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Effect of pharmaceutical effluent on soil phosphatase, dehydrogenase and urease activities: linking ecotoxicity to soil infertility

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ABSTRACT:

There are different ways of affecting the soil micro-flora, starting from the crude oil spill to industrial effluent. This scenario usually impacts negatively on plant growth indices, with the assumption that the soil fertility is impacted in a negative direction. Special attention is given to the effect of medicament effluent on the soil microflora or enzymes. The discharge of pharmaceutical waste is often accompanied with antimicrobial, antifungal and antiviral agents. In this study, effects of pharmaceutical effluents on the activities of some soil enzymes (*in situ*) - phosphatases, dehydrogenases and ureases were determined using spectrophotometric technique and other standard methods. Samples were collected from the point of discharge. The uncontaminated soil samples were spiked with pharmaceutical effluents. After thirty days, the soil samples were assayed for soil enzymes activities. The results showed significant de-regulation in activities of phosphatases, dehydrogenases and ureases on the effluents contaminated soil samples compared to the control. The soil obtained outside at the industrial site did not show a decrease in the urease activity. Comparing the results with the control samples, the present investigation suggests that industrial effluents if not treated before discharged may cause disruption and destruction of some soil enzymes.

Keywords:

Pharmaceutical effluent, Soil enzymes, Soil pollution, Soil ecosystem and Infertility.

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INTRODUCTION

Far back sixteenth century, Paracelsus recognized that "dosage makes the poison". Environmental concentration of pharmaceutical metabolites excreted by people is constrained on the grounds that a characterized portion is administered to a small amount of the populace. Paradoxically, ongoing examinations have recognized direct discharges from drug manufacturers to frame a source of a lot higher ecological discharges that surpasses toxic threshold some times.

. Production is concentrated in specific locations, the risks are not linked to usage patterns as per the standards. The ecological risks related with the manufacturing of drugs involved a more extensive set of therapies related with those dangers from excretions. Despite the fact that pollution from manufacturing is less broad, discharges that enhance the improvement of drug-resistant microbes can at present have worldwide consequences (Larsson, 2014). The discharge of untreated pharmaceutical effluents into agricultural farmlands have been linked with a negative effect on soil enzymes. This constituted the major problem faced by industries, due to the generation of the high volume of effluents, coupled with limited space for land-based treatment and discharge. It's influence on the soils and crops growth indices became soil pollution issue to individuals, when the irritant is capable of exerting adverse effects on the soil, thereby deregulating its fertility potentials (Kumar and Chopra, 2010).

Pharmaceutical industries are important in health and economy development of any nation. Effluents released from pharmaceutical industries constitute a higher degree of organic pollution in both aquatic and terrestrial ecosystems, which alter the physicochemical properties of the receiving cell or organisms and affect soil ecosystem (Kumar and Chopra, 2010). Research has implicated pharmaceutical pollutants in the soil microbial community (Nagaraju *et al.,* 2007). The discharge of industrial effluents especially without treatment may have profound effects on soil enzymes, physicochemical and biological properties (Aishwarya *et al.,* 2014). Soil enzymes play functional roles in catalyzing reactions associated with organic matter and decomposition (Sinsabaugh, 1994). Osaigbovo and Orhue (2006) asserts that pharmaceutical effluent had an effect on the soil chemical properties and maize growth. Soil enzyme activities have a direct expression of soil community to metabolize available nutrients.

The diversity of soil organisms, the capacity of soil microbial communities to maintain functional diversity is important. Additionally, stress or succession may at last be essential to eco-system efficiency and steadiness than taxonomic diversity (Caldwell, 2005). Sulaiman *et al.* (2015) reported the soil enzymatic activities and microbial biomass carbon (comic) as two important soil biological activities influenced by contamination taking place in the soil ecosystem. Because of the complex drug combinations and other synthetic pollutants in nature, it progresses toward becoming errand task to set up a clear link between specific ecological drug contamination and unfavorable consequences on the soil environment .

However, exposure experiments with different drugs have been investigated. For instance, exposure studies recommended that naturally relevant concentrations of levonorgestrel and oxazepam influence fish reproduction and behavior (Zeilinger *et al*., 2009; Brodin *et al*., 2013). This sort of exposure studies might be valuable for expanding the knowledge about the impacts of ecological pollution, to distinguish substances of particular environmental concern. This study focused on changes in the selected soil enzyme activities as a result of the potential inhibitory effects of soil contaminated by pharmaceutical effluents.

Project site

Nemel pharmaceutical industry in Emene

Enugu, Enugu State Nigeria is an industrial layout with a scattered settlement. It is located on 6' 25" North and 7'30' East. The Emene area has a population of 1,00,000 persons. The soil sample was collected from this site, 15 cm beneath the earth using soil auger.

MATERIALS AND METHODS

Test sample

The pharmaceutical effluent was collected on January 10th, 2016 from the industrial plant discharge point at Nemel pharmaceutical company, Enugu, Nigeria.

Collection of effluent and soil samples

The raw effluents were collected at the point of discharge from a pharmaceutical company at Enugu Nigeria. The company produces analgesics, anti-malarial drugs, multivitamins, antibiotics and other lines of drugs. Sample collection was with a two-liter plastic container rinsed with deionized water. Before use, the container was rinsed twice with the sample. The pH of the sample was determined at the point of collection. The sample was labeled and transported to the laboratory where it was refrigerated at 4°C until the commencement of analysis. Contaminations were checked by running blanks of all determinations. Two different soil samples were collected from the pharmaceutical industry and within the surroundings of the company. The soil from the effluent pit was collected with a plastic container to prevent contamination with other metals 15 cm deep. The second soil sample was collected from a distance 1 km away from the effluent pit at 15 cm depth.

Assay of soil enzymes activity

Dehydrogenases activity was determined using the method of Tabatabai (1982). Soil urease activity was determined according to the spectrophotometric method of Brosdbent *et al.* (1958). Soil phosphatase activity was determined according to the method of Tabatabai and Bremner (1969).

Statistical analysis

Results are expressed in mean± SD at ($P<0.05$), using one-way and two-way analysis of variance (ANOVA). The data obtained were analyzed using Statistical Package for Social Sciences (SPSS), version 8 (SPSS, 2017).

RESULTS AND DISCUSSION

Contamination of soils is a particularly serious

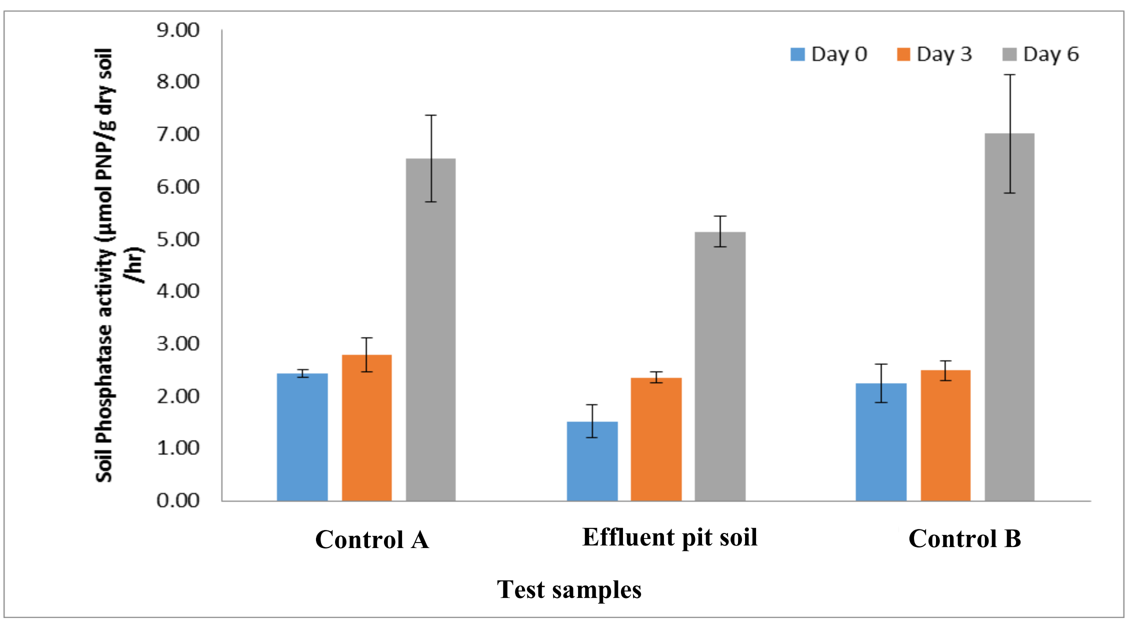

Figure 1. Phosphatase activity in the soil

problem because of the impact it exerts on soil functioning, and on the ecosystem. Though some contamination is due to natural processes (e.g. volcanic eruptions and weathering of the soil parent materials), many others are generated by the daily human activity, such as industrial processes, transportation, construction, uncontrolled discharges of effluents, waste generation and agriculture. Agricultural soils which are continually exploited to produce food and fodder are sensitive to contamination. The filtering and degrading functions of soils enzymes are intensive and irreversibly modified (Diana *et al.,* 2014). Agricultural soil enzymes usually show poor resilience, *i.e.* they are incapable of recovering from any type of aggression, insult and other types of contamination that they suffer and may probably lead to their complete degradation.

Soil phosphatase activity presented in Figure 1 shows an increase in phosphatase activity in all the soil samples with a sharp and significant ($P<0.05$) increase from day three to day six. It was observed that the phosphatase activity in the effluent pit sediment was significantly ($P<0.05$) low compared to control A (control soil) and B (control treated soil).

The most outstanding finding with regard to soil contamination with chlorophenols is the decrease and vanishing of dehydrogenase activity. This impact has been seen by many scientists, even in soils with very different physical and chemical characteristics (Bello *et al*., 2008; Diez *et al*., 2006; Bello *et al*., 2013). Dehydrogenase is an oxidoreductase catalyst which acts intracellularly and influences the microbial activity (Skujins and Burns, 1976; Nannipieri, 1994). The solid decrease in activity, in this way, mirrors the death of a few or the majority of the soil microorganisms. The mortality is related with the toxicity of chlorophenols to the edaphic microbiota (Scelza *et al*., 2008), brought about by the presence of chlorophenol anion. This anion is produced by the dissociation of chlorophenol. Because of its negative charge and small size, it goes through cell membranes into the cytoplasm to cause cell death (Packham *et al*., 1982). Soil dehydrogenase activity is shown in Figure 2. The dehydrogenase action decreased with time. This decrease was significant ($P<0.05$), concerning the control group without

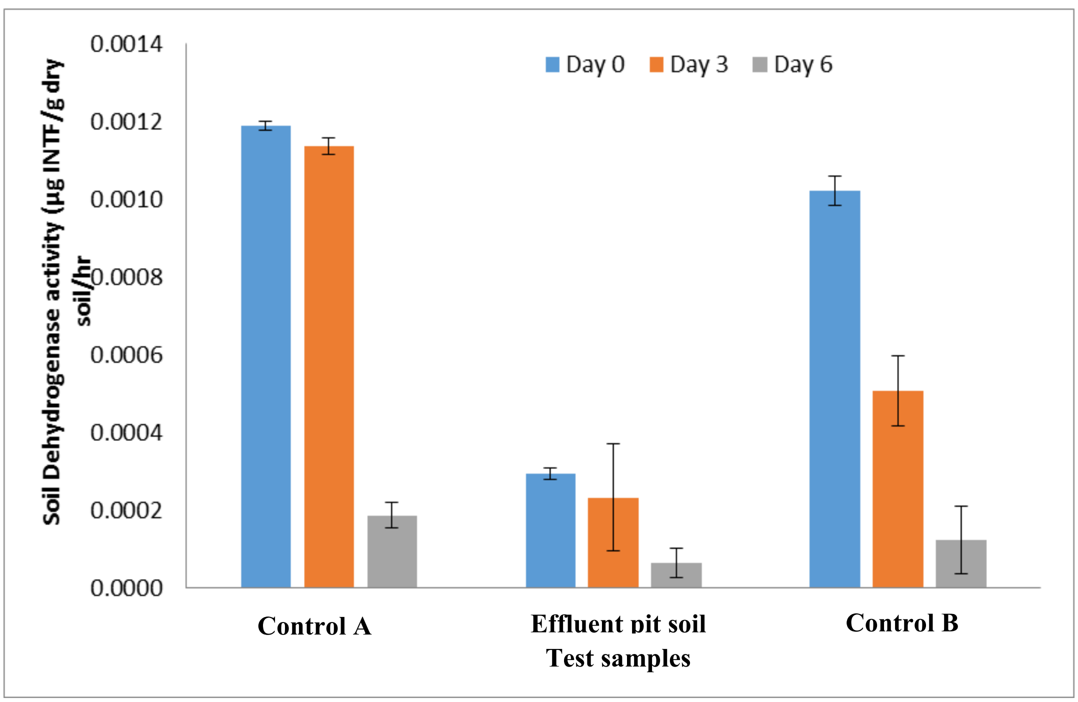

Figure 2. Dehydrogenase activity in the soil

treatment. The effluent pit sediment demonstrated the most low action for dehydrogenase compared with the controls while the control A had a higher dehydrogenase activity contrasted with control B .

In addition to the pharmaceutical effluents, other Persistent Organic Pollutants (POPs) can occur in soils (both agricultural and natural soils) in significant amounts; these include phenolic compounds, mainly chlorophenols, and Polycyclic Aromatic Hydrocarbons (PAHs) (Diana *et al.,* 2014). Both are stable groups of compounds that persist in the environment due to their structural properties. Many of these compounds are volatile and do circulate widely *via.* a process known as the "grasshopper effect".

Once released repeatedly into the soil, processes such as evaporation and deposition of these compounds are transported to far distances through the atmosphere (Rao *et al*., 2014). The recalcitrant nature of these compounds enables them to accumulate in living organisms and later reach humans through the food chain and cause health effect (environmental recharging). Figure 3 represents the soil urease activity. The pattern of enzyme activity was not consistent. However, the urease activity was shown to be highest in the effluent sediment at day three. There were no statistical differences when compared with other soil treatments. In the control A, there was a decrease in urease activity which latter increase on the day six. The same was observed in the control B. But, on the contrary, the urease activity increased in the effluent pit soil from day zero to day three but decreased by day six. The observed changes in mean in the different group were not statistically significant (P>0.05).

Results of urease activity in the soil showed that the effluents' pit soil has low phosphatase activity (Figure 1); this may be due to low available phosphorus concentration in the soil. We can infer that the effluent has negative effects on the enzymes activities in the soil. Soil extractable phosphorous was significantly (P< 0.05) related to acid phosphatase activity in the arid soils (Sardans *et al.,* 2008) because acid phosphatase activity is associated with soil organic phosphorous mobilization (Conn and Dighton, 2000; Dick *et al.,* 2000). Soil dehydrogenase test indicated total microbial activity in the soil because an increase in soil microbial biomass concentration increases dehydrogenase activity (Chu *et al.,* 2007). The result in figure 2 showed that dehydrogenase activity is very low in the effluent pit sediment and its activity decrease with time. The dehydrogenase activity in the control is found to be

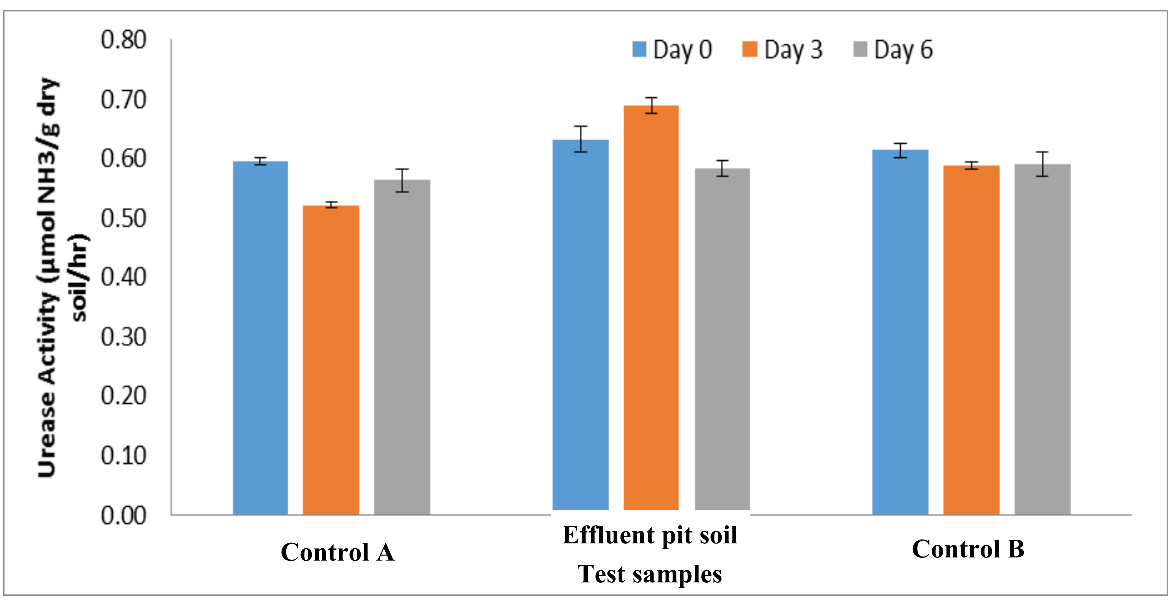

Figure 3. Urease activity in soil

higher compared to effluents.

The chart in Figure 3 represents urease activity in soil. The pharmaceutical effluent did not affect the urease activity of the three soil samples examined. This is reflected in the percentage of nitrogen found in the soil. Soil urease hydrolysis urea to release NH_3^+ and altered urease activity may increase NH_3 loss in the soil (Haynes and Williams, 1999; Singh and Kumar, 2008). Though, urease is an indicator of plant nitrogen availability in different environments (He *et al*., 2010), results of the correlation showed that, when urease concentration was increasing, dehydrogenase and phosphatase activities decrease. While dehydrogenase activity was increasing, the toxic effects of contaminants in the soil are greatly affected by the reciprocal synergism fractions of both organic and inorganic substances, which favor sorption processes and removal of the contaminant from the soil. These properties are diminished in the agricultural soils, relative to natural soils, as a result of pollution. Agricultural soils are sensitive to contamination, indicating the urgent need for reliable indicators of degradation. Although soil enzymes are suitable contamination indicators, while pharmaceutical effluents are applicable to surrogate markers, therefore methods of determining enzyme activities require high throughput technology to enable researchers to select the best indicator (s).

CONCLUSION

The effects of pharmaceutical effluents on the activities of some soil enzymes are profound. This investigation revealed that the effluents had a negative effect on the enzyme activities. The decrease in the activities of the soil enzymes are indications of effluents effects. They either deregulate the soil microbial load or have directly inhibited some enzymes that are involved in mineral cycling in the soil. We suggest that untreated effluents should not be discharged into farmlands and environment.

CONFLICT OF INTEREST

The author (s) hereby declare that no research or financial conflict of interest exists on this manuscript.

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