ISSN: 2319-8753

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 10, October 2014

Effect of Paper Industry Effluents on Soil Protease and Urease Activity

P. Gouthami [1], M. Sahana [2], L. Saida Naik [3], K. Venkateswar Reddy [4]

P.G. Student, Centre for Biotechnology, Institute of Science and Technology, Jawaharlal Nehru Technological University, Hyderabad, Telangana State, India[1]

P.G. Student, Centre for Biotechnology, Institute of Science and Technology, Jawaharlal Nehru Technological University, Hyderabad, Telangana State, India[2]

Assistant Professor, Centre for Biotechnology, Institute of Science and Technology, Jawaharlal Nehru Technological University, Hyderabad, Telangana State, India[3]

Lecturer, Centre for Biotechnology, Institute of Science and Technology, Jawaharlal Nehru Technological University, Hyderabad, Telangana State, India[4]

ABSTRACT: Release of paper industry effluents on to the agricultural lands causes indicative changes in nutrient cycling and organic matter processing. In the present study, paper industry effluent discharged soil (test) and undischarged soil (control) were collected from the surrounding areas of paper industry. The physicochemical, biological properties and soil enzyme activities such as protease and urease were examined. The experimental results indicated that, most of the physicochemical properties such as silt, clay, electrical conductivity, water holding capacity, organic matter and total nitrogen, phosphorous and potassium, 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 upto 21 days over 0 days, however, activities were adversely affected at 28 days. Furthermore, relatively higher activities were observed in soil incubated in the presence of substrate than in the absence of substrate.

KEYWORDS: Paper industry, effluents, physicochemical, biological, soil enzymes.

I. 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), paper industry (3), paper industry (4), dairy industry (5), and dairy wastewater (6). Thus, determination of enzyme activity and microbial biomass, soil physico-chemical 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. Nevertheless, 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), chemical (organic matter, total nitrogen, phosphorus and potassium), biological (bacterial and fungal populations) properties and selected soil enzyme activities.

II. MATERIALS AND METHODS

A.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.

B. Physico-chemical properties of soil

The physico-chemical 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 soluble phosphorous of soil samples were determined by the methods of a Johnson & Ulrich(9), Walkey-black (10), Microkjeldhal (11) and Kurrevich and Shcherbakova (12), respectively. Electric conductivity and pH were determined by Elicoconductivitymeter and pH meters, respectively.

C.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 plates for the isolation of bacteria and fungi, respectively. Nutrient agar plates were incubated at 37 ºC for 24 h, where as Czapeck-Dox agar plates were at room temperature for 7d. After incubation period, colonies formed on the surface of the medium were counted by colony counter (2).

D.Enzyme assays

Five grams of soil samples contaminated with/without effluents of paper and board mills 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 thesoil enzyme activities as detailed earlier by Tu (13). The method employed for the assay of protease and urease were essentially the same developed by Speir and Ross (14), Pancholy and Rice (15), and Zantua and Bremner (16), respectively. The soil samples were transferred to 250mL of Erlenmeyer flasks and one mL of toluene was added. After 15min, 10mL of 2% casein(protease) in 0.1 M tris buffer at ph(7.5) was added and flasks were incubated at 30ºC for 24hrs.After incubation,soil extracts were passed through whatmann filter paper,then tyrosine(protease) contents in the filtrate were determined by the method of Folin-Lowry(17). For urease the method comprises release of ammonia up on incubation of soil with 4mL of 0.2 M sodium phosphate buffer (pH 7.0), 1mL of 1 M urea solution incubated for 30 min and 10ml of 2M KCl was added and kept at 4 ºC for 15 min and centrifuged, then 0.5mL of Nesslers reagent followed by 3.5mL of distilled water were added and the color was read at 495nm, in an digital spectrophotometer.

III. RESULTS AND DISCUSSION

Soil samples of both with and without effluents discharge were analysed for their physico chemical properties and their results were represented in table 1. Soil samples with paper and board mills industry effluent underwent changes in all measured parameters of physical and chemical properties in comparison to control. There was no noticeable change in the pH of the test soil over control. However, soil texture in terms of percentage of clay, silt, sand were 34,32,34 in test and 33,32,35 in the control soils, respectively. Higher water holding capacity was observed in test soil than control, values were found to be 2.5 and 1.3 mL g^{-1}, respectively. The electrical conductivity of both test and control soils were 275 and 151 µMhos 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.

ISSN: 2319-8753

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TABLE 1
PHYSICO-CHEMICAL CHARACTERISTICS OF SOIL SAMPLES

	Control a	**Test b**
Colour	Black	Brown
Odour	Normal	Unpleasant
PH	4	5
Texture Clay(%) Slit(%) Sand(%)	 33 32 35	 34 32 34
Electrical Conductivity(µmhos/cm)	151	275
60% Water holding capacity(mLg^{-1})	1.3	2.5
Organic matter (%)	876	11.8
Total Nitrogen	0.16	0.24
Available Phosphorous(P_2O_5) in (kg/ha)	368	428
Available Potassium(K_2O) in (kg/ha)	628	723

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

The results were in conformity with the studies of Sparling et al (18), Narasimha et al (2), Poonkothai and Parvatham (19), and Xiao et al (20) had increased electrical conductivity in soil contaminated by the effluents of dairy, cotton ginning, automobile, and black liquor for straw pulping 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 11.8 %, 0.24 gkg^{-1}, 428 kg ha^{-1}, 723 kg ha^{-1}, and control soils were 8.76 %, 0.16 g kg−1, 368 kg ha−1, 628 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 (2) and Kaushik et al (21) made similar reports on the discharge of effluents from cotton ginning and distillery industries, respectively. 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 (22). Similarly, discharge of effluents from various industries like sugar industry, dairy factory (5) and petrochemical industry (23) 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 (24).

The microorganisms play a vital role in nutrient cycling and soil fertility. Bacteria and fungi synthesize and secrete enzymes such as amylase, cellulose, ureases, proteases, phosphates, pectinase 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 (25), because such activities may reflect the potential capacity of a soil to form certain biological transformation of importance to soil fertility (26). 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).

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TABLE 2
MICROFLORA* OF SOIL SAMPLES

Micro flora	**Control a**	**Test b**
Bacteria	$28x10^6$	$46x10^6$
Fungi	$12x10^6$	$24x10^6$

* Colony forming units per g of soil

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

Protease Activity

The enzyme protease plays a crucial role in catalyzing the hydrolysis and solubilising the substrates containing $_{N2and}$ C, respectively. The activity of the protease in polluted and non polluted soils was determined and results listed in table 3. The activity of protease, as evidenced by the accumulation of tyrosine from casein was considerably greater in the soils polluted with effluents at all incubations over control. Furthermore, both the samples showed increased activity up to 21 d of interval and then the activity was declined at further incubation. For instance, test sample exhibited 0.05 mg of tyrosine equivalents per gram of soil per 24 h against 0.02 mg TE g^{-1} 24 h^{-1} of control at 0 days, later it was increased in both soils up to 21 days and declined at 28 days interval. However, the increased protease activity in polluted soil over control may be due to availability of substrate and or casein degrading micro flora in polluted soil (Table 3). Similar results were reported by Reddi Pradeep and Narasimha (27) that cotton ginning effluents increased the soil protease activity.

TABLE 3

PROTEASE ACTIVITY* IN SOIL AFTER 24 H INCUBATION AS INFLUENCED BY PAPER INDUSTRY EFFLUENTS

Incubation in days	**Protease activity**			
	Test a		**Control b**	
	WS	**WOS**	**WS**	**WOS**
0	0.05± 0.005	0.03± 0.01	0.03± 0.00	0.02± 0.01
7	0.75± 0.01	0.65± 0.003	0.62± 0.04	0.53± 0.01
14	1.09± 0.01	0.83± 0.01	0.69± 0.01	0.58± 0.01
21	1.21± 0.05	1.16± 0.18	0.93± 0.06	0.64± 0.01
28	0.34± 0.02	0.26± 0.01	0.25± 0.07	0.20± 0.01

*mg tyrosine g_{-1} 24 h_{-1}
a.soil polluted with paper industry effluents
b.soil polluted without paper industry effluents

Control - Soil without paper and board mills industry effluents Test – Soil polluted with paper and board mills industry effluents

WS – with substrate WOS – without substrate

All entries are average mean of triplicate values

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Urease Activity

Urea is an organic chemical complex used mainly as nitrogenous fertilizer in agriculture. Conversion of this nitrogen to inorganic nitrogen-ammonia and carbon 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 and results were shown in table 4. Urease activity also increased up to 21days of incubation and later declined. For instance the urease activity in test soil with substrate at 0 days was 0.50 mg $NH4^{+}$- N g−1 30 min−1, it was increased to 1.23 mg NH4+-N g−1 30 min−1 at 21 days and declined to 0.21 mg $NH4^{+}$- N g−1 30 min^{-1} at 28 days (Table 4).

TABLE 4
UREASE ACTIVITY* IN SOIL AFTER 30 MIN INCUBATION AS INFLUENCED BY PAPER INDUSTRY EFFLUENTS

Incubation In Days	Urease activity			
	Test a		Control b	
	Ws	Wos	ws	wos
0	0.50± 0.03	0.41± 0.03	0.39± 0.01	0.27± 0.01
7	0.71± 0.01	0.60± 0.02	0.55± 0.2	0.42± 0.05
14	0.87± 0.01	0.74± 0.03	0.58± 0.07	0.49± 0.03
21	1.23± 0.01	1.19± 0.01	0.46± 0.04	0.33± 0.01
28	0.21± 0.01	0.15± 0.01	0.12± 0.005	0.06± 0.01

*mg NH4+-N g−1 30 min−1
Refer table 3 foot note for other details
a.soil polluted with paper industry effluents
b.soil polluted without paper industry effluents

Similar trend was also noticed in other samples also. Similar results were noticed by Narasimha et al (24) that urea's activity was increased in the first week of incubation, there after declined in soil contaminated with cotton ginning mill effluents.

IV. 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 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).

ACKNOWLEDGEMENT

Authors are thankful to the staff of Andhra Pradesh paper Mills, Rajmandry, Andhrapradesh, India, for their timely help and encouraging us throughout this study.

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ISSN: 2319-8753

International Journal of Innovative Research in Science, Engineering and Technology

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