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IMPACT OF PAPER INDUSTRY EFFLUENTS ON SOIL CELLULASE ACTIVITY

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ABSTRACT

Release of industrial effluents causes indicative changes in nutrient cycling and organic matter processing. In view of importance of soil enzymes in biochemical functioning of natural resource - soil system for recycling of nutrients, impact of industrial effluents on enzyme activities in soil such as cellulase enzyme were examined in this study. In this direction, soil samples were collected from Andhra Pradesh Paper Mills, Rajmandry, and Andhra Pradesh, India. The experimental results indicated that, most of the physicochemical properties such as silt, clay, electrical conductivity, water holding capacity, organic matter and total nitrogen contents, microbial population and selected enzyme activities were significantly higher in the test sample than in the control. Additionally, activities were increased with increasing the incubation period up to 21 d over 0 d, however, activities were adversely affected at 28 d. Furthermore, relatively higher activities were observed in soil incubated in the presence of substrate than in the absence of substrate.

1. INTRODUCTION

Soil is an important system of terrestrial ecosystem. There is a direct impact of pollutants on minerals, organic matter and microbial community of soil [1]. The discharge of industrial effluents especially without treatment may have profound influence on physico-chemical and biological properties of soil related to soil fertility. A wealth of information on occurrence of changes in properties of soils due to discharge of effluents from other industries is available such as cotton ginning mill [2], sugar industry [3], paper mill [4], dairy industry [5], and dairy wastewater [6]. Thus determination of enzyme activity and microbial biomass, chemical soil parameters seems to be the best approach for evaluating the state of microbial activity. Alarmingly, effluents from paper industry, a major industry that produces huge volume of waste water, contains several toxic and non-biodegradable organic materials, which include sulphur compounds, pulping chemicals, organic acids, chlorinated lignin's, resin acids, phenolics, unsaturated fatty acids and terpenes, eventually these may affect soil enzyme activities, which in turn soil fertility. In reality, the soil enzymes occupy a vital role in catalyzing reactions associated with organic matter decomposition and nutrient cycling [7].

In the present study, an attempt has, therefore been made to find out the impact of effluents of paper industry on soil physical [pH, EC, water holding capacity, soil texture], chemical [organic matter, total nitrogen,], biological [bacterial and fungal populations] properties and selected soil cellulase activity.

2. MATERIALS AND METHODS

2.1 Collection of soil samples

Soil samples were collected from the surrounding areas [1/4 km] of Andhra Pradesh Paper Mills, Rajmandry, and Andhra Pradesh, India. Soil sample without effluent discharges served as control was collected from adjacent site [1 km away] of industry. Soil samples both with and without effluents were used for determination of physico-chemical, biological and enzyme activities. These two soil samples were air dried and mixed thoroughly to increase homogeneity and shifted to < 2 mm sieves for determination of soil texture.

2.2 Physico-chemical characters

The physical, chemical and biological properties of test and control soils were determined by the following standard procedures. The soil particles like sand, silt and clay contents were analyzed with the use of different sieves by the method of Alexander [8]. Whereas water holding capacity, organic carbon, total nitrogen, and soil samples were determined by the methods of a [9] [10], Walkley-black [11], and Microkjeldhal [12] and [13] [14], respectively. Electric conductivity and pH were determined by Elico conductivity meter and pH meters, respectively.

2.3 Biological parameters

Micro flora such as bacterial and fungal populations of both soil samples were enumerated by serial dilution technique. One gram of each soil sample was serially diluted and 0.1 ml was spread with a sterile spreader on nutrient agar medium and Czapeck-Dox agar medium for the isolation of bacteria and fungi respectively. Nutrient agar plates were incubated at 37º C for 24 h, where as Czapeck-Dox plates were at room temperature for 7 d. After incubation period, colonies formed on the surface of the medium were counted by colony counter [2].

2.4 Cellulase activity

Cellulase activity of two soil samples was determined by placing 5g of each soil sample with 60% water holding capacity in separate boiling test tubes (25×200mm) at 28±4ºc. The activity of cellulase was assayed as described by [15]. Triplicate samples (5g) of each soil were withdrawn after 0, 7, 14, 21 and 28days of incubation, placed in 50ml Erlenmeyer flasks and 0.5ml of toluene was added. Contents in the flasks were mixed thoroughly, after 15min,10 ml of acetate buffer of 0.5M (p^H 5.9) was added and followed by 10ml of 1% carboxyl methyl cellulose (CMC). After 30 min of incubation, approximately 50ml of distilled water was added. Then the suspension was filtered by Whatman No.1 filter paper and the volume of the filtrate was made upto 100ml with distilled water. The resultant filtrate was used for the determination of reducing sugar content by [5] in Elico digital spectrophotometer. Finally, cellulase activity was expressed in terms of mg of Glucose Equivalents per g of soil per 30min (mg GE g^{-1} $30m^{-1}$).

2.5 Statistical Analysis

The activities of the cellulase were calculated on the basis of soil weight (oven dried). Data were analyzed using one-way ANOVA and the differences contrasted using Duncan's multiple range test (DMRT) [12]; [3]. All statistical analysis was performed at ($P < 0.05$) using SPSS statistical software package.

3. RESULTS AND DISCUSSION:

3.1 Physicochemical characters:

Effluent discharged soil samples underwent significant changes (Table 1) in all measured parameters in comparison to control. Soil texture in terms of percentage of Clay, Silt and Sand were 54, 38 and 8 in the test; 32, 21and 47 in the control soils, respectively. The above results indicated that test samples had relatively lower Sand and higher Clay and Silt contents than control samples. The pH of the test sample was decreased to 7.02 from 8.30 upon the release of effluents. Water holding capacity, Electrical conductivity, Organic matter and total nitrogen contents were higher in the test samples over the control samples and they were 0.34ml/gm, 1.71µS/cm (microsiemens cm^{-1}), 6.432g/kg (grams $Kilograms^{-1}$) and 0.22g/kg of the test against 0.2ml/g, 0.24µS/cm, 3.6g/kg and 0.14g/kg of the control, respectively.

Table 1. Physico-chemical characteristics of soil as affected by paper industry effluents.

Character	Control[a]	Test[b]
Color	Black	Thick black
Odor	Normal	Normal
pH [1:1.25 soil–water slurry]	8.30	7.02
Texture:		
Clay [%]	32	54
Silt [%]	21	38
Sand [%]	47	8
Electrical conductivity [μS/cm]	0.24	1.71
60% Water-holding capacity [ml g^{-1}]	0.2	0.34
Organic matter [%]	3.6	6.432
Total nitrogen [g kg^{-1} soil]	0.14	0.22

a. soil polluted without paper industry effluents
b. soil polluted with paper industry effluents

3.2 Biological characters:

The micro flora of both soil samples were enumerated and listed in table 2. Three fold higher bacterial and two fold higher fungal populations were observed in the test soil over the control soil.

Table 2. Biological characters of soil as affected by Paper industry effluents.

Microflora	Control[a]	Test[b]
Bacteria	64×10^4	192×10^4
Fungi	7×10^4	15×10^4

a. soil without paper industry effluents
b. soil polluted with paper industry effluents
c. Microbial population in terms of colony forming units g^{-1} of soil.

3.3 Cellulase activity:

The present results clearly indicate that the activity of cellulase was greatly enhanced in test soil over the control. By increasing the soil incubation period, the cellulase activity was increased up to 21 days interval, and was declined in both samples. For example, cellulase activity of the test sample at 0day was 0.42 mg GE g^{-1} 30 min^{-1}, it was increased by two fold 0.74 mg GE g^{-1} 30 min^{-1} at 21day , and later declined by 0.27 mg GE g^{-1} 30 min^{-1} at 28day. Same trend was also noticed in the control soil. Furthermore, higher activity was recorded in test sample than in control sample at all incubation periods. For instance, the test sample exhibited more cellulase activity over the control at 0 day interval; it was 0.42 mg GE g^{-1} 30 min^{-1} against 0.30 mg GE g^{-1} 30 min^{-1} of the control soil and same trend was continued at the rest of the incubation period.

Table 3 Cellulase activity* in soil [with and without substrate] after 30 min incubation as influenced by paper industry effluents

Incubation in days	Activity of Cellulase			
	Test		Control	
	With substrate	Without substrate	With substrate	Without substrate
0	0.42 ± 0.04	0.30 ± 0.05	0.40 ± 0.05	0.22 ± 0.01
7	0.55 ± 0.05	0.50 ± 0.04	0.50 ± 0.06	0.32 ± 0.01
14	0.68 ± 0.04	0.57 ± 0.05	0.58 ± 0.04	0.47 ± 0.06
21	0.74 ± 0.03	0.66 ± 0.04	0.67 ± 0.04	0.58 ± 0.06
28	0.27 ± 0.06	0.22 ± 0.04	0.16 ± 0.06	0.14 ± 0.05

*mg glucose g^{-1} 30 min^{-1}

The microorganisms play a vital role in nutrient cycling and soil fertility. Bacteria and fungi synthesize and secrete enzymes such as amylase, cellulase, ureases, proteases, phosphatases, pectinases are extracellular. Those microbial secreted enzymes constitute an important part of soil matrix as extra cellular enzymes [7]. Thus, there is a considerable interest in the study of enzyme activities of soil [16], because such activities may reflect the potential capacity of a soil to form certain biological transformation of importance to soil fertility [17]

Little information is available on the effect of industrial effluents on soil cellulase activity. In this direction, cellulase activity was enhanced in soils treated with the effluents of textile and sugar industry [18], cotton ginning mills [19], paper mill effluent and amendment addition [20], solid urban waste [21], and sodium based black liquor from fiber pulping for paper making [22] over untreated soils. Similarly, urban expansion into wild lands significantly increased the cellulase activity [23], Contrary to this, soil contaminated with cement dust, the cellulase activity was ceased [24].

4. CONCLUSION

The results of the present investigation clearly indicated that discharge of effluents from paper industry has altered the physico-chemical properties, affected the micro flora and enhanced the cellulase activity of the soil, but it was declined with the time. Furthermore, by increasing the effluents concentration, the enzyme activity was improved up to 50% and later decreased. This observation, therefore, greatly warrants a prior treatment of paper industry effluents before discharging into a water body or on to agricultural land and additional research will be necessary to discriminate the type of cellulase producing microorganisms (genera and species).

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