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Impact of paper industry effluents on physico-chemical, microbiological and enzyme activities of soil

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Abstract

In a comparative study of various parameters between a paper industry effluent affected soil (test sample) and a control soil, it was formed that physico-chemical changes like pH was higher (7.2) in affected test soil than the control (6.3) and electrical conductivity was more in the former (1.1) than the later (0.8). There were increased levels of water holding capacity, organic carbon, nitrogen, potassium and phosphorus in the test soil as compared to control which indicated the fertility of the former. There was two fold increase in the population of both bacteria and fungi; and also the enzyme levels (cellulase, xylanase, pectinase and invertase) were significantly higher in the test sample than the control, as these are indicators of microbial activity. These results indicate that paper industry effluents may increase the fertility of treated soil for plant growth.

INTRODUCTION

Soil is a rich source of microorganisms and their enzymes [1], the activities of which will decompose complex organic crop residues into simpler organic compounds and form an ecosystem [2, 3] for plant growth.

Industrialization has led to the generation of effluents. Dumping of rich organic toxic effluent into soil will pollute the soil and it leads to several legislations to protect the soil and the environment in general. It also depends upon the changes caused by human use and management of land [4]. However, there is an urgent need to recover the rich pool of nutrients from such effluents [5]. These are in generated or left untreated in many cases composing bio-gas production and other methods are need for utilization of these effluents [6].

Paper industry is one of the oldest and the largest industries in India, as it has a capacity of 3.0 million metric tons of finished product per annum. It consumes about 300 m^3 of water per ton of paper produced and as much generates large volumes of colored and toxic effluents. The sludge contains high amounts of plant nutrients [7] and about 500 different chlorinated organic compounds have been identified in the effluent [8].

It has been formed that changes in microbial populations is their enzymatic activities (bio-indicators) can precede detectable changes in soil physico-chemical properties that provide an early sign of soil improvement or early warming of soil degradation [9,10] and their estimation will aid in the assessment of soil quality [11]. Hence a preliminary study was conducted to determine the impact of paper mill effluents in physico-chemical and microbial extra cellular enzymes in the soil as compared to a control soil, and the results are presented in this paper.

MATERIALS & METHODS

Study site:

Kurnool city is the gate way of Rayalaseema in Andhra Pradesh (India), is going rapid industrialization. Rayalaseema paper industry is located in the huge expanse of 625 acre area northern bank of river Tungabhadra near the Kurnool city. The soil samples were collected during the spring season.

Sample collection:

Soil sample was collected from the paper mill surroundings aseptically in closed polythene covers. The sample used as control was collected from one kilometer far away from the industry. Prior to testing the soil was air dried and sieved through 2 mm sieve and stored at freezing conditions for further analysis.

Physico-chemical analysis of soil:

Soil pH and conductivity was determined by pH meter (Elico) and conductivity meter respectively. The water holding capacity of the soil was determined by taking 10 g of sample, drying in a hot air oven at 80°C for 24 h and then weighed to determine difference in weight. The soil contents like, nitrogen, phosphorus and potassium were determined by the method described by Watanabe and Olsen [12].

Microbiological analysis:

Soil microorganisms including fungi and bacterial population were enumerated by serial dilution technique. One gram of soil sample was serially diluted and 0.1 ml of soil sample was spreaded on the nutrient agar medium and malt extract agar medium with a sterile spreader for the growth of bacteria and fungal strains respectively. The total bacterial count was determined after 24 h of incubation at 37°C by the method of Olutiola [13] and the total fungal count was determined after 5 days of the incubation at 37°C.

Cellulase assay:

The cellulase activity in the soil was determined by the incubating 2 g of sieved soil for 7 days in 60 ml of 50 mM Na-acetate buffer (pH-6.0) with 2% carboxy methyl cellulose (CMC) and 1 ml of toluene. After the completion of incubation period, the sample was centrifuged for 20 min at 12,000 rpm and filtered through the Whatman filter paper. The filtrate was analyzed for reducing sugars released during the incubation period hydrolysis of CMC by soil cellulases. Reducing sugars in the medium filtrate were measured at 520 nm in spectrophotometer, using the dinitro salicylic acid (DNS) method [14]. One unit of β-glucodiase activity was defined as amount of enzyme required to liberate 1 µ mol glucose equivalents in 1 ml enzyme solution in 1 min.

Xylanase assay:

Five grams of soil sample were taken and placed in Erlenmeyer flasks and 6 ml of 2 M acetate buffer (pH-5.5) containing 1% birch wood xylan (Hi-Media) was added and incubated for 24 h, and the suspension was intensively mechanically shaken in orbital shaker. Blank was incubated without substrate (xylan). After incubation period, immediately filtered and liberated end product, reducing sugars were measured at 520 nm in spectrophotometer, using the DNS method [15]. Xylanase activity was expressed as the number of µmoles of reducing sugars produced per minute of hydrolysis per ml of enzyme used.

Invertase assay:

The invertase activity was determined by the method of using Schinner and Von Mersi (1990). In this method five grams of soil incubated with sucrose solution (50 Mm) as a substrate on water bath with shaking at 55°C for 3 h, reducing sugars were measured at 520 nm in spectrophotometer. One unit of invertase (IU) was defined as the amount of enzyme which liberates 1µ mole of glucose/min/ml under the assay condition.

Pectinase assay:

Initially 1 g of pectin substrate in 0.1 M acetate buffer (pH-4.5) was incubated at 40°C for 20 min. Suspended substrate (2 ml) was added to the 5 g of soil in Erlenmeyer flask and the mixture was incubated at 40°C for 10 min. After that 1 ml of DNS reagent was added to the above mixture and it was boiled for 5 min at 90°C. The reaction was stopped by adding 1 ml of Rochelle's salt. Then the mixture was diluted by adding 2 ml of de-ionized water. The absorbance was measured spectrophotometrically at 520 nm. One unit of pectinase activity was defined as the amount of enzyme which liberated 1µ mole glucose per min.

Table-1: Physico-chemical properties of soil contaminated with/without paper effluents

Physico chemical properties	Control soil	Test soil
Soil	Black	Light brown
Odour	Normal	Unpleasant
Soil nature	High weight	Low weight
Electrical conductivity (Mmhos/cm)	0.8	1.1
pH	6.3	7.2
Water holding capacity (ml/g of soil)	0.4	0.6
Nitrogen (mg/kg)	0.03	0.06
Phosphorus (mg/kg)	0.01	0.03
Potassium (mg/kg)	0.02	0.05
Organic carbon (%)	0.41	0.49

Table- 2 : Microbial populations (CFU/g) in soil contaminated with/without paper effluents

Microorganisms	Test soil	Control soil
Fungi	106×10^5	62×10^5
Bacteria	145×10^5	96×10^5

RESULTS

Physico-chemical properties of soil:

The soil polluted with paper effluents decreased soil pH 7.2 to 6.3, water holding capacity increased 0.4 to 0.6 ml/g of soil, electrical conductivity decreased to 1.1 to 0.8 Mmhos/cm. Similarly higher organic carbon and high amount of nitrogen, phosphorus and potassium were observed in the test soil (Table 1).

Soil fungal and bacterial strains:

Due to the presence of high organic carbon in the soil, the microorganisms increased in test soil compared to control soil. For instance, the bacterial and fungal populations in test and control soil were 145×10^5, 96×10^5 and 106×10^5, 62×10^5 cfu/g gram of soil respectively (Table 2).

Enzymes activities in soil:

The cellulase activity in test soil increased as compared to control soil up to 12 days incubation period beyond which the cellulase activity decreased (Fig 1). In the same way, the xylanase activity also increased but up to 16 days, beyond this period the activity decreased. In control soil, the activity of the xylanase was very low (Fig 2). Similarly the invertase activity in the test soil increased up to 12 h, after which the enzyme activity decreased (Fig 3). Pectinase activity in the test soil gradually increased at low rate. In the control soil, the pectinase activity was negligible (Fig 4).

DISCUSSION

The results show that the effect of paper industry effluents on soil leads to increase in soil pH of the test soil (Table 1). This indicates the acid nature of the contaminants disposed in the test soil. Increased soil pH by industrial effluents was also reported in the contaminated soils [16]. Increased water holding capacity and electrical conductivity observed in the test soil. It was reflected by the chemical and biological activities of the industrial effluent in the soil. Similar results were observed in long-term discharge of sewage wastes into the soil [17]. The higher organic carbon matter showed in Table 1, indicates the alkaline substances like cellulosic and paper material were added to the soil. A similar result was observed in the long- term disposal of municipal waste into the soil [18] and soil contaminated with pesticide industrial wastes [19]. Higher population of bacterial and fungal population in test soil may be due to the presence of high organic matter present in the soil. Bacterial count was higher than the fungal count.

The microbial count would depend upon the soil properties such as basal soil respiration rate, enzyme activities, the soil pH, organic matter content and other chemical properties [20]. Soil microbial biomass plays a critical role in ecosystem processes, such as carbon cycling and the enzymes are strongly connected with it and enzymes are the sensitive indicators of soil quality. In

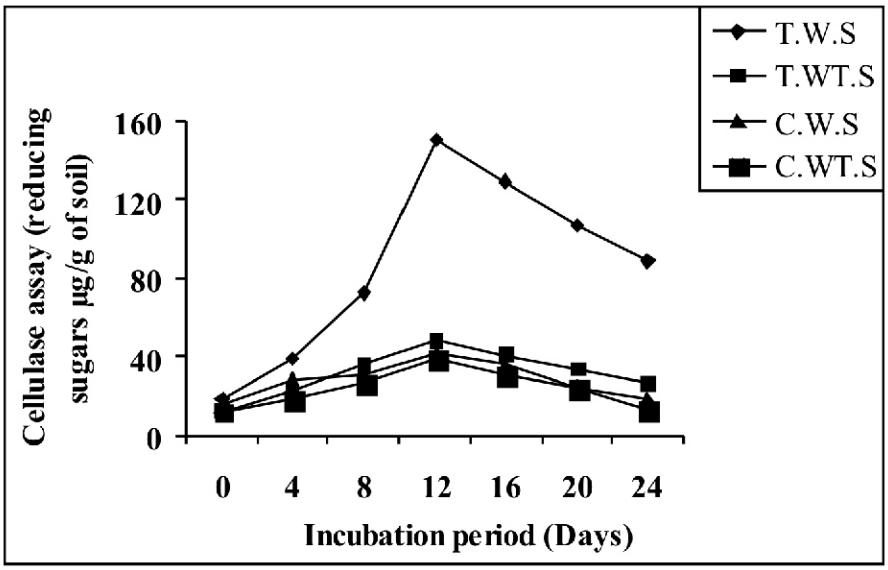

Fig. 1 : Cellulase activity of soil contaminated with/without paper industrial effluents. T.W.S: Test with substrate, T.Wt.S; Test without substrate; C.W.S: Control with substrate; C.Wt.S: Control without substrate.

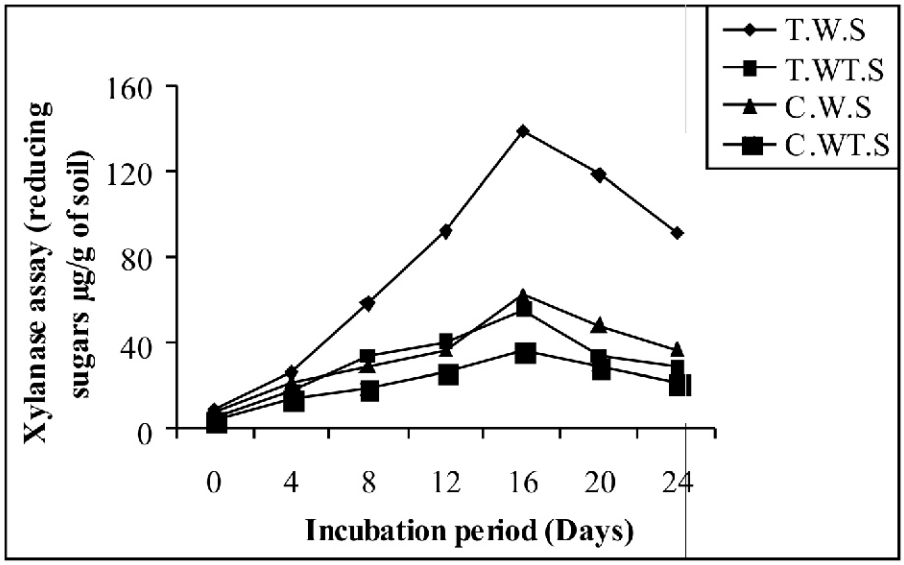

Fig. 2 : Xylanase activity of soil contaminated with/without paper industrial effluents. T.W.S: Test with substrate, T.Wt.S; Test without substrate; C.W.S: Control with substrate; C.Wt.S: Control without substrate.

the present study, predominant enzymes produced from microbes were cellulase followed by xylanase, pectinase and invertase, because the lignocellulosic material present in the soil was very high. Time taken to reach the maximum activity of cellulase and pectinase enzymes was lower (12 d) than that of xylanase enzyme activity (16 d), perhaps due to the complex nature of substrate-enzyme interaction. But in case of invertase the maximum activity was observed with in 12 h. The components like nitrogen, potassium and phosphorus also increased in the test soil, which would add on to the fertility of the soil that may aid in improved crop production. Land filling with or without incineration or pre–composting is the most common effluent disposal method. Composted paper mill wastes have been proposed as good soil conditioners because of their high organic matter (OM) content and low toxicity. Results also show that the enzymes present in the test soil are higher than the control soil due to the presence of higher microbial activity, which is influenced by the nutrients of the paper industry effluents.

CONCLUSION

Based on the above studies it can be suggested that the paper industrial effluents may be used for the enhancement of the soil fertility. Effluents can change the physico-chemical and microbiological properties along with microbial enzymes that would aid in the soil fertility. However experiments on crop production in such effluent treated soil on long term basis are to be conducted to prove the effluent benefits in the soil.

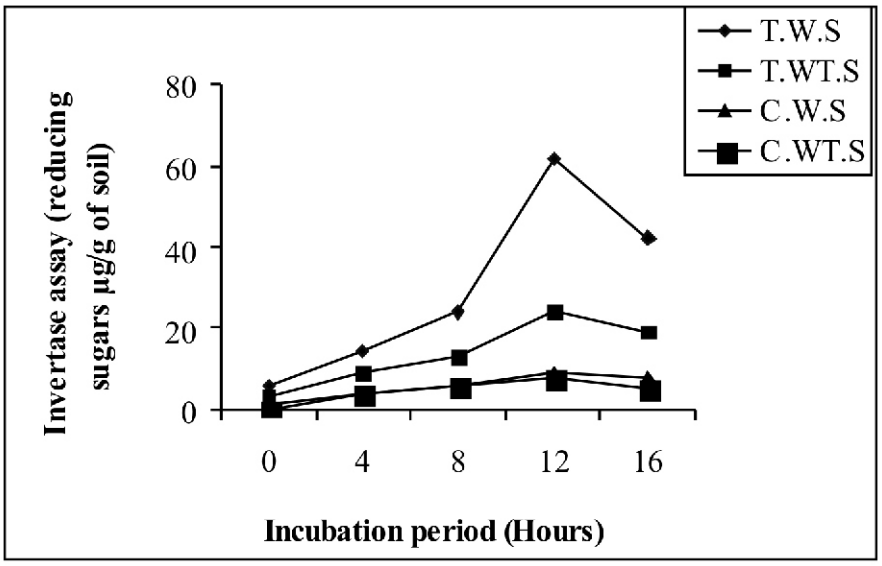

Fig. 3 : Invertase activity of soil contaminated with/without paper industrial effluents. T.W.S: Test with substrate, T.Wt.S; Test without substrate; C.W.S: Control with substrate; C.Wt.S: Control without substrate.

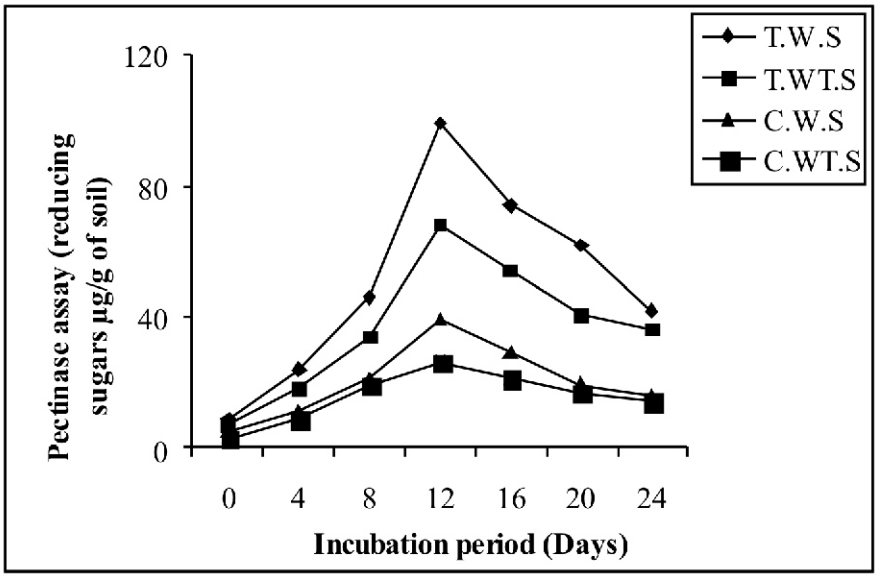

Fig. 4 : Pectinase activity of soil contaminated with/without paper industrial effluents. T.W.S: Test with substrate, T.Wt.S; Test without substrate; C.W.S: Control with substrate; C.Wt.S: Control without substrate.

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