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# Effect of enriched sewage sludge on soil urease activity

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## Abstract

*Current increases in producing considerable amount of organic matter such as, sewage sludge has resulted in their application on agricultural lands. Soil biological and biochemical properties are influenced by organic matter application. The effect of nitrogen and phosphorus enriched sewage sludge on urease activity was investigated in this study. Different rates of sewage sludge (0, 200 and 350 t ha$^{-1}$) were enriched using nitrogen (0, 170 and 250 kg ha$^{-1}$ in the form of urea) and phosphorus (0 and 150 kg ha$^{-1}$ in the form of potassium phosphate) fertilizers. This experiment was performed using a factorial model in completely randomized design. Prepared soil samples were incubated at 25 $^{o}$C and 70% of water holding capacity. Urease activity was measured colorimetrically. The results showed that application of sewage sludge and nitrogen fertilizer increased urease activity markedly within the first 15 days of incubation, although its activity dropped down sharply by the end of the experiment. Also, the results of phosphorus application showed a decrease in enzyme activity. In conclusion increasing time decreased the available substrate for enzyme activity and resulted in the reduction of enzyme activity reduction.*

**Key words:** Urease activity, nitrogen, phosphorus, sewage sludge

## Introduction

Decomposition in the soil is nature's way to recycle the organic products of biological activity. As long as population density remained sparse, waste products of human activity could be similarly accommodated. The growth of cities and industries, however, produced quantities of solid and liquid wastes in excess of the soil's ability to reprocess in the immediated domain of human habitation. Some societies (notably in China and other parts of Asia) continued to transport human wastes from cities to agricultural land for the purpose of fertilizing crops and replenishing soil nutrients. In many other places, the uncontrolled discharge of sewage ended up polluting streams and wells, to threat the environment and public health. With the advent of integrated sewerage systems in the European cities at the beginning of the modern age, interest in land application increased (Schuval *et al.*, 1986). Interest in the disposal of sewage sludge, which contain a range of valuable nutrients (N, P, Fe, Ca, Mg) and various essential macro and micro nutrients for plant growth on agricultural land has increased during the last decades (Singh 1998). Most papers concerned with the results of sewage sludge studies deal with the influence of sewage sludge on soil biological and enzymatic activity. Soil enzymes undoubtedly perform critical functions to the cycling of nutrients. This would eliminate problems such as seasonal changes and inherent differences on activities to be early indicators of management-induced changes in the soil. Konig *et al.* (1966) observed a decrease of soil biological activity such as microbial biomass and enzyme activities, due to sewage sludge application. Conversely, (Sastre *et al*., 1996; Banjeree *et al*., 1999) found that the sewage sludge amendment increased soil enzyme activities. Soil enzymes play an essential role in catalyzing reactions necessary for the decomposition of organic matter and nutrient cycling in ecosystems, involving a range of plants, microorganisms, animals and their debris (Johansson *et al*., 2000). Therefore, changes in enzymes could alter the availability of nutrients for plant uptake and these changes are potentially sensitive indicators of soil quality (Ajwa *et al*., 1999; Albiach *et al*., 2000). Dick and Tabatabai (1992) expressed that measurements of several enzymatic activities have been used to establish indices of soil biological activity. Enzymes in soil play a dominant role in transformation of organically bound nutrients into inorganic plant available forms (Speir and Ross, 1975; Skujins 1976; Stevenson, 1986).

In the present study, soil enzyme representative of the nitrogen cycle was selected. Urease is a constitutive intracellular enzyme with three subunits of α, ß and γ and two nickel ions. Urease catalyzes the hydrolysis of urea and amides to carbon dioxide and ammonia. It acts on carbon-nitrogen (C-N) bounds other than peptide linkage (Bremner and Mulvaney, 1978; Karaca *et al*., 1999). Application of organic wastes such as sewage sludge as a source of organic matter is a common practice, especially to soils containing little organic matter, to maintain or improve soil quality and soil enzymatic activities (Giusquiani *et al*., 1995). The objective of this study was to study the effect of different application rates of sewage sludge, nitrogen and phosphorus

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on Urease activity and available metals (Zn and Pb) in a calcareous soil under laboratory conditions.

## Materials and Methods

The soil sample was collected from experimental field, located in Mashhad,, North-East of Iran. The soil was classified as *Typic Haplocambids*. Sampling site's climate is aridic; mean annual rainfall and temperature are 300 mm and 25 °C, respectively. It contained 36.5% clay, 28.5% silt and 35% sand. The homogenized soil sample was sieved to 2 mm and stored at 4 °C until used. Dried sewage sludge was obtained from central waste water treatment located in Mashhad city. It was sieved to less than 2 mm and stored in polyethylene bags at the laboratory temperature until used. The amount of bicarbonate-extractable inorganic phosphorus was determined colorimetrically after the extraction of soil (Olsen and Sommers, 1982). Organic matter was analyzed by Wlakley-Black (1934) method. Nitrogen was determined by Kjeldahl method. The content of some metals of interest is given in Table 1. This experiment was performed using a factorial model, with three rates of sewage sludge (0, ($S_0$), 200 ($S_{200}$), and 300 ($S_{350}$) t $ha^{-1}$), three rates of nitrogen in the form of urea [0 ($N_0$), 170 ($N_1$) and 250 ($N_2$) kg $ha^{-1}$] and two rates of phosphorus in the form of potassium phosphate [0 ($P_0$) and 150 ($P_1$) kg $ha^{-1}$] in completely randomized design with three replications. Treated sewage sludge-amended soils were moistened to 70% soil water holding capacity and incubated for 90 days at 25°C. The moisture content was maintained through out the experiment. Sub-samples were removed at time intervals of 0, 15, 30, 45, 60 and 90 days to determine the changes of Urease activity. Urease (EC 3.5.1.5) activity was measured using Hoffmann and Teicher (1961) method. Citrate buffer (0.75 mL) of pH=6.7, 1 mL of 10% urea substrate solution and 0.25 mL toluene were added to 1 g sample and the samples were incubated for 3 h at 37° C. The formation of ammonium was determined spectrophotometrically at 578 nm and the results were expressed as µg $NH_4^+$ $g^{-1}$ dry soil. The availability of Zinc and Lead in soil samples were determined using DTPA solution (0.005µ DTPA+ 0.01 µ TEA buffered at pH=7.3) according to Lindsay and Norvell (1978). The data were analyzed using MSTAC statistics software and the means were compared using least significant difference (LSD) test ($P<0.05$).

## Results and Discussion

Physico-chemical properties and available heavy metals content of soil and sewage sludge are given in Table 2. Different rates of sewage sludge significantly affected the levels and distribution of available (DTPA-extractable) Zn and Pb in the soil when compared with the control treatment (Figure 1, 2). The figures express that the Zn and Pb concentrations of high rates of sewage sludge application were higher at all sampling times. By increasing rate of sewage sludge application the amount of available Zn and Pb has increased drastically, also available amount of Zn and Pb increased in control treatments (blank) and it shows the existence of small amount of heavy metals in control treatment (Figures 1, 2). Doelman and Haanstra (1984) pointed out that organic wastes such as sewage sludge are the most important factors that control the availability of heavy metals in soil. Also, the concentration of Zn and Pb in the soil can be attributed to the high affinity of the metals to organic matter (Tyler and McBride, 1982; McGrath and Lane, 1989).

**Table 1. Available metal contents of the sewage sludge**

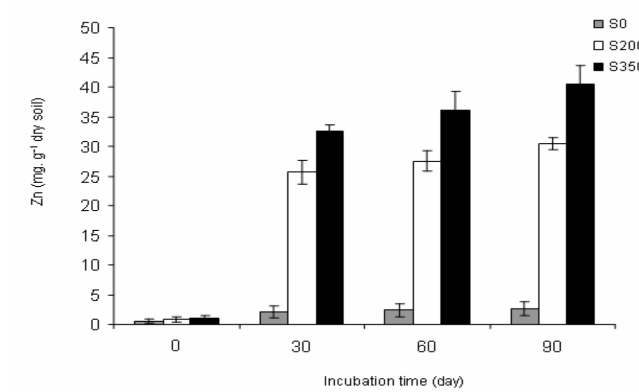
| Metals | Concentration (µg $g^{-1}$) |
|---|---|
| Ni | 29.3 |
| Pb | 45.8 |
| Cd | 4.2 |
| Zn | 66.5 |
| Cu | 19.4 |

**Table 2. Soil and sewage sludge properties**

| Property | Soil | Sewage sludge |
|---|---|---|
| Clay | 37.5% | - |
| Silt | 27.5% | - |
| Sand | 35% | - |
| pH | 7.5 | 7.32 |
| EC (1:5) | 1.8 dS $m^{-1}$ | 2.31 dS $m^{-1}$ |
| Organic carbon | 0.26% | 8.25% |
| Organic phosphorus | 64.8 mg $kg^{-1}$ | 0.17% |
| Total nitrogen | 77.69 mg $kg^{-1}$ | 0.55% |
| Potassium | 413 mg $kg^{-1}$ | 0.04% |
| Nickel† | - | 29.3 mg $kg^{-1}$ |
| Lead† | - | 45.8 mg $kg^{-1}$ |
| Cadmium† | - | 4.2 mg $kg^{-1}$ |
| Zinc† | - | 66.5 mg $kg^{-1}$ |
| Copper† | - | 19.4 mg $kg^{-1}$ |

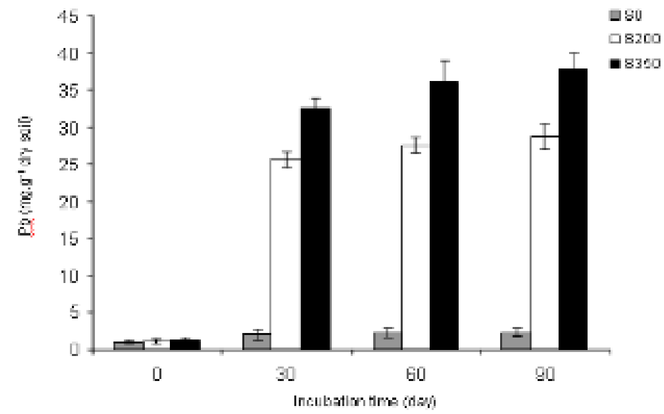
†available concentration

Urease activity was significantly ($p<0.05$) changed after the application of different rates of enriched sewage sludge during incubation period (Figure 3). Its activity increased rapidly and reached the maximum level after 15 days in sludge treated soils, although it remained constant in control treatment (Figure 3). The highest activity was in soil treated with the highest rate of sludge ($S_{350}$). After 15 days, its activity decreased significantly but the sludge-amended soil activity was higher than control treatment (Figure 3). However, more interestingly at 60 and 90 days urease activity of sludge-amended soil was lower than control treatment. The highest values of urease activity were observed in high rates of sludge application, therefore we can express it to urease characteristic as an intracellular enzyme and consequently it increases or decreases proportional to microbial biomass which would increase by high organic matter (Nannipieri, 1994). In general, urease activity can be divided in 2 stages, in the first stage its activity was dramatically upward and it was as a result of adding organic matter (sewage sludge) to the soil and the second stage lasting to the end of the incubation time in which a notable reduction in enzyme activity in soil treated with sludge was observed. For this stage we can express the effect of heavy metals in sewage sludge. Enzymatic activity diminished with increasing available concentration of metals (Tyler, 1974; Kizilkaya *et al*., 2004). Increased levels of heavy metals will react with sulphydral groups of enzymes causing inhibition or inactivation of the enzymatic activity (Nannipieri, 1994). Metals also indirectly affect soil enzymatic activities by altering the microbial community which synthesizes enzymes (Kandeler *et al.,* 1996). The rapid decomposition of organic matter which occurs after the application of sewage sludge to soil increases the proportion of available metals as a result of mineralization of organically complexed metals (Dudley *et al*., 1986). The organic matter-heavy metal fractions which are readily available for plant uptake, occur in organic matter and soil solutions. This would prevent the heavy metal from interacting directly with the active sites of enzyme, thus affecting the enzyme's activity (Doelman and Haanstra, 1984). There was a significant increase in urease activity within 15 days after the application of different rates of nitrogen, even though its activity diminished up to the end of the period noticeably (Figure 4). It is evident that nitrogen can have different effects on decomposition rates depending on its stage. The initial stage of decomposition will often be nitrogen limited and consequently addition of nitrogen will enhance the degradation rate and will cause an increase in enzyme activity. By increasing time the available substrate for enzyme activity was reducing and as a result its activity diminished gradually. Increasing levels of applied phosphorus reduced urease activity (Figure 5). On the whole, phosphorus application has affected urease activity and increased its activity within 15 days, although its activity dropped down by the end of the experiment in both trearments and control. Hass *et al.* (1992) demonstrated that application of phosphorus may cause an increase in inorganic phosphorus as an available substrate for microorganism's activity. Hence, its activity reduced by introducing phosphorus to the treatments.



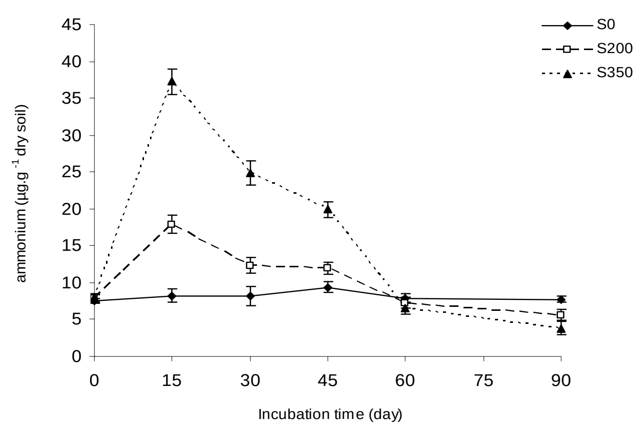

Vertical bar shows SE of means

**Figure 1. Sewage sludge effect on Zn availability**



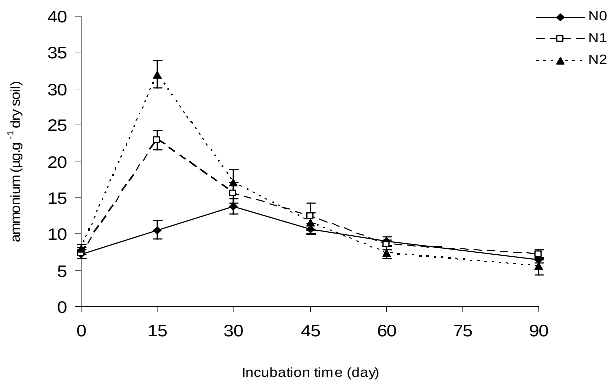

Vertical bar shows SE of means

**Figure 2. Sewage sludge effect on Pb availability**



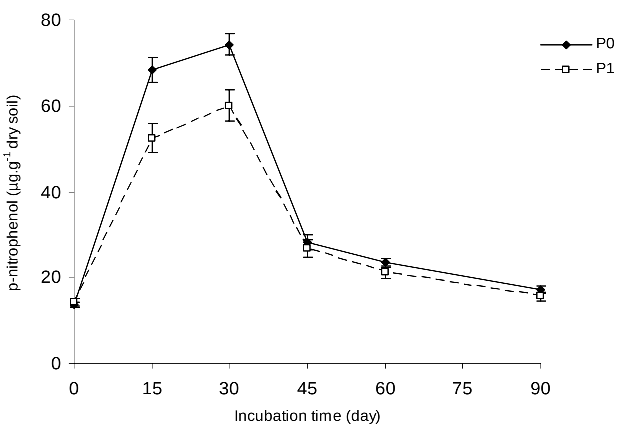

Vertical bar shows SE of means

**Figure 3. Sewage sludge effect on urease activity**




Vertical bar shows SE of means

**Figure 4. Nitrogen effect on urease activity**




Vertical bar shows SE of means

**Figure 5. phosphorus effect on urease activity**

## Conclusions

In this study considerable attention has been focused on estimating the fate of enzymatic activity in the enriched sludge-amended soil. Also, a suitable balance of nutrients (nitrogen and phosphorus) and organic matter is critical in any land management program. High amounts of nitrogen and phosphorus in the initial sludge or application of nitrogen and phosphorus in sludge-amended soil can result in enzyme activity reduction or inhibition. Our present study can not completely represent the relationship between the negative influence of available heavy metals and enzyme activitiy. Thus, further studies are needed to investigate their relationships.

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