRODUCTION:

Pharmaceutical effluents are wastes generated by pharmaceutical industries during the process of drug manufacturing (Idris *et al*., 2013). Their risk to human health and environmental species cannot be overstated (Chimezie *et al*., 2011). In developing countries like Nigeria, the increase in demand for pharmaceutical has resulted in a consequent increase in pharmaceutical manufacturing companies in the countries and hence increased pharmaceutical waste which most times contain substantial amount of heavy metals. Some of the most representative pharmaceutical product found in the environment include antibiotics, lipid regulators anti-inflammatories, tranquilizers, antioxidants, etc (Lateef, 2004). These effluents are usually discharged into the environment and when they are not properly handled and disposed, they affect both human health and the environment (Osaigbovo & Orhue, 2006). Most pharmaceutical effluents are known to contain varying concentrations of organic compound and heavy metals such as mercury, nickel, chromium, etc which, when discharged from pharmaceutical industries affect the environment (Foess & Ericson,1980). As a result of the carcinogenic as well as mutagenic properties of heavy metals, much attention have been given to them since they have direct exposure to human and other organisms (Momodu & Anyakara,2010). Therefore, the discharge of these effluents constitutes biohazard to man and other living organism in the environment because they contain toxic substances detrimental to health (Adebisi *et al*., 2007).

Soil enzymes are group of enzymes whose usual inhabitants are the soil. They play an important role in maintaining soil ecology, physical and chemical properties, fertility and soil health and also play key biochemical functions in the overall process of organic matter decomposition in the soil systems (Sinsabagh *et al*., 1991). Soil enzymes increase the rate at which plants residues decompose and release plant available nutrients. There are lots of enzymes in soil environment such as the oxidoreductases, hydrolases, isomerases, ligases and lyases and each of them play key biochemical roles in the overall process of energy and material conversion (Gu *et al*., 2009).

Soil dehydrogenases (EC 1.1.1.1) are the major representatives of the oxidoreductase enzymes class and among all enzymes in the soil environment, dehydrogenases are one of the most important, and are used as an indicator of overall soil microbial activity (Salazer *et al.*, 2011). This is because they occur intracellularly in all living microbial cells (Yuan & Yue, 2012). Dehydrogenases are tightly linked with microbial oxidoreduction processes and more importantly, they do not accumulate extracellularly in the soil (Moeskop *et al*., 2010). Dehydrogenases play a key role in the biological oxidation of organic matter by transferring hydrogen (proton and electron) from organic substrate to inorganic acceptors (Zhang *et al*., 2010). Many specific dehydrogenases act by transferring hydrogen to either nicotinamide adenine dinucleotide (NAD^+) or nicotinamide adenine dinucleotide phosphate

($NADP^+$) (Subhani *et al*., 2001). Throughout mentioned co-enzymes, hydrogen atoms are involved in the reductive processes of biosynthesis and as a result of this, the overall dehydrogenase activity of the soil depend on the activities of various dehydrogenases, which are fundamental part of the enzyme system of all living microorganisms, like enzymes of the respiratory metabolism, the citrate cycle as well as nitrogen metabolism (Subhani *et al*., 2001). Therefore, dehydrogenase serves as an indicator of the microbiological redox systems and could be considered a good and adequate measure of microbial oxidative activities in soil. Active dehydrogenases can utilize both oxygen and other compounds as terminal electron acceptors, although anaerobic microorganisms produce most dehydrogenases (Brzezinka *et al*., 2007). As a result of these, dehydrogenases reflect metabolic ability of the soil and its activity is considered to be proportional to the biomass of the microorganisms in soil.
This study is aimed at examining the effect of pharmaceutical effluents on soil dehydrogenase activity

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: 2,3,5-triphenyl tetrazolium chloride (TTC) (Hopkins and Chadwell health essex, England), Ethanol, Buffer solution (Na_2HPO_4), Formazan 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 REAGENT

2.4.1 Preparation of 3% aqueous (w/v) 2,3,5-triphenyl tetrazolium chloride (TTC):
This was prepared by weighing out 3 grams of 2,3,5-triphenyl tetrazolium chloride and dissolving it in phosphate buffer and then made up to 100ml mark on volumetric flask with the buffer.

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 DEHYDROGENASE ACTIVITY

Dehydrogenase activity was determined using the method described by Tabatabai (1982).

PRINCIPLE:

Dehydrogenases converts 2,3,5-triphenyl tetrazolium chloride to formazan. The absorbance of formazan was read spectrophotometrically at 485 nm.

PROCEDURE:

0.5 g of sieved soil was weighed and placed in test tubes, mixed with 0.5ml of 3% aqueous (w/v) 2,3,5-triphenyl tetrazolium chloride and stirred with a glass rod. After 96 hours (4 days) of incubation (27^{o}C), 10 ml of ethanol was added to each test tube and the suspension was vortexed for 30 seconds. The tubes were then incubated for 1 hour to allow suspended soil to settle. The resulting supernatant (about 2.5 ml) was carefully transferred to clean test tubes using pipettes. Absorbance was read spectrophotometrically at 485nm. The concentration of formazan was evaluated using extinction coefficient of 15433Mol cm $^{-1}$ (Dushoff *et al*., 1965).

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 DEHYDROGENASE ACTIVITY IN THE SOIL SAMPLES

Table 2. Result of dehydrogenase activity in the soil samples

	1 km from site		500m from site		School compound		Discharge Point	
	Week 1	Week 2	Week 1	Week 2	Week 1	Week 2	Week 1	Week 2
Sample	Dehydrogenase Activity (x10-5 mol/L/hr)							
HDSB	4.300± 0.559	1.200 ± 0.156	1.700 ± 0.221	1.100 ± 0.143	1.400 ± 0.052	0.600 ± 0.078		
NDSB	2.900 ± 0.377	2.200 ± 0.286	1.300 ± 0.169	0.600 ± 0.078	1.900 ± 0.247	1.000 ± 0.130		
LDSB	2.600 ± 0.338	2.100 ± 0.273	1.100 ± 0.143	2.700 ± 0.351	1.100 ± 0.143	0.600 ± 0.078		
HDSF	3.600 ± 0.468	2.500 ± 0.325	1.600 ± 0.208	0.500 ± 0.065	0.700 ± 0.091	0.600 ± 0.377		
NDSF	2.000 ± 0.260	1.700 ± 0.221	1.200 ± 0.156	0.900 ± 0.117	0.600 ± 0.078	0.500 ± 0.104		
LDSF	1.400 ± 0.182	0.900 ± 0.117	1.100 ± 0.195	1.000 ± 0.130	0.500 ± 0.065	0.400 ± 0.078		
Control	2.000 ± 0.260	1.100 ± 0.143	1.930 ± 0.169	0.600 ± 0.078	1.910 ± 0.052	1.100 ± 0.143		
Point of Discharge, Dry Syrup							1.900 ± 0.247	1.900 ± 0.247
Point of Discharge, Liquid Syrup							1.800 ± 1.040	1.700 ± 1.014

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 result of the dehydrogenase activity in the soil samples were shown in Table 2 above. The results were expressed as mean± standard deviation. The dehydrogenase activity was shown to be very low at the point of discharge, liquid syrup ($1.800 \pm 1.040 \times 10^{-5}$ mol/L/hr). The activity of soil dehydrogenase in all samples showed a decrease when monitored within 14 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 ($C_{19}H_{19}ClN_3NaO_6S$) as its active pharmaceutical ingredients and is therefore, a potential source of N, Na and S to the effluents.

The result of the dehydrogenase activity in the soil samples showed that the activity of the enzyme was low at the point of discharge, liquid syrup compared to the other points. This could be attributed to the fact that the pharmaceutical effluent tends to inhibit the action of this enzyme by reducing the activities of microorganisms in the contaminated surrounding. Also the activity of the dehydrogenase in the spiked soil samples showed a decrease when monitored in week 1 and 2. The dehydrogenase activity in the spiked soil samples at different doses was higher on the soil samples spiked with Barbiclox dry syrup than on the ones spiked with Flu-j liquid syrup. This suggests that the pharmaceutical effluents containing liquid syrup has higher inhibitory effect on soil dehydrogenase than the one containing the dry syrup. It was also observed that as the distance away from the points of

discharge increases, the activity of the enzyme increases. The activity of soil dehydrogenase in the spiked soil samples was observed to increase as the doses of the drugs increase. The was a significant differences in the activity of soil dehydrogenase in the soil sample obtained from school compound and the other sampling points and this may be attributed to the differences in the nature of the soil.

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 activity of soil dehydrogenases 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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