International Journal of Bioassays
ISSN: 2278-778X
CODEN: IJBNHY
OPEN ACCESS

Original Research Article

INFLUENCE OF COTTON GINNING MILL EFFLUENTS ON SOIL PROTEASE AND UREASE ACTIVITY

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Received for publication: August 05, 2014; **Revised:** August 11, 2014; **Accepted:** September 20, 2014

Abstract: Release of cotton ginning mill effluents on agricultural lands causes changes in nutrient cycling and organic matter processing. In the present study, cotton ginning industry mill discharged soil (test), and undischarged soil (control) were collected from surrounding area of cotton ginning mill. The physicochemical, biological properties and soil enzyme activities such as protease and urease were examined. These physicochemical, biological properties effect up on soil fertility. The experimental results indicated that, most of the physicochemical properties such as silt, clay, water holding capacity, organic matter and total nitrogen, phosphorous, potassium and microbial population and selected soil enzyme activities were significantly higher in the test sample than control sample. Additionally, activities were increased with increasing incubation period up to 21 days over 0 days, however, activities were adversely effected at 28 days. Furthermore, relatively higher activities were observed in soil incubated in the presence of substrate than in the absence of substrate.

Key Words: Cotton Ginning mill, Effluents, Physicochemical, Biological, Soil Enzymes.

INTRODUCTION

Soil is one of the most vital natural resources. It produces food for teeming million sand supplies raw materials for a large number of industries on which the world economy is sustained. Agro-industries include pulp, paper, sugar, ginning, textile, dairy, dyes and fruit processing and generate large volume of solid/liquid effluents and release them into environment. A perusal of the literature on the discharge of effluents on the soil (Monanmani *et al.*, 1990; Kannan and Oblisami, 1990a; Narasimha *et al.*, 1999) strongly indicates that, they cause marked changes in physicochemical, biological and enzymatic properties.

As a result of this chemical boom, many hazardous compounds are being added on a regular basis in our biosphere. Modern cotton ginning industrial process is a continuous process spanning from the unloading of raw cotton to bailing of processed cotton fibers. In order to get clear cotton for raising crop in the next season the residual cotton fiber on seeds by ginning process is usually removed by acid treatment ginning of cotton produces large quantities of solid waste in the form of cotton seeds, gin trash are released in to environment and effect the soil microbial ecological processes, such as biogeochemical cycling of elements, mineralization of carbon, nitrogen, sulfur and phosphorous Paul and Clark (1996). Any disturbance in soil ecosystem can disturb the microbial activity and effect the availability of nutrients. Changes in physicochemical and biological properties in black soil occurred due to release of cotton ginning mill effluents (Narasimha, 1999). The present study is aimed at monitoring health status of soil under the influence of effluents of cotton ginning industry by examining to further compare activities of enzymes urease & protease of wide importance in recycling of nutrients in soil with/without effluents from cotton ginning mill.

MATERIALS AND METHODS

Collection of Soil Sample

Soil samples were collected from surrounding areas of (1/4KM) of GAJALAKSHMI ginning mill located at Nandyal mandal, Kurnool district of Andhra Pradesh, India. Soil samples without effluent discharges served as control was collected from adjacent site (1km away) from mill. Soil sample both with and without effluents used for physicochemical, 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 parameters

The physico-chemical and biological properties of test and control soils were determined by following standard procedures. The soil particles like sand, silt, clay contents were analyzed with the use of different sieves by the method of Alexander (1961). Whereas water holding capacity, organic carbon, total nitrogen, soluble phosphorous of soil sample were determined by the method of Johnson and Ulrich (1960); walkey-black (1996); Microkjeldhal (1971) and kurrevich and shcherbakova (1972), respectively. Electrical conductivity and P^H were determined by Elico-conductivity meter and P^H meter respectively.

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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 medium and Czapeck-Dox agar medium agar for the isolation of bacteria and fungi respectively. Nutrient agar plates were incubated at 37°c for 24 h, whereas Czapeck-Dox agar plates were incubated at room temperature for 7d. After incubation period colonies formed on the surface of the medium were counted by colony counter (Narasimha, 2011).

Enzyme assays

Five g of soil samples contaminated with/without effluents of cotton ginning mill effluents were transferred to test tubes. Soil samples were maintained at 60% water holding capacity at room temperatures (28 c° to 32° c). Triplicate soil samples of each waste water treated and control were withdrawn at periodic intervals to determine the soil enzyme activities as detailed earlier by *Tu (1982). The method employed for the* assay of protease and urease were essentially developed by Spier and Ross (1975) and Zantua and Bremner *(2005)* respectively. The soil samples were transferred to 250 ml of Erlenmeyer flask and 1ml of toluene was added. After 15 min, 6mL of 0.2M acetate phosphate buffer (PH 5.5) containing 2% casein (protease) was added to soil samples and flasks were plugged with cotton and held for 24h at 30°c for protease enzyme activity. After incubation soil samples were passed through whatman filter paper then tyrosine content in the filtrate were determined by the method of Lowry method (1951). For urease enzyme activity 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°c for 15 min and centrifuged, then 0.5 mL of Nesslers reagent followed by 3.5 mL of distilled water were added and color was read at 495 nm, in an elico- digital spectrophotometer.

RESULTS AND DISCUSSION

Soil samples of both with &without effluents discharge were analyzed for their physicochemical properties and their results were represented in Table 1. Soil samples with cotton ginning mill effluents underwent changes in all measured parameters of physical and chemical properties in comparison to control. There was increase in the PH of the test soil over control. However soil texture in terms of percentage of clay, silt and sand were48, 27, 25 in test and 38, 26, 36 in the control soil respectively. Higher water holding capacity is observed in the test soil than the control, values were found to be 2.8 and 1.2 mL.g^{-1}, respectively. The electrical conductivity of both test and control soils were 1.88 and 0.79µmhos.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 cotton ginning mill effluents.

The results were in conformity with the studies of Sparling, *et al.,* (2001); Narasimha, *et al.,* (2011); Poonkothai and Parvatam (2005) had increased electrical conductivity in soil contaminated by the effluents of dairy, paper industry, automobile, black liquor for straw pulping industries, respectively. The parameters like organic matter percentage, total nitrogen, phosphorous, potassium were higher in the test soil than control soil. The values of above properties of test sample were 0.46gkg^{-1}, 700kg/ha, 1000 kg/ha, and control soil were 0.24gkg^{-1}, 500kg/ha, 750 kg/ha, 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.,* (2011) and kaushik *et al.,* (2005) made similar reports on the discharge of effluents from cotton ginning and distillery industries, respectively. Thus the 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 (1999). Similarly discharge of effluents from various industries like sugar industry (Narasimha, 2007), dairy factory (Nizamuddin, 2008) and petrochemical industry (2002) influenced the physico-chemical properties of soil. This is due to organic waste that may contribute to maintain or increase the organic matter nutrient content in the soil (Bollag, 2002).

Table 1: Physico-chemical characteristics of soil samples

Character	A Control	B Test
Color	Black	Thick black
Odor	Normal	Un pleasant
PH (1:1.25 soil-water slurry)	7	8.5
Texture		
Clay (%)	38	48
Silt (%)	26	27
Sand (%)	36	25
Electrical conductivity(µmhos/cm)	0.79	1.88
60%Water holding capacity(mL .g^{-1})	2.8	1.2
Organic matter (%)	3.8	5.8
Total nitrogen in(g kg^{-1})	0.24	0.46
Available phosphorous in (kg/ha)	500	700
Available potassium in(kg/ha)	750	1000

a. Soil without polluted cotton ginning mill effluents
b. Soil polluted with cotton ginning mill effluents.

The microorganisms play a vital role in nutrient cycling and soil fertility. Bacteria and fungi synthesize and secrete enzymes such as cellulases, proteases, ureases etc. Those enzymes play an important role in soil texture (Sinsabaugh, 1994), There is considerable interest in the study of enzyme activities of soil (Burns, 1978), because such activities may reflect the potential capacity of a soil to form certain biological transformation of importance to soil fertility (chandrayan, 1980). Micro floras of both soil samples were enumerated and are listed in Table 2. Polluted soil caused two fold increases in bacterial and fungal population compared to control soil (Table 2).

Table 2: Biological characters of soil as affected by cotton ginning mill effluents

Micro flora	A Control	B Test
Bacteria	$40x10^6$	$78x10^{6\,c}$
Fungi	$20x10^6$	$39x10^6$

a. Soil without polluted cotton ginning mill effluent.
b. Soil polluted with cotton ginning mill effluent.
c- Colony forming units per gm soil.

The enzyme proteases play an important role catalyzing the hydrolysis and solubilizing the substrates containing N2. The activity of protease in polluted and non-polluted soil was determined and results listed in Table 3. The activity of protease, as evidenced by the accumulation of tyrosine from casein was considerably in the soil polluted with effluents at all incubations over control. Furthermore both the soil samples showed increased activity up to21 d of interval and activity was declined further incubation. For instance the test sample exhibited 2.06 mg of tyrosine equivalents per gram of soil per 24h against 0.17 mg TE g^{-1} 24 h^{-1} of control at 0 d, later it was increased in both soil up to 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 casein degrading micro flora in polluted soil (Table 3). Similar results were reported by Reddi pradeep and Narasimha (2012) that cotton ginning effluents increased the soil protease activity.

Table 3: Protease activity* in soil after 24hr incubation as influenced by cotton ginning mill effluents

Incubation day	Protease activity			
	Test		Control	
	Ws	Wos	Ws	Wos
0	2.06±0.56	1.66±0.27	0.17±0.01	0.02±0.008
7	2.11±0.48	1.77±0.14	0.26±0.04	0.08±0.005
14	2.20±0.26	1.68±0.53	0.43±0.23	0.12±0.06
21	3.60±0.25	3.12±0.27	0.90±0.70	0.16±0.12
28	1.07±0.08	0.77±0.03	0.51±0.05	0.33±0.17

*mg Tyrosine g^{-1} 24 h^{-1}
Control-soil without polluted cotton ginning mill effluents
Test-soil with polluted cotton ginning mill effluents
WS-with substrate; WOS-without substrate
All entries are avg. mean of triplicate values.

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 on hydrolysis of urea. Urease activity also increased up to 21 d of incubation and later declined. The urease activity in test soil with substrate at 0 d was 3.56 mg $NH4^+$-N g^{-1}30 min^{-1} it was increased to 4.3 mg $NH4^+$-N g^{-1} 30 min^{-1} at 21 d and declined to 1.32 $NH4^+$-N g^{-1} 30 min at the 28 d.

Table 4: Urease activity *in soil after 30 min incubation as influenced by cotton ginning mill effluents

Incubation day	Urease activity			
	Test		Control	
	Ws	Wos	Ws	Wos
0	3.56±0.34	3.45±0.2	3.0±0.17	1.05±1.59
7	3.78±0.56	3.77±0.15	3.2±0.28	1.96±0.47
14	4.03±0.11	3.68±0.05	3.2±0.36	1.93±0.15
21	4.3±0.58	4.1±0.1	3.2±0.9	2.23±0.322
28	1.32±0.22	0.77±0.23	0.07±0.09	0.04±0.01

*mg $NH4^+$- N g^{-1} 30 min^{-1}
Control- soil without polluted cotton ginning mill effluents
Test-soil polluted with cotton ginning mill effluent
WS- with substrate
WOS-Without substrate

Same thing noticed in other samples also. Similar results showed by Narasimha *et al.*, (2011) that urease activity was increased up to 21 d of incubation there after declined in soil contaminated with cotton ginning mill effluents.

CONCLUSION

Present study clearly indicates that the disposal of effluents from cotton ginning mill changes in physico-chemical, biological properties and enzyme activities. The enzyme activities were increased up to 21 d of incubation after it shows adverse effect on it. By this observation greatly warrants a prior treatment of cotton ginning mill 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.

ACKNOWLEDGEMENT

Authors are thankful to the staff of M/s. Gajalakshmi ginning mill, Kurnool, A.P., India for their timely help and encouraging us throughout this study.

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Source of support: Nil
Conflict of interest: None Declared