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SOIL MICROBIAL RESPONSE TO PAPER INDUSTRY EFFLUENTS

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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, protease, amylase and urease were examined in this study. In this direction, soil samples were collected from Andhra Pradesh Paper Mills, Rajmandry, 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, activites were increased with increasing the incubation period upto 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.

KEYWORDS: Effluents, Paper industry, Soil enzymes.

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 (Lowry *et al*.,1951). 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 (Nagaraju *et al*.,2007), sugar industry (Megharaj *et al*.,1999), paper mill (Nelson, D.W and Sommers.,1996), dairy industry (Nelson, 1944), and dairy wastewater (David shyam Babu, 2010). 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 lignins, 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 (Poonkothai. M and R. Parvatham, 2005).

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

MATERIALS AND METHODS

Collection of soil samples

Soil samples were collected from the surrounding areas [1/4 km] of Andhra Pradesh Paper Mills, Rajmandry, 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.

Physico-chemical and biological properties of soil

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 (Alexander, 1961). Where as water holding capacity, organic carbon, total nitrogen, and soluble phosphorous of soil samples were determined by the methods of a Johnson & Ulrich (Jackson, M.L, 1971), Walkley-black (Narasimaha *et al*., 2011), and Microkjeldal (Gooty Jaffer mohiddin et al., 2011) and Kurrevich and Shcherbakova (Kaushik et al., 2005), respectively. Electric conductivity and pH were determined by Elico conductivitymeter and pH meters, respectively.

Biological parameters

Micro flora such as bacteria 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 (Nagaraju *et al*., 2007).

Enzyme assays

Five grams of soil samples contaminated with/without effluents of paper industry effluents were transferred to test tubes. Soil samples were maintained at 60% water holding capacity at room temperature in the laboratory [28 ± 4 °C]. Triplicate soil samples of each waste water treated and controls were withdrawn at periodic intervals to determine the soil enzyme activities as detailed earlier by Tu (Sparling *et al*., 2001). The method employed for the assay of amylase, protease, cellulase and urease were essentially the same developed by Cole (Cole, 1977), Speir and Ross (Sinsabaugh, 1994), Pancholy and Rice (Nelima and Madhuri, 2005), and Zantua and Bremner (Tu. C.M, 1982), respectively. The soil samples were transferred to 250 mL of Erlenmeyer flasks and one mL of toluene was added. After 15 min, 6 mL of 0.2 M acetate phosphate buffer [pH 5.5] containing either 2% starch [amylase], 2% casein [protease] and 1% CMC [cellulase] were added to soil samples and flasks were plugged with cotton and held for 48 h [amylase], 24 h [protease], and 30 min [cellulase] at 30 °C. After incubation, soil extracts were passed through whatman filter paper, then glucose [amylase, cellulase] and tyrosine [protease] contents in the filtrate were determined by the methods of Nelson-Somagyi (Narasimha *et al*., 1999), Lowry (Kuprevich and Shecherbakova, 1972), respectively. For urease the method comprises release of ammonia up on incubation of soil with 4 mL of sodium phosphate buffer [pH 7.0], 1 mL of 1 M urea solution incubated for 30 min and 10 ml of 2 M KCl was added and kept at 4 ºC for 15 min and centrifuged, then 0.5 mL of nesslers reagent followed by 3.5 mL of distilled water were added and the color was read at 495 nm, in an digital spectrophotometer.

Statistical Analysis

The activities of the cellulase, amylase, protease and urease was 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) (Gooty Jaffer Mohiddin *et al*., 2011; Megharaj *et al*., 1999). All statistical analysis was performed at (P# 0.05) using SPSS statistical software package.

RESULTS AND DISCUSSION

Soil samples of both with and without effluents discharge were analyzed for their physico-chemical properties and their results were represented in table 1. Soil samples with paper industry effluent underwent changes in all measured parameters of physical and chemical properties in comparison to control. Threre was no noticible change in the pH of the test soil over control. However, soil texture in terms of percentage of sand, silt, clay was 8, 38, 54 in test and 47, 21, 32 in the control soils, respectively. Higher water holding capacity was observed in test soil than control, values were found to be 0.34 and 0.2 mL g^{-1}, respectively. The electrical conductivity of both test and control soils were 1.71 and 0.24 µ Mhos cm^{-1}, respectively. Increased water holding capacity and electrical conductivity in contaminated soil may be due to the accumulation of organic waste such as amino acid residues, acids and alkalis in the paper industry effluents. The results were in conformity with the studies of Sparling et al (Reddi Pradeep and Narasimha, 2012), Narasimha et al (Nagaraju *et al*, 2007), Poonkothai and Parvatham (Nizamuddin *et al*., 2008), and Xaiao et al (Speir and Ross, 1975) had increased electrical conductivity in soil contaminted by the effluents of dairy, cotton gining, automobile, and black liquor for straw pupling industries, respectively. The parameters like organic matter percentage, total nitrogen, phosphorus, potassium were higher in test soil than the control soil. The values of above properties of test sample were 6.432%, 0.22 g kg^{-1}, 197 kg ha^{-1}, 1755 kg ha^{-1}, and control soil were 3.6%, 0.14 g kg^{-1}, 194 kg ha^{-1}, 1301 kg ha^{-1}, respectively [Table 1]. Higher organic matter of the polluted soil may be due to the discharge of waste water in organic nature. Also, increased organic matter enhanced soil enzyme activity. Narasimha et al (Nagaraju et al, 2007) and Kaushik et al (Johnson and Ulrich, 1960) made similar reports on the discharge of effluents from cotton ginning and distillery industries, respectivley. Thus, soil is a potent system of terrestrial ecosystem, and direct discharge of industrial effluents especially that without treatment may have profound influence on physico chemical and biological properties of soil related to soil fertility (Nagaraju *et al*, 2009). Similalry, discharge of effluents from various industries like sugar industry (Lowry *et al*., 1951), dairy factory (Nelson, 1944) and petrochemical industry (Andrade, 2012) influenced the physico-chemical properties of soil. This is due to organic waste that may contribute to maintain or increase the organic matter and nutrient content in the soil (Bollag *et al*., 2002).

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 (Poonkothai and Parvatham,

2005). Thus, there is a considerable interest in the study of enzyme activities of soil (Burns et al., 1978), becasue such activities may reflect the potential capacity of a soil to form certain biological trasformation of importance to soil fertility (chandrayan *et al*., 1980).

Table 1. Physico-chemical characteristics of soil samples

Character	Control	Test
Color	Black	Thick black
Odor	Normal	Normal
pH [1:1.25 soil–water slurry]	7.01	7.02
Texture: Clay [%] Silt [%] Sand [%]	 32 21 47	 54 38 8
Electrical conductivity [µmhos/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
Available phosphorus [P_2O_5] in [kg/ha]	194	197
Available potassium [K_2O] in [kg/ha]	1301	1755

Table 2. Microflora* of soil samples

Microflora	Control	Test
Bacteria	6.4×10^6	1.92×10^8
Fungi	7×10^4	15×10^5

* colony forming units per g of soil

Table 3 Cellulase activity* in soil [with and with out substrate] after 30 min incubation as influenced by paper industry effluents

Incubation in days	Activity of Cellulase			
	Test		Control	
	With substrate	With out substrate	With substrate	With out 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}

Table 4. Amylase activity* in soil [with and without substrate] after 48 h incubation as influenced by paper industry effluents

Incubation in days	Amylase activity			
	Test		Control	
	With substrate	Without substrate	With substrate	Without substrate
0	0.60 ± 0.04	0.36 ± 0.05	0.30 ± 0.02	0.24 ± 0.03
7	0.64 ± 0.05	0.45 ± 0.04	0.43 ± 0.04	0.34 ± 0.01
14	0.67 ± 0.02	0.48 ± 0.04	0.50 ± 0.04	0.38 ± 0.04
21	0.81 ± 0.03	0.54 ± 0.01	0.57 ± 0.03	0.47 ± 0.04
28	0.51 ± 0.01	0.31 ± 0.03	0.35 ± 0.04	0.22 ± 0.05

*Glucose g^{-1} 48 h^{-1}

Table 5. Protease Activity* in soil [with and without substrate] after 24 hr incubation as influenced by paper industry effluents

Incubation in days	Protease activity			
	Test		Control	
	With substrate	With out substrate	With substrate	With out substrate
0	0.83 ± 0.01	0.60 ± 0.04	0.69 ± 0.01	0.64 ± 0.02
7	0.91 ± 0.02	0.82 ± 0.03	0.90 ± 0.01	0.78 ± 0.02
14	1.45 ± 0.03	1.12 ± 0.02	1.12 ± 0.01	0.97 ± 0.02
21	1.49 ± 0.05	1.47 ± 0.04	1.40 ± 0.07	1.13 ± 0.04
28	0.71 ± 0.04	0.57 ± 0.06	0.66 ± 0.01	0.59 ± 0.05

*mg tyrosine g^{-1} 24 hr^{-1}

Table 6. Urease Activity* in soil [with and without substrate] after 30 min incubation as influenced by paper industry effluents

Incubation in days	Urease activity			
	Test		Control	
	With substrate	With out substrate	With substrate	With out substrate
0	2.62 ± 0.08	1.68 ± 0.08	1.42 ± 0.07	0.93± 0.05
7	2.76 ± 0.09	1.88 ± 0.05	1.60 ± 0.09	1.22 ± 0.07
14	2.93 ± 0.09	2.00 ± 0.07	1.82 ± 0.07	1.62 ± 0.05
21	3.00 ± 0.10	2.45 ± 0.09	2.16 ± 0.08	1.98 ± 0.08
28	2.63 ± 0.09	1.67 ± 0.09	1.40 ± 0.08	1.09 ± 0.07

*mg NH_4^+-N g^{-1} 30 min^{-1}

Micro flora of both samples were enumerated and listed in table 2. Polluted soil caused two fold increases in bacterial and fungal population compared to control soil [Table 2]. Majorly, the enzymes protease and amylase play a crucial role in catalysing the hydrolysis and solubilising the substrates containing N_2 and C, respectively. The activity of the protease in polluted and non polluted soils was detremined and results listed in table 5. The activity of protease, as evidenced by the accumulation of tyrosine from caesin was considerably greater in the soils polluted with effluents at all incubations over control. Furthermore, both the samples showed increased activity upto 21 d of interval and then the activity was declined at further incubation. For instance, test sample exhibited 0.83 mg of tyrosine equivalents per gram of soil per 24 h against 0.69 mg TE g^{-1} 24 h^{-1} of control at 0 d, later it was increased in both soils upto 21 d and declined at 28 d interval. However, the increased protease activity in polluted soil over control may be due to availability of substrate and or caesin degrading microflora in polluted soil [Table 5]. Similar results were reported by Reddi Pradeep and Narasimha (Pancholy and rice., 1973) that cotton ginning effluents increased the soil protease activity. The amylase activity was measured in terms of release of glucose from starch. There was an increase in activity upto 21 d incubation, there after activiteis were adversely affected. For intance, amylase activity in polluted soil increased from 0.60 to 0.81 mg glucose equivalents g^{-1} 48 h^{-1} from 0 to 21 d. Later it was decreased to 0.51 mg GE g^{-1} 48 h^{-1} at 28 d. Comparision of soil amylase activity in soil samples with/without effluents discharged revealed that the soil polluted with effluents stimulated the amylase activity by ~ 2 fold than control soil. Narasimhan et al (Nagaraju et al., 2007) made a similar observation in soils polluted with cotton ginning mill effluents stimulated the soil amylase activity

The cellulase activity was measured in terms of release of glucose from CMC. There was an increase in the formation of glucose with increasing soil incubation such as 0, 7, 14, and 21 d. The cellulase activity was decreased after 21 d of incubations [Table 3]. For intance, the cellulase activity in test soil increased from 0.42 mg GE g^{-1} 30 min^{-1} to 0.74 mg GE g^{-1} 30 min^{-1} 0 to 21 d. Later it was decreased to 0.27 mg GE g^{-1} 30 min^{-1} at 28 d incubation. Same was reported by Narasimhan et al (Nagaraju et al., 2007) in soils polluted with effluents of cotton ginning mills stimulated the soil cellulase activity at early d of incubation.

Urea is an organic chemical complex used mainly as nitrogenous fertiliser in agriculture. Conversion of this nitrogen to inorganic nitrogen-ammonia and carbaon dioxide takes place due to activity of urease enzyme, secreted by certain microorganisms and is responsible for supply of nitrogenous demand to growing crops. Assay of urease activity in soil samples involves quantification of ammonia released upon hydrolysis of urea. Urease activity in soils with/without effluents discharges was measured. Urease activity also increased upto 21 d of incubation and later declined. For instance the urease activity in test soil with substrate at 0 d was 2.62 mg NH_4^+-N g^{-1} 30 min^{-1}, it was increased to 3.0 mg NH_4^+-N g^{-1} 30 min^{-1} at 21 d and declined to 2.63 mg NH_4^+-N g^{-1} 30 min^{-1} at 28 d. Similar treand was also noticed

in other sampels also. Similar results were noticed by Narasimha et al (Nagaraju et al., 2009) that urease activity was increased in the first week of incubation, there after declined in soil contaminated with cotton ginning mill effluets.

CONCLUSION

The present study clearly indicates that the disposal of effluents from paper industry alters the physic-chemical, biological properties and activities of enzymes such as protease, amylase, cellulase and urease were stimulated in soil over control. Nonetheless, prolonged incubation causes adverse effects. Thus, 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 these extra cellular enzyme producing microorganisms [genera and species].

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