



## Effects of Tetracycline on Soil Enzyme Activities in an Alluvial Soil

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### Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

### Article Information

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### ABSTRACT

**Aims:** To study the effect of various doses of tetracycline (TC), oxytetracycline (OTC) and chlortetracycline (CTC) on the enzymatic (DHA, acid phosphatase, alkaline phosphatase, urease and catalase) activity in sewage amended and un-amended alluvial soil.

**Methodology:** A laboratory incubation study was conducted during 2012-2013 on an Aligarh farm alluvial soil. The enzymatic activity was studied in presence of various doses of three tetracycline antibiotics in soil; soil amended with sewage sludge; and mixture of sewage sludge and tetracyclines at different time intervals [(0 (4h), 7, 14, 21, 35, 56, 70,91d].

**Results:** Activity of all the studied enzymes was significantly inhibited for up to 14-21 days of incubation (14 d for DHA and acid phosphatase, 21 d for alkaline phosphatase, urease and catalase) and thereafter inhibition got weaker. The activity of all the studied enzymes decreased with increase in the doses of tetracyclines. Higher enzymatic activity was observed in sewage sludge amended soil than in the un-amended soil. Tetracyclines in presence of sewage sludge were found to have no appreciable effect on enzymatic activities.

**Conclusions:** Dehydrogenase, acid and alkaline phosphatase, urease and catalase activity in tetracycline free soil was superior to soil containing antibiotics in the period of 2-3 weeks of incubation. The inhibition of soil enzyme activity was directly proportional to tetracycline concentration. In presence of sewage sludge, the studied enzymes activity initially increased up to

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3 weeks and decreased thereafter. In presence of sewage sludge and antibiotics, the activity of enzymes remains almost unchanged. The activity of studied enzymes in soil was positively correlated to soil organic content.

**Keywords:** Antibiotics; tetracycline; oxytetracycline; chlortetracycline; sewage sludge; soil enzyme activity.

## 1. INTRODUCTION

The most important function of agricultural land is considered to be its ability to serve as a habitat for plants, microorganisms and soil-living animals [1]. Soil enzyme activity is involved in nutrient cycling and availability of nutrients present in the soil and this may be used as an index of soil functioning [2]. Soil enzymes are essential not only for plant growth but are equally important for soil fertility too. Anthropogenic activities leading to intentional or unintentional deposition of contamination may be harmful to soil environment; they also affect the amount and activity of soil enzyme at different functional levels, and reduce the growth of plant and crop yield. Enzyme specificity is mostly dictated by the nature of the groups attached to the susceptible bonds. The total activity of enzymes in soil is composed of the activities of accumulated enzymes and enzymes of proliferating microorganisms. Pharmaceutical compounds are of potential concern as they are highly adsorbable and may get accumulated in the soil, sediments and in the tissues. In the past decade, pharmaceutical antibiotics, mainly tetracyclines were recognized as emerging soil pollutants. They reach agricultural land mostly through contaminated manure used as fertilizer and sewage effluent. Gu and Karthikeyan [3], during their study found that 80% of wastewater effluent contains tetracyclines (TCs). TCs are poorly adsorbed by humans and animals after intake; about 50-80% is eventually excreted as unmetabolised parent compounds into domestic sewage. In the past decade, a number of workers have found TC in surface and ground water [4,5]. Antibiotics use perturbs the composition of the micro biota. The excessive use of antibiotics caused an increase in antibiotic resistant pathogens. In the absence of appropriate microbial signals, the immune system does not develop normally which can predispose the host to infections [6].

The present experiment was planned to study the effect of various doses of tetracycline (TC), oxytetracycline (OTC) and chlortetracycline

(CTC) on the enzymatic (DHA, acid phosphatase, alkaline phosphatase, urease and catalase) activity in a sewage amended and unamended alkaline soil.

## 2. MATERIALS AND METHODS

The soil sample selected for this study was collected from a farm of Aligarh district at plough layer (0-30 cm). The soil sample was air dried at room temperature and passed through a 2mm sieve and analysed for physico-chemical properties. The pH and electrical conductivity (EC) (1:2.5 water) of the soil were 8.1 and 7.6dS  $m^{-1}$  respectively. Its cation exchange capacity (CEC)  $cmol(p^+) kg^{-1}$  and organic carbon were 83, 27.9gkg $^{-1}$  respectively. The soil contained 61% sand, 26% silt and 13% clay; the surface area of soil was 224  $m^2g^{-1}$ .

To study the effect of different doses of antibiotics, sewage sludge and their mixture on enzymatic activity, three experiments were conducted. In the first experiment, 1 kg of soil was fortified with 0, 5, 10 and 50 mg of antibiotics (in 5mL of methanol) (T<sub>1</sub>-T<sub>4</sub>) in several glass bottles separately. In the second experiment, 1 kg of soil was fortified with 0, 2.5 and 5 g of sewage sludge (T<sub>5</sub>-T<sub>7</sub>). In the third experiment, 1 kg of soil was fortified with 2.5 g of sewage sludge and 5, 10 and 50 mg of antibiotics (in 5mL of methanol) (T<sub>8</sub>-T<sub>10</sub>) in glass bottles separately. The studies were made at 60% of water retention capacity maintained on the basis of the initial soil analysis. Moisture was regularly maintained based on the difference between two consecutive days. Sample bottles were incubated at 30±2°C for 91 days. The soil samples were drawn at different time intervals [(0 (4h), 7, 14, 21, 35, 56, 70 and 91 d] after incubation and stored in plastic vials at 4°C to evaluate the enzyme activity.

*Dehydrogenase activity (DHA)* activity was measured by Casida et al. [7] method as: Air dried 20 g soil was mixed with 0.2 g of CaCO<sub>3</sub> and 6 g of this mixture was placed in each of the three test tubes. After adding 1 mL of 3%

aqueous solution of TTC (Triphenyltetrazolium chloride) and 2.5 mL of deionized water, samples were then incubated at 37°C for 24 hours. Triphenylformazon (TPF) formed was extracted with 10 mL of methanol by shaking vigorously for 2 h and filtered. The red color intensity was measured spectrophotometrically at 485nm. The DHA activity was expressed as  $\mu\text{g TPF d}^{-1} \text{g}^{-1}$  soil.

*Acid and alkaline phosphatase activity* were measured by Tabatabai and Bremner [8] method as: Air dried 1 g soil sample was mixed with 0.2 mL of toluene, 4 mL of modified universal buffer (pH 6.5 for acid phosphatase and at pH 11 for alkaline phosphatase) and 1 ml of *p*-nitro phenyl phosphate solution. After incubation at 37°C for 1 h, the extinction of the PNP (*p*-nitro phenol) produced was measured spectrophotometrically at 420 nm. The activity was expressed as  $\mu\text{g of PNP hydrolysed h}^{-1} \text{g}^{-1}$  soil.

*Urease activity* was measured by Tabatabai and Bremner [9] method as: Five g of soil was incubated with 5 mL of 10% urea solution and 20 mL of citric acid buffer (pH 6.7) at 37°C for 24 h. The amount of N-NH<sub>4</sub> produced was estimated in the filtrate at 580 nm spectrophotometrically using mixed reagent (phenol+ NaOH) and sodium hypochlorite. The results were expressed as  $\mu\text{g NH}_4^+ \text{d}^{-1} \text{g}^{-1}$  soil.

*Catalase activity* was measured by back-titrating residual H<sub>2</sub>O<sub>2</sub> with KMnO<sub>4</sub> [10] as: Two g of soil sample was added to 40 mL distilled water with 5 mL of 0.3% hydrogen peroxide solution. The mixture was shaken for 20 min and then 5 mL of 1.5 M H<sub>2</sub>SO<sub>4</sub> was added. Afterwards the solution was filtered and titrated using 0.02 M KMnO<sub>4</sub>. The reacted amount of 0.02 M KMnO<sub>4</sub>, calculated per g of dry soil. Catalase activity was expressed as mL of 0.1 mol/L KMnO<sub>4</sub> g<sup>-1</sup> soil.

All the chemicals used were of analytical grade and all the experiments were done in triplicate.

### 3. RESULTS AND DISCUSSION

The effect of different doses of three tetracycline antibiotics (TC, OTC and CTC) on the enzymatic (dehydrogenase, acid and alkaline phosphatase, urease and catalase) activity in soil alone and in soil amended with sewage sludge at different time intervals [0 (4h), 7, 14, 21, 35, 56, 70 and 91 d] are shown in Figs. 1, 2, 3, 4 and 5.

#### 3.1 Dehydrogenase Activity

Soil dehydrogenase (DHA) representing metabolic oxidative activity of soil organism, was found influenced significantly by application of tetracyclines, sewage sludge and their mixture. The dehydrogenase activity in tetracyclines free soil was superior to soil containing antibiotics in the period of 2 weeks of incubation (Fig. 1) and this inhibition increased with increase in tetracycline concentration. The reduction in dehydrogenase activity after 14 days of application values was 53% for TC, 56% for CTC and 62.5% for OTC of their initial values and the inhibition for all the studied concentrations of tetracyclines got weaker after 14 days of application. The increase in the dehydrogenase activity after 14 days of antibiotics application might be due to dissipation of antibiotics causing an increase in soil organic matter. With amendment of sewage sludge, the dehydrogenase activity increased up to 14 days of application and thereafter fell down (Fig. 1). Fig. 1 also denotes that the activity of dehydrogenase is directly proportional to amount of sewage sludge amended. The dehydrogenase activity in presence of 2.5 g of sewage sludge and lower amount of antibiotics remains almost unchanged during the course of study; while it decreased significantly up to 14 days of treatment with higher doses of antibiotics. These results indicate that antibiotic tetracyclines incorporation in soil decreases the microbial activity up to 2 weeks of treatment. The results also denote that dehydrogenase activity is correlated significantly with soil organic matter content, as organic matter is the seat of microbial population and activity. Similar results are also reported by other workers [11,12].

#### 3.2 Acid and Alkaline Phosphatase Activities

As the mineralization of organic phosphorous by the activity of phosphatase in soils makes one of essential elements-phosphorous available in soil for plant growth, the phosphatase activity in soils with/ without tetracyclines was measured. In presence of various doses of three tetracyclines under study, the alkaline phosphatase activity initially decreased significantly up to 21 d of application (Fig. 2), while activity of acid phosphatase decreased up to 14 d of application (Fig. 3), and thereafter the activity of both acid and alkaline phosphatase increased till the end of the experiment.

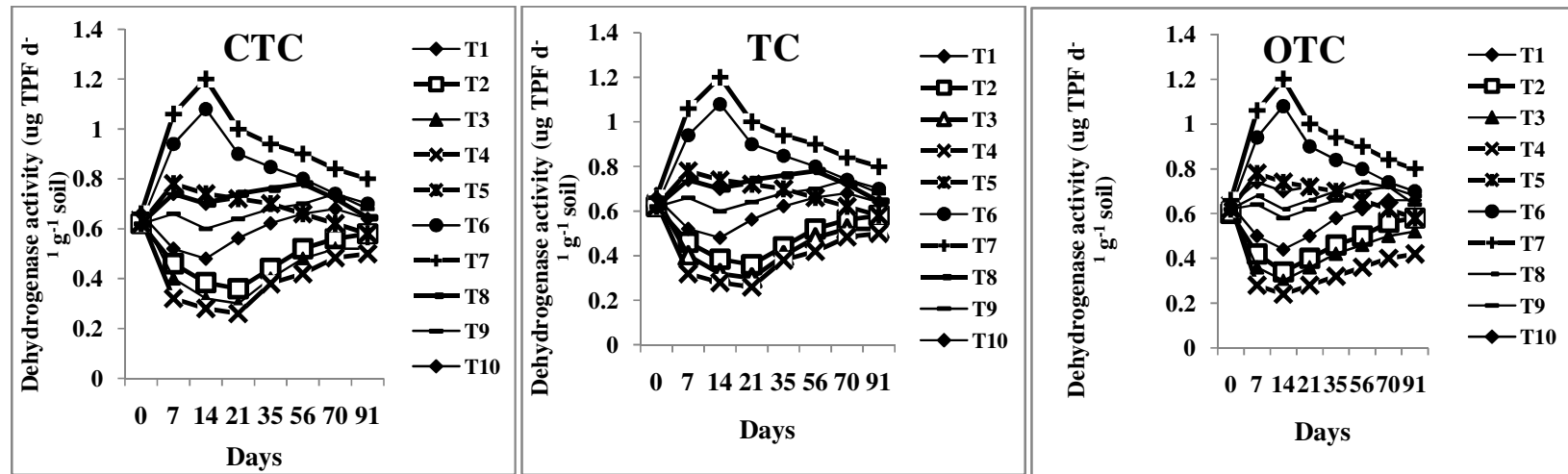


Fig. 1. Effect of different doses of tetracyclines (T1= 0 mg; T2 =5 mg; T3= 10 mg and T4= 50 mg kg<sup>-1</sup> soil), sewage sludge (T5= 0 g; T6= 2.5 g and T7= 5g kg<sup>-1</sup> soil) and 2.5 g of sewage sludge with (T8= 5 mg; T9= 10 mg and T10= 50 mg antibiotics kg<sup>-1</sup> soil) on Dehydrogenase activity in the soil. The DHA activity was expressed as ug TPF d<sup>-1</sup> g<sup>-1</sup> soil

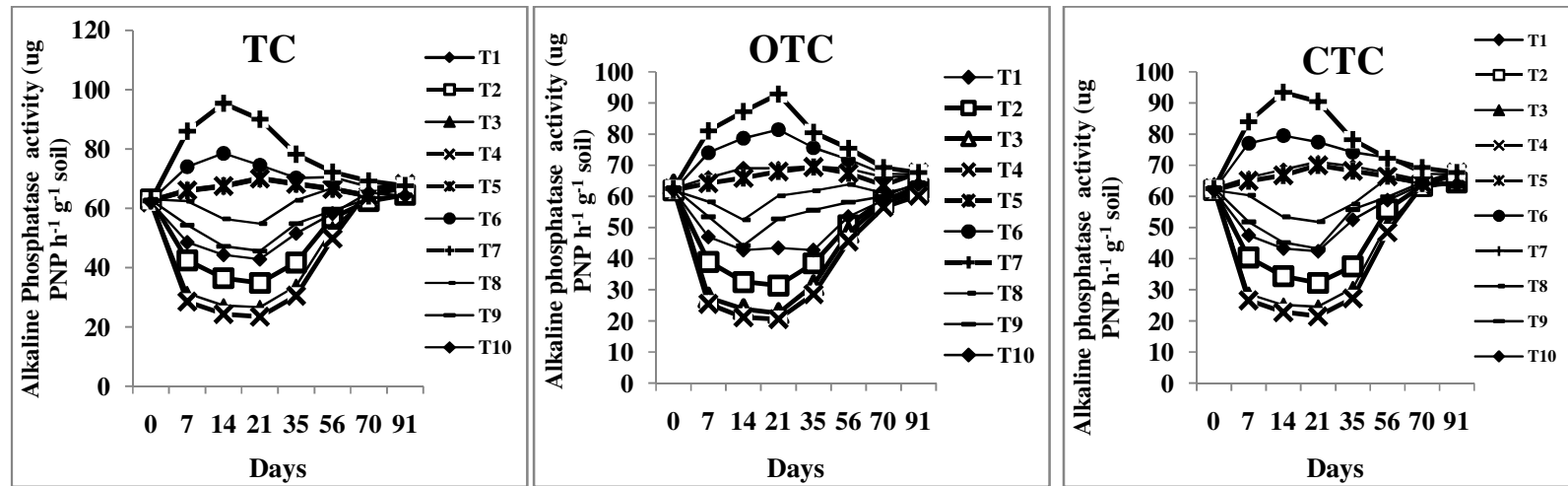


Fig. 2. Effect of different doses of tetracyclines (T1= 0 mg; T2 =5 mg; T3= 10 mg and T4= 50 mg kg<sup>-1</sup> soil), sewage sludge (T5= 0 g; T6= 2.5 g and T7= 5g kg<sup>-1</sup> soil) and 2.5 g of sewage sludge with (T8= 5 mg; T9= 10 mg and T10= 50 mg antibiotics kg<sup>-1</sup> soil) on Alkaline phosphatase activity in the soil . The activity was expressed as ug of PNP hydrolysed h<sup>-1</sup> g<sup>-1</sup> soil

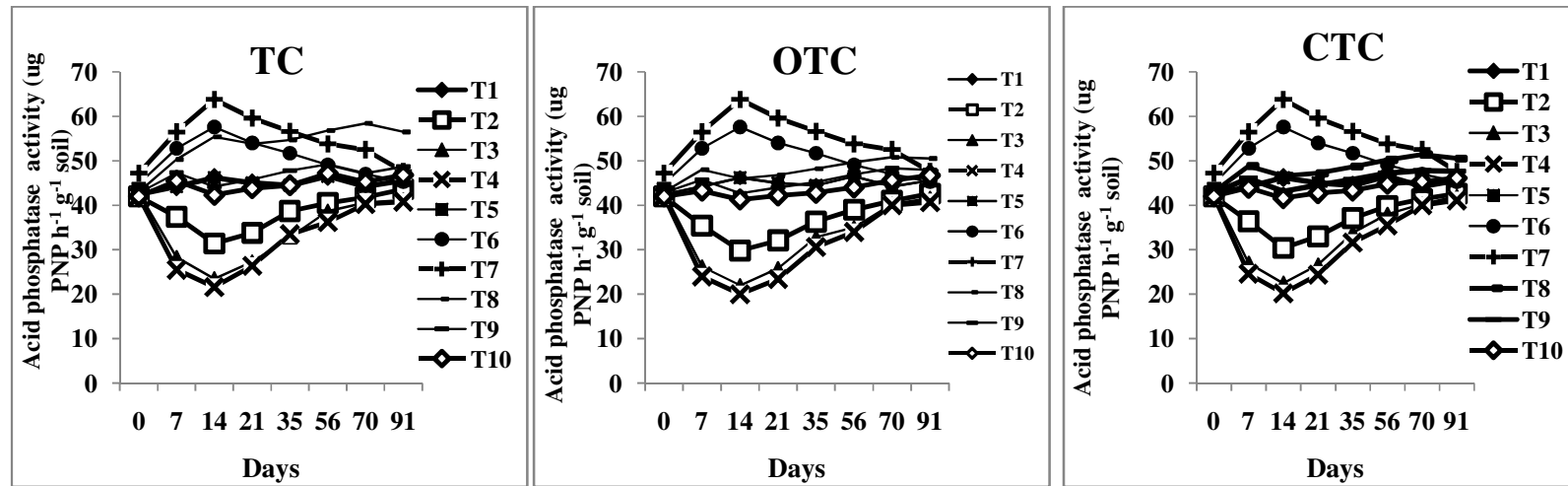


Fig. 3. Effect of different doses of tetracyclines (T1= 0 mg; T2 =5 mg; T3= 10 mg and T4= 50 mg kg<sup>-1</sup> soil), sewage sludge (T5= 0 g; T6= 2.5 g and T7= 5g kg<sup>-1</sup> soil) and 2.5 g of sewage sludge with (T8= 5 mg; T9= 10 mg and T10= 50 mg antibiotics kg<sup>-1</sup> soil) on Acid phosphatase activity in the soil. The activity was expressed as ug of PNP hydrolysed h<sup>-1</sup> g<sup>-1</sup> soil

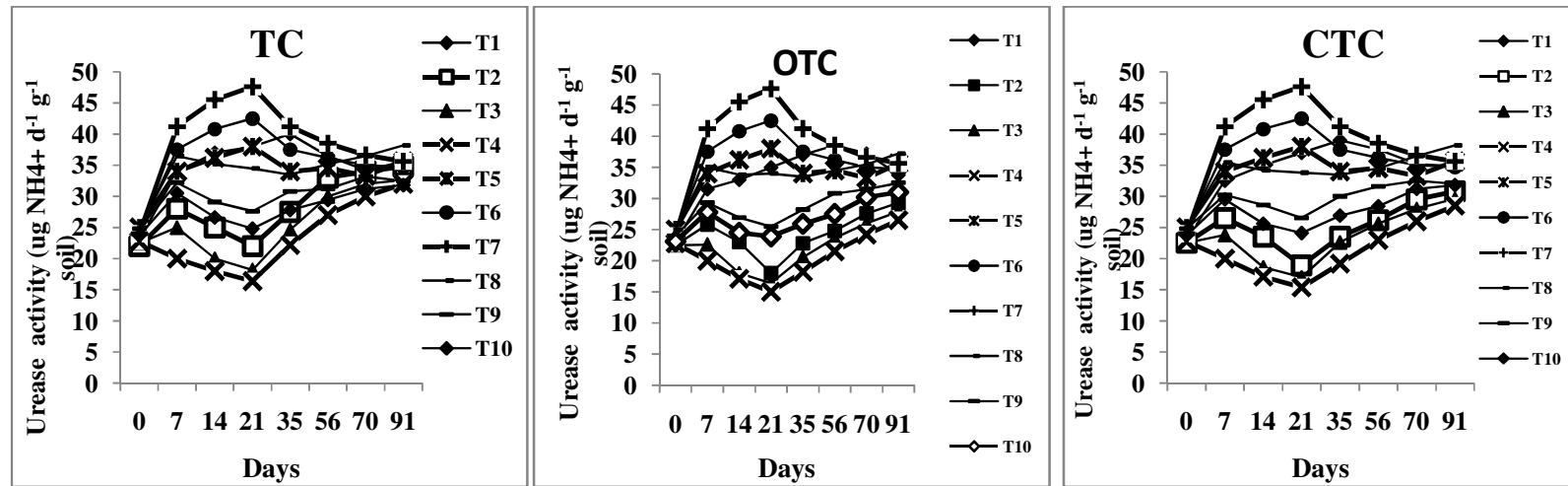


Fig. 4. Effect of different doses of tetracyclines (T1= 0 mg; T2 =5 mg; T3= 10 mg and T4= 50 mg kg<sup>-1</sup> soil), sewage sludge (T5= 0 g; T6= 2.5 g and T7= 5g kg<sup>-1</sup> soil) and 2.5 g of sewage sludge with (T8= 5 mg; T9= 10 mg and T10= 50 mg antibiotics kg<sup>-1</sup> soil) on Urease activity in the soil. The results were expressed as ug NH<sub>4</sub><sup>+</sup> d<sup>-1</sup> g<sup>-1</sup> soil

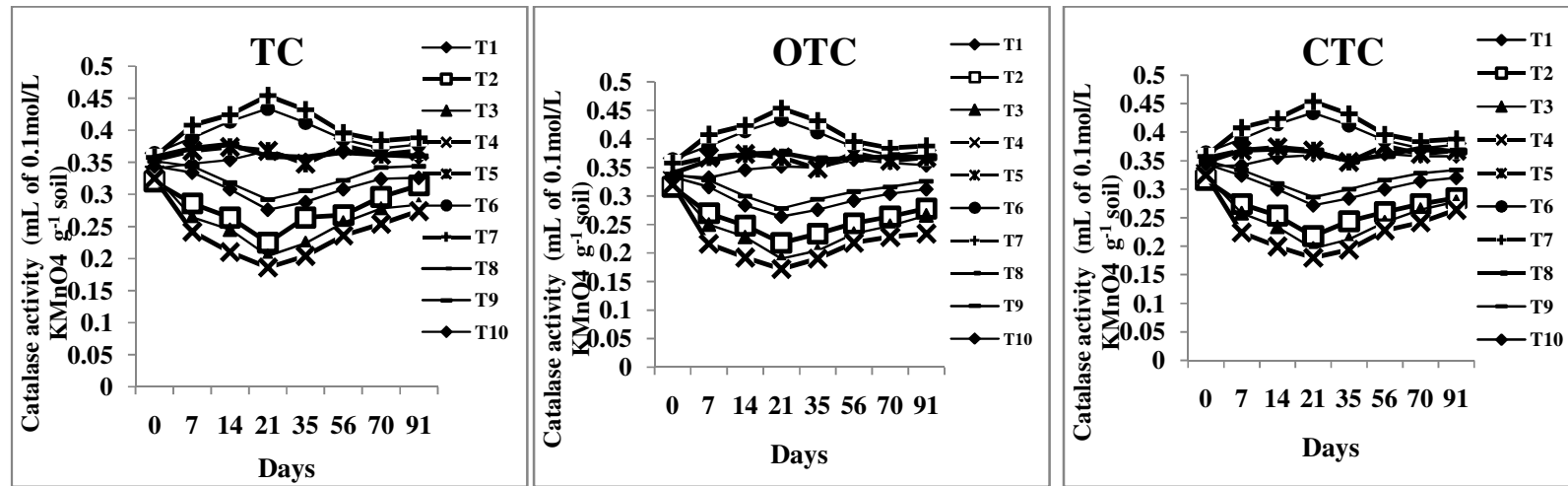


Fig. 5. Effect of different doses of tetracyclines (T1= 0 mg; T2 =5 mg; T3= 10 mg and T4= 50 mg kg<sup>-1</sup> soil), sewage sludge (T5= 0 g; T6= 2.5 g and T7= 5g kg<sup>-1</sup> soil) and 2.5 g of sewage sludge with (T8= 5 mg; T9= 10 mg and T10= 50 mg antibiotics kg<sup>-1</sup> soil) on Catalase activity in the soil. Catalase activity was expressed as mL of 0.1 mol/L KMnO<sub>4</sub> g<sup>-1</sup> soil



The rate of inhibition for both acid and alkaline phosphatase was significantly positively correlated with the doses of tetracycline added. The initial decrease in activities of both phosphatases might be due to the release of orthophosphates from lysed cells, which may act as competitive inhibitor of phosphates in soil [6,13]. The increase in phosphatase activity after 14 or 21 days of application and/or in presence of sewage sludge (Figs. 2 and 3) may be due to increase in amount of organic matter and rich microbial population in soil samples via sewage sludge /or increase in soil organic matter due to dissipation of antibiotics [14]. The activity of both acid and alkaline phosphatase in presence of 2.5 g sewage sludge  $\text{kg}^{-1}$  soil and different doses of tetracyclines remain almost unchanged (Figs. 2 and 3).

### 3.3 Urease Activity

The urease enzyme plays an important role in the efficient use of urea fertilizer. Urease breaks the C-N bonds other than peptide bonds in linear amides and releases  $\text{NH}_3$ . The urease activity after application of different doses of the three tetracyclines (Fig. 4) became 32-45% in comparison to control. The inhibition of soil urease activity got weaker for all the concentrations of tetracyclines after 21 d of application. The inhibition percentage was proportional to the concentration of tetracyclines applied. The increase in activity after 21 days of treatment might be due to dissipation of antibiotics, nitrogen fixation and increase in soil organic matter. The urease activity increased with addition of sewage sludge up to 28 days of treatment and fell down thereafter. In presence of 2.5 g of sewage sludge and lower amount of antibiotics, it remained almost unchanged during the course of study (Fig. 4). These results indicate that application of tetracyclines in soil decreases microbial activity for up to 3 weeks of treatment and the urease activity is significantly correlated with nitrogen fixation and soil organic matter content. Similar results are also reported by other workers [15,16].

### 3.4 Catalase Activity

The catalase split hydrogen peroxide into molecular oxygen and water and thus prevents cells from damage by reactive oxygen species [17]. This enzyme can be found in all the aerobic microorganisms, plant and animal cells. Response of catalase activity to tetracycline contamination in Aligarh soils are given in Fig. 5.

The higher values of enzymatic activity of controls were associated with biotic and abiotic natural processes which took place in soil. Conversely, the introduction of tetracyclines to soil samples must have clearly disturbed the metabolic homeostasis of microorganisms which led to reduction in enzymatic activity [18]. The inhibition of catalase activity by addition of different concentration of tetracyclines got weaker after 21 d of application. The inhibition percentage was proportional to the concentration of tetracyclines. The addition of lower dose of sewage sludge to soil had no appreciable effect on catalase activity with respect to non-amended soil, while high dose of sewage sludge lead to significant increase in catalase activity (Fig. 5). This suggests that the presence of a minimum amount of fresh organic matter is needed to activate soil catalase activity [19]. The activity of catalase in presence of 2.5 g sewage sludge  $\text{kg}^{-1}$  soil and different doses of tetracyclines remains almost constant (Fig. 5).

## 4. CONCLUSION

The results of present study suggests that the activity of dehydrogenase, acid and alkaline phosphatase, urease and catalase was superior in tetracycline free soils than in soil containing antibiotics in the period of 2-3 weeks of incubation. Inhibition of activity got weaker after 2-3 weeks of treatment. The inhibition of soil enzyme activity increased with tetracycline concentration. In presence of sewage sludge, initially the studied enzymes activities increased and decreased after 3 weeks. In presence of sewage sludge and antibiotics, the activity of enzymes remains almost unchanged. The activity of studied enzymes in soil was positively correlated to soil organic content.

## CONSENT

Not applicable.

## ETHICAL APPROVAL

Not applicable.

## ACKNOWLEDGMENTS

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## COMPETING INTERESTS

Author has declared that no competing interests exist.

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