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# EFFECT OF WATER LOGGING AND WEEDS AS ORGANIC MANURES ON ENZYME ACTIVITIES UNDER TYPIC PALEUSTALF SOIL

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**ABSTRACT**
Nutrient cycling in soils involves biochemical, chemical and physicochemical reactions, with biochemical process mediated by microorganisms, plant roots and soil animals. It is well known that all biochemical reactions are catalyzed by enzymes, which are protein with catalytic properties owing to their power of specific activation. Enzymes are catalyst that is they are substances that without under going permanent alteration cause chemical reactions to proceed at faster rates. Laboratory incubation was conducted to study the invertase, cellulase, urease and acid phosphatase using parthenium and chromolaena as compost as well as green manure to Mudigere soil under flooded condition. The invertase, cellulase and urease activity increased up to 60$^{th}$ day of flooding and then decreased. The acid phosphatase activity increased up to 30$^{th}$ day of flooding and then decreased. The increasing level of C application increases the enzyme activity.

**KEYWORDS:** Invertase, Cellulase, Urease, Acid phosphatase

## INTRODUCTION
In Residue recycling and sustainability of the rice cropping system centers around the maintenance of soil organic carbon, which is difficult, if not impossible in tropical agriculture. Due to high soil temperature organic carbon gets oxidized and sequestration of carbon (C) becomes often impossible. There is increasing evidence that microbial biomass and soil enzyme activities could be used as sensitive indices not only for the biological status of the soil, but for a variety of factors such as organic carbon input to the systems, presence of certain toxic elements and polluting organic compounds. The ultimate measure of soil health would be the ability of the soil to support and sustain plant growth in a given situation.
Nutrient cycling in soils involves biochemical, chemical and physicochemical reactions, with biochemical process mediated by microorganisms, plant roots and soil animals. It is well known that all biochemical reactions are catalyzed by enzymes, which are protein with catalytic properties owing to their power of specific activation. Enzymes are catalyst, are substances that without undergoing permanent alteration cause chemical reactions to proceed at faster rates. In addition, they are specific for the types of chemical reactions in which they particulate. Most soil enzymes studies have been confined to arable agricultural and forest soils, but a flooded rice soil is predominantly anaerobic and the study was restricted. Despite extensive studies on the chemistry (Ponnamperuma, 1972) and microbiology (Yoshida, 1975) of flooded soils, over knowledge of these enzyme activities are limited. Weeds such as parthenium and chromolaena have spread menacingly threatening agriculture and environment. These weeds can be used as green manure and also compost can be prepared from these weeds and can be used for agriculture. In this context experiment was conducted to study the invertase, cellulase, urease and acid phosphatase activities of flooded soils with the incorporation of parthenium and chromolaena as green manure and their compost.

## MATERIALS AND METHODS
### Study site
The soil used for this study was Typic Paleustalf, collected from Mudigere taluk, Chickmagalur district, Karnataka, India. The study was conducted at Department of Soil Science and Agricultural Chemistry, College of Agriculture, University of Agricultural Sciences, Bangalore, Karnataka, India. Soil samples were air dried, sieved (< 2mm) and analyzed for physico-chemical properties *viz*, pH 5.38, EC 0.04 $dSm^{-1}$, total organic carbon 0.98%, $NH_4^+$ - N 61.10 mg $kg^{-1}$, $NO_3$ - N 10.0 mg $kg^{-1}$, olsen - P 6.71 mg $kg^{-1}$ and $NH_4OAc$ - K 68.91 mg $kg^{1}$.
### Soil Incubation
Based on the total C content of the plant materials, amount of organic material materials *i.e*, parthenium and chromolaena as green manure and their compost were required for soil incorporation was calculated and added to 500 g of soil contained in polythene pots. Appropriate amount of organic Excess amount of water was added and mixed to create puddle and flooded conditions. There were three levels of organic material application to the soil to achieve 0.5, 1.0 and 2.0 per cent level of organic carbon. The following treatments in duplicate were set out. There are 13 treatments and 3 replications, T1: Soil (Control), T2: Chromolaena as green manure @ 0.5 % C, T3: Chromolaena as green manure @ 1.0 % C, T4: Chromolaena as green manure @ 2.0 % C,T5: Parthenium as green manure @ 0.5 % C,T6: Parthenium as green manure @ 1.0 % C,T7: Parthenium as green manure @ 2.0 % C, T8: Chromolaena compost @ 0.5 % C, T9: Chromolaena compost @ 1.0 % C, T10: Chromolaena

compost @ 2.0 % C, T11: Parthenium compost @ 0.5 % C, T12: Parthenium compost @ 1.0 % C and T13: Parthenium compost @ 2.0 % C. At periodic intervals, destruction sampling was done for analyzing enzyme activities at 15, 30, 60 and 90 days after flooding. Results were expressed on oven dry weight basis after taking account of moisture per cent.

## ENZYME ASSAYS

### Invertase

Invertase activity was determined by Prussian blue method (Schinner, 1990). For invertase activity each 5 g sample was treated with 15 ml of 0.2 M acetate buffer (pH 5.5) was added and further 1.2 per cent (w/v) sucrose, substrate solution prepared in 0.2 M acetate buffer was added and incubated for 3 hours at 50$^o$ C. Contents were filtered reducing sugar content was measured.

### Cellulase

Cellulase activity was also determined by Prussian blue method (Schinner, 1990). Similar to invertase activity, soil was incubated with 0.7 per cent (w/v) carboxy methyl cellulose prepared in 0.2 M acetate buffer solution (pH 5.5) and incubated for 24 hours at 50$^o$ C. Filtered and diluted extracts were measured for increase in reducing sugar content analyzed at 690 nm.

### Urease

The sample was incubated with Tris Hydroxy Amino Methane (THAM) buffer (pH 9.0) containing 2 per cent urea for 2 hours at 37$^o$C in presence of toluene. Extra $NH_4^+$ formed as a hydrolysis product of urea over a control which did not receive any urea solution was extracted with $Ag_2SO_4$ - KCl solution and $NH_4^+$ content in the aqueous extract was measured by steam distillation (Zantua and Bremner, 1975).

### Acid phosphatase

The sample was treated with modified universal buffer at a pH value of 6.5. P - Nitro phenyl phosphate disodium salt was used as the substrate and yellow colour developed due to hydrolysis of ester bonds was measured against a blank at 600 nm (Evazi and Tabatabai 1977).

## RESULTS AND DISCUSSION

**TABLE 1.**Changes in invertase and cellulase activity (mg glucose produced g$^{-1}$ oven dry soil, hr$^{-1}$ at 50$^0$C) in soils Incorporated with different organic manures under flooded condition

| Treatments | Invertase activity | | | | | | Cellulase activity | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Days after flooding | | | | | | Days after flooding | | | | | |
| | 7 | 15 | 30 | 60 | 90 | 120 | 7 | 15 | 30 | 60 | 90 | 120 |
| $T_1$:Soil alone (Control) | 0.62 | 0.59 | 0.75 | 0.97 | 0.61 | 0.32 | 9.60 | 13.80 | 29.70 | 32.70 | 22.10 | 7.80 |
| $T_2$:Chromolaena as green manure @ 0.5 % C | 0.63 | 0.57 | 0.78 | 0.62 | 0.63 | 0.49 | 11.70 | 65.10 | 79.20 | 110.80 | 140.70 | 26.10 |
| $T_3$:Chromolaena as green manure @ 1.0 % C | 0.72 | 0.61 | 0.69 | 0.82 | 0.68 | 0.53 | 17.60 | 82.70 | 119.70 | 136.10 | 153.70 | 36.70 |
| $T_4$:Chromolaena as green manure @ 2.0 % C | 0.97 | 0.83 | 1.13 | 1.26 | 0.97 | 0.62 | 22.70 | 98.10 | 126.80 | 144.70 | 168.70 | 72.80 |
| $T_5$: Parthenium as green manure @ 0.5 % C | 0.64 | 0.59 | 0.73 | 0.81 | 0.70 | 0.59 | 12.80 | 56.70 | 73.90 | 98.70 | 129.70 | 149.80 |
| $T_6$: Parthenium as green manure @ 1.0 % C | 0.66 | 0.64 | 0.99 | 1.25 | 0.90 | 0.56 | 18.70 | 63.10 | 75.80 | 80.10 | 138.10 | 96.70 |
| $T_7$: Parthenium as green manure @ 2.0 % C | 0.87 | 0.73 | 1.15 | 1.31 | 0.98 | 0.63 | 21.00 | 76.10 | 83.10 | 96.10 | 125.70 | 93.10 |
| $T_8$: Chromolaena as compost @ 0.5 % C | 0.68 | 0.57 | 0.97 | 1.27 | 0.68 | 0.52 | 19.70 | 75.10 | 85.60 | 99.10 | 121.70 | 90.10 |
| $T_9$: Chromolaena as compost @ 1.0 % C | 0.76 | 0.69 | 0.97 | 1.32 | 0.79 | 0.43 | 26.30 | 76.80 | 96.10 | 126.00 | 149.70 | 90.70 |
| $T_{10}$: Chromolaena as compost @ 2.0 % C | 0.36 | 1.13 | 1.88 | 1.39 | 1.47 | 0.66 | 30.80 | 99.80 | 146.80 | 160.10 | 196.00 | 142.70 |
| $T_{11}$: Parthenium as compost @ 0.5 % C | 0.71 | 0.69 | 0.98 | 1.27 | 0.99 | 0.53 | 14.10 | 76.20 | 96.30 | 133.70 | 148.70 | 96.70 |
| $T_{12}$: Parthenium as compost @ 1.0 % C | 0.86 | 0.71 | 1.26 | 1.67 | 1.06 | 0.61 | 20.70 | 99.50 | 114.60 | 166.70 | 180.60 | 88.10 |
| $T_{13}$: Parthenium as compost @ 2.0 % C | 1.27 | 0.98 | 1.46 | 1.66 | 1.21 | 0.82 | 28.10 | 86.50 | 126.50 | 171.70 | 163.10 | 93.10 |
| SEm ± | 0.03 | 0.04 | 0.01 | 0.02 | 0.04 | 0.02 | 2.69 | 10.24 | 9.34 | 11.16 | 6.98 | 2.14 |
| LSD (P = 0.05) | 0.09 | 0.12 | 0.03 | 0.06 | 0.12 | 0.06 | 8.01 | 30.98 | 28.02 | 33.48 | 18.94 | 6.31 |

### Invertase

Increasing organic C load from 0.5 per cent to 2.0 per cent C level maintained higher invertase activity. Lowest invertase activity (0.32 mg glucose production, g$^{-1}$ oven dry soil hr$^{-1}$) was noticed in T1 (control). Invertase activity showed a peak value on 60$^{th}$ day of flooding and later on it decreased. Highest invertase activity noticed in $T_{13}$: Parthenium compost @ 2.0 % C and $T_{10}$: Chromolaena compost @ 2.0 % C was 0.74 mg glucose production, g$^{-1}$ oven dry soil, hr$^{-1}$ at day 120$^{th}$ of flooding. Invertase activity is directly related to labile fraction is directly related to labile fraction of residues, which are positively, correlated (Rajashekara Rao, 2000). The high activity noticed during initial stages indicates rapid microbial turn over of C compounds. Invertase enzyme exists in soil, bound to all constituents such as disintegrated cells and viable but non proliferating cells, not as enzyme released from cells and then adsorbed (Kiss *et al*., 1975). Invertase activity seems to be a potential indicator to show the existence of easily degradable C compounds (Balasubramanian *et al*., 1972).

**Cellulase**

In general, flooding the soils resulted in increased cellulase activity. In control, initial cellulase activity was 9.60 mg glucose production, increased to 32.70 mg glucose production, $g^{-1}$ soil, $hr^{-1}$, at day 60 of flooding, but later decreased to 7.80 mg glucose production $g^{-1}$ soil, $hr^{-1}$ at day120 of flooding. The peak cellulase activity was observed, when maximum quantities of substrate cellulose are released from lingo-cellulose complex. The relation observed between cellulase activity and cellulose contents of the material used for the incorporation (Rajashekara Rao, 2000). It implies that content of cellulose *sensu stricto* may not induce cellulase enzyme production in soil. Cellulase is an inducible enzyme and where ever there is accessibility to free cellulose its production will be triggered. Linkins *et al.* (1990) concluded that activity of cellulase is directly related to the mass loss of decomposing organic materials and inversely related to the lignin content. In the present study, since carboxy methyl cellulose is used as substrate, which can not be taken up inside the microbial cells, the assay should give an indication of extras cellular cellulase, which are accumulated and stabilized on clay

**TABLE 2.** Changes in the urease and acid phosphatase activity (mg glucose produced, $g^{-1}$ oven dry soil, at $50^0$ C) in soils incorporated with different organic manures under flooded conditions

| Treatments | Urease | | | | | | Acid phosphatase | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Days after flooding | | | | | | Days after flooding | | | | | |
| | 7 | 15 | 30 | 60 | 90 | 120 | 7 | 15 | 30 | 60 | 90 | 120 |
| $T_1$:Soil alone (Control) | 19.80 | 56.10 | 63.70 | 54.80 | 16.80 | 18.10 | 5.20 | 6.40 | 26.80 | 16.90 | 13.70 | 4.10 |
| $T_2$:Chromolaena as green manure @ 0.5 % C | 23.10 | 62.70 | 39.10 | 43.10 | 36.70 | 28.70 | 9.80 | 13.70 | 29.30 | 18.70 | 14.60 | 9.60 |
| $T_3$:Chromolaena as green manure @ 1.0 % C | 29.80 | 66.70 | 76.70 | 83.40 | 59.40 | 33.60 | 12.10 | 16.10 | 32.10 | 16.60 | 12.40 | 10.10 |
| $T_4$:Chromolaena as green manure @ 2.0 % C | 38.40 | 79.30 | 86.80 | 96.70 | 66.10 | 44.30 | 14.40 | 18.90 | 36.70 | 20.00 | 16.20 | 11.20 |
| $T_5$: Parthenium as green manure @ 0.5 % C | 22.90 | 59.60 | 23.40 | 96.60 | 114.50 | 61.40 | 7.70 | 11.20 | 26.30 | 12.30 | 9.60 | 3.60 |
| $T_6$: Parthenium as green manure @ 1.0 % C | 30.60 | 66.70 | 96.70 | 112.60 | 163.50 | 76.40 | 9.10 | 13.40 | 29.40 | 16.20 | 11.40 | 6.70 |
| $T_7$: Parthenium as green manure @ 2.0 % C | 36.40 | 71.30 | 92.10 | 121.70 | 156.40 | 63.40 | 13.20 | 16.10 | 32.10 | 18.40 | 14.60 | 5.10 |
| $T_8$: Chromolaena as compost @ 0.5 % C | 29.80 | 96.10 | 126.50 | 136.40 | 144.30 | 93.20 | 12.80 | 19.10 | 28.10 | 13.80 | 12.10 | 9.10 |
| $T_9$: Chromolaena as compost @ 1.0 % C | 36.10 | 68.70 | 139.40 | 151.60 | 137.70 | 86.70 | 13.90 | 17.10 | 30.20 | 16.70 | 14.30 | 9.00 |
| $T_{10}$: Chromolaena as compost @ 2.0 % C | 42.70 | 99.10 | 123.10 | 156.70 | 169.70 | 119.20 | 15.10 | 20.30 | 33.10 | 22.10 | 17.10 | 12.70 |
| $T_{11}$: Parthenium as compost @ 0.5 % C | 26.30 | 66.70 | 88.20 | 144.70 | 156.10 | 63.70 | 9.20 | 16.30 | 23.30 | 14.30 | 9.30 | 7.70 |
| $T_{12}$: Parthenium as compost @ 1.0 % C | 32.20 | 78.60 | 96.10 | 129.80 | 146.10 | 76.70 | 10.70 | 19.60 | 26.50 | 16.60 | 10.70 | 8.80 |
| $T_{13}$: Parthenium as compost @ 2.0 % C | 39.80 | 86.70 | 113.70 | 149.50 | 166.70 | 99.70 | 12.10 | 22.10 | 27.00 | 19.30 | 13.70 | 11.30 |
| SEm ± | 2.08 | 3.11 | 1.94 | 4.28 | 1.94 | 1.04 | 0.09 | 1.10 | 2.19 | 1.98 | 7.09 | 0.98 |
| LSD (P = 0.05) | 6.11 | 10.01 | 4.98 | 12.84 | 4.18 | 3.63 | 0.27 | 3.00 | 6.57 | 5.95 | 3.13 | 2.94 |

power. Similar results were found by Rajashekara Rao, 2000.

**Urease**

Higher urease activity was noticed in all the treatment under different intervals it ranged from 19.80 to 169.70 mg $NH^+_4$ production, $g^{-1}$ soil. $hr^{-1}$. In T1 urease activity was 63.70 mg $NH^+_4$ produced, $g^{-1}$ soil $hr^{-1}$, ($15^{th}$ day of flooding). Fluctuating trend was noticed in all the treatments which received organic manures. Increasing the C load form 0.5 to 2.0 per cent C level increased the urease activity in soils. Increasing carbon load from 0.5 per cent 2.0 C level increased the urease activity in soils. Urease being an extra cellular enzyme will not be produced in response to the presence of urea molecule (Zantua and Bremner, 1975). The application of chromolaena compost and parthenium compost at 0.5, 1.0 and 2.5 per cent C level maintained higher urea hydrlytes

**Acid Phosphatase**

A wide fluctuation was noticed with respect to acid phosphatase activity, when a range of organic manures were incorporated to flooded soil and monitored over a period of 120 days. The acid phosphatase activity ranged from 5.20 to 32.10 µg PNP produced $g^{-1}$ soil, $hr^{-1}$. Increasing the rate of C application through different sources increased the phosphatase activity. Peak enzyme activity was observed at $30^{th}$ day of flooding and then a steady decline in enzyme activity was observed in all the treatments. In T1 phosphatase activities were recorded 5.20, 6.40, 26.80, 16.90, 13.70 and 4.10 µg PNP production $g^{-1}$ soil, $hr^{-1}$ on 7, 15, 30, 60, 90 and $120^{th}$ day of flooding. Addition of C at 20 per cent level maintained higher phosphatase activity than 1.0 and 0.5 per cent application of same material. During initial stages of flooding, phosphatase activity is related to P content of the organic material used. The decreases in phosphatase activity in the later half of the incubation period may be due to increase in pH and acid phosphomonoesterace activity are negatively correlated (Eivazi and Tabatabai, 1977). Phosphatase activity had a positive relation ship with P supply and negatively related to inorganic P availability (Pan, 1985). Flooding has been reported to decrease phosphatase activity as available phosphorous content increases by reduction process (Pulford and Tabatabai, 1988). It is evident in the present study that continuous supply of easily degradable carbon and availability of organic P compounds may illicit phosphate production, even though; inorganic P supply is in high quantities. Therefore it can be concluded that supply of readily degradable C and organic P compounds could over weigh, end product depression of phosphatase enzyme. The application of chromolaena compost and parthenium compost at 2.0 C level maintained higher activities of invertase, Cellulase, urease and acid Phosphatase enzyme.

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