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Impact of Cotton Ginning Mill Effluents on Physico-Chemical & Biological Properties of Soil

Anusha B[1] , Harshini T[2], Saida Naik L[3] and Venkateswar Reddy K[4*]

[1,2,3] Centre for Biotechnology, Institute of Science and Technology,
Jawaharlal Nehru Technological University, Hyderabad – 500085, A.P., India
[4]Centre for Environment &Biotechnology, Institute of Science and Technology,
Jawaharlal Nehru Technological University, Hyderabad – 500085, A.P., India
[*]Corresponding author: e.mail: vkvenkat07@gmail.com, [1]bottla.anusha@gmail.com,
[2]harshinicool15@gmail.com, [3]lavudisaida@jntuh.ac.in Tel: +918977375801

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ABSTRACT:

Effect of cotton ginning mill effluents on soil was assessed for Physico-chemical & Biological properties of soil in the present study. Discharge of cotton ginning mill effluents on to the soil caused changes in Physico-chemical &biological properties of soil. These changes include increase in clay and silt percentages, Electrical conductivity, Water Holding capacity, and Organic matter, total N, available K, and P in test over control sample. However, there was a less sand, higher bacterial and fungal populations were recorded in test soil. Also, cellulose degrading bacteria were isolated from cotton ginning mill effluent contaminated soil by Carboxy methyl cellulose (CMC) Agar Medium.

Keywords: Cotton ginning industry, Effluents, Physicochemical, Biological, cellulolytic bacteria

I .INTRODUCTION

Soil is one of the most vital natural resources. It produces food for teeming millions and supplies raw materials for a large number of industries on which the world economy is sustained. In fact, on the other hand, progress of civilization and rapid industrialization brought with it danger of soil pollution. Agro-industries include pulp, paper, sugar, ginning, textile, dairy, dyes, edible oil and fruit processing and generate large volume of liquid/solid effluents and release them into the environment. A perusal of the literature on the discharge of effluents on to the soil (14) strongly indicates that, they cause marked changes in physicochemical, biological and enzymatic properties. Thus, the determination of specific enzyme activity and microbial biomass, together with the use of chemical soil parameters, seems to be the best approach for evaluating the state of microbial activity and understanding its response to cultivation practices and environmental factors.

As a result of this chemical boom, many hazardous compounds are being added on a regular basis in our biosphere, the list being endless. Modern cotton ginning industrial process is a continuous process spanning from the unloading of raw cotton to bailing of processed cotton fibres. In order to get clear cotton for raising crop in the next season the residual cotton fibre or lint left on seeds by ginning process is usually removed by acid treatment. Ginning of cotton produces large quantities of liquid/solid wastes in the form of cotton seeds [which may be used in animal feed] and gin trash, and released in to the environment and affect the soil microbial ecological processes, such as the biogeochemical cycling of elements, and the mineralization of carbon, nitrogen, sulphur and phosphorous (i.e. organic matter decomposition) .Any disturbance in the soil ecosystem can disturb the microbial activity and thereby affect the availability of nutrients. Changes in physicochemical and biological properties in black soil occurred due to release of cotton ginning industry effluents (14). 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- cellulose, protease, amylase and Urease of wide importance in recycling of nutrients in soil with/without effluents from cotton ginning mill**.**

II. MATERIALS AND METHODS

2.1.Collection of soil samples

Soil samples were collected from the surrounding areas (1/4 km) of M/s. 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 (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 and biological properties of the soil

The physico-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 (2).Whereas water holding capacity, organic carbon, total nitrogen, and soluble phosphorous of soil samples were determined by the methods of a Johnson an Ulrich(7),Walkeyblack (22); Microkjeldhal (8) and Kuprevich and Shcherbakova (10), respectively. Electric conductivity and pH were determined by Elicoconductivity 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, whereas 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 (15).

2.4.Isolation of cellulolytic Bacteria

Ten grams of soil sample was suspended in 90 ml sterile distilled water and serial dilutions were prepared by transferring 1ml of diluted suspension to 9ml of sterile distilled blanks. A 0.1ml suspension of 10^4 and 10^5 dilution were spread onto carboxy methyl cellulose (CMC) agar and then the plates were incubated at 28ºC for 2 days. The incubation temperature focused on mesophiles. The initial medium pH was adjusted to 7.0 if not specified . A few bacterial colonies were harvested and transferred to fresh CMC agar plates containing trypan blue. The plates were incubated at 28ºC for 2 days, and the cellulolytic clones were detected by clear halos around the colonies [3]. Three clones were finally chosen based on their relatively higher cellulolytic activities among 10 cellulase - positive clones that showed good colonial development and visible clearing zones and were maintained on CMC agar.

Screening of cellulolytic bacteria

The Colonies grown on CMC agar media were not considered as pure even though only one type of colony appeared and exhibited the zone of clearing. These bacterial colonies were purified by taking single colony each time in a streak plate method on cellulose agar medium repeatedly at least seven times until plate contained uniform one type of colonies. The purified colonies were checked for cellulolytic activity by the method described (21). The cellulose hydrolysis zones were visualized. The plates were further stabilized at least for 2 weeks by flooding with 1M HCl, which changes the dye colour blue into red and inhibited further enzyme activity. The Bacterial isolates were maintained on nutrient agar plates for routine use and stored at 4° C.

Identification of cellulolytic bacteria:

Identification of cellulolytic bacteria was carried out by method as described by Cowan and Steel [5] and Cullimore [6], which was based on morphological and biochemical tests.

III. RESULTS AND DISCUSSION:

The analytical results of physico-chemical and biological parameters of both the test and control soils were presented in table I. Test soil samples underwent changes in all the measured parameters of physico-chemical and biological parameters in comparison to control soils. The soil texture in terms of percentages of clay, silt and sand were56,34,10 and 36,24 and 40 in test and control soil, respectively as shown in Table I. The above results indicated that soils contaminated with the effluents had relatively lower sand content and higher clay and silt contents than control soil. This may be due to the micronutrients discharged into the soil through effluents reduce the porosity of the soil resulting in poor yields. More recently, similar results were also noticed by Pradeep and Narasimha [18] in soil polluted with leather industry effluents caused drop in sand content and hike in clay content. Surprisingly, in their observation, sand content was adversely affected with increasing the quantity of effluent in soil. Also, Nizamuddin et al.,[16] reported that discharge of dairy factory effluents decreased the soil sand content. Numerous results reported that the soils treated with long term sewage effluents [1], effluents of cotton ginning mills [14] and sugar industry [13] increased the clay content of soil and subsequently improved the soil texture and fertility. Higher water holding capacity and electrical conductivity were observed in test soil than control (Table I) may be due to accumulation of organic wastes and salts in paper industry effluents. Likewise, soil discharged with effluents from cotton ginning mills [14], paper mills[12], and sugar industry[13] increased the water holding capacity and electrical conductivity. In contrast, soils polluted with cement industries had low water holding capacity and higher electrical conductivity [19,20]. Surprisingly, there was no observable change in soil pH upon discharging of effluents. This could be due to neutral nature of paper industry effluents. Furthermore, half of the higher content of organic matter was recorded in test soil over control. This may be due to the discharge of effluents in an organic nature. The contents of total nitrogen and phosphorous in soils polluted with effluent were generously higher in test soil than control soil (Table I). Kannan and Oblisami [9] observed that irrigation of sugarcane crops with combined pulp and paper mill effluent increased soil pH, organic C, N, P, and K. A perceptible change in bulk density, pH, EC, OC was observed under continuous paper mill effluent irrigation [4]. Similarly, discharge of effluents from cotton ginning mills [14] and sugar industry [13] increased the total nitrogen and phosphorous contents compare to the control soil. Furthermore, micro flora of both soil samples were enumerated and listed in the table II. One fold higher bacterial and fungal population noticed in test soil over control sample. Same was reported by Kannan and Oblisami [9] that the irrigation practices for a period of 15years with contaminated effluents, resulted in increase in soil microbial population, which also represent populations were directly proportional soil organic C and to the available nutrient status of the soils . Chinnaiah et al., [4] reported that rhizosphere micro flora increased in amended plots receiving paper mill effluent irrigation. Similarly, Monanmani et al., [11], Narasimha et al., [14], and Nagaraju et al., [13] observed higher microbial population in soil polluted with effluents from alcohol, cotton ginning mills, and sugar industries, respectively. In another study, Pourcher et al., [17] observed high number of total cultivable heterotrophic bacteria in fresh refuse and 1 year old refuse area soils. Subsequently, two bacteria were isolated from the effluent polluted soil by enrichment method and morphological and biochemical characteristics of the two cultures were analyzed. One isolate (Isolate 1) was a gram positive, cocco *bacilli* and spore former (Table III), while another one (Isolate 2) was gram negative, rod shape, and non spore forming bacterium. Furthermore, isolate 1 has shown positive reaction in casein hydrolysis, Catalase, H-L test, lipase and citrate utilization (Table IV). Whereas isolate 2 has shown positive result in the hydrolysis of starch, gelatin, and casein. Additionally, it has shown positive reaction in Catalase, H-L, lipase, amylase, nitrate reduction and citrate utilization tests. Based on morphology & Biochemical characterization our strain is similar to *pseudomonas*

Character	Control	Test
Colour	Black	Thick black
Odour	Normal	Normal
pH [1:1.25 soil–water slurry]	8.0	9.2
Texture: Clay [%] Silt [%] Sand [%]	 36 24 40	 56 34 10
Electrical conductivity [µmhos/cm]	0.28	1.62
60%Water-holding capacity [ml g^{-1}]	0.4	0.8
Organic matter [%]	3.2	6.8
Total nitrogen [g kg^{-1} soil]	0.18	0.26
Available phosphorus [P_2O_5] in [kg/ha]	190	289
Available potassium [K_2O] in [kg/ha]	1290	1689

Table no-I: Analysis of Physico-Chemical Properties:

Table no-II: Biological Parameters:

Micro flora	Control	Test
Bacteria	40×10^7	18×10^7
Fungi	20×10^5	12×10^5

Table -III: Morphological Characteristics of Bacteria

SN	Characters Tested	Isolate-1	Isolate-2
1	Colony Morphology	Whitish round non slime	Whitish round non slime
2	Cell Morphology	Rod Shape	Rod Shape
3	Gram's Stain	-	-
4	KOH Test	+	+
5	Spore Stain	-	-
6	Speculation	-	-
7	Motility Test	+	+

Table-IV: Biochemical Characteristics of Bacteria

SN	Name Of The Test	Isolate-1	Isolate-2
1	Amylase Production	+	+
2	Gelatin Hydrolysis	+	+
3	Catalane hydrolysis	+	+
4	Nitrate Reduction Test	+	+
5	Insole Test	-	-
6	Methyl Red Test	-	-
7	V-P Test	+	+
8	Simmons citrate utilization Test	+	+
9	Carbohydrate fermentation Test	-	-
10	Unease Test	-	-
11	Starch Hydrolysis	+	+
12	Casein Hydrolysis	+	+
13	H-L Test	+	+
14	Lipase Activity	+	+

V. CONCLUSION:

Results of the present study clearly indicate that the discharge of effluents from paper industry altered the physic chemical biological properties of soil. Additionally, polluted soil contained bacteria utilizing cellulose as their sole carbon source. However, additional research is necessary for the characterization of isolated cellulolytic bacteria

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