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INFLUENCE OF PAPER AND PULP INDUSTRY EFFLUENTS ON PHYSICO-CHEMICAL & BIOLOGICAL PROPERTIES OF SOIL

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Abstract: Effect of Paper and Pulp Industry effluents on soil was assessed for Physico-chemical & Biological properties of soil in the present study. Discharge of Paper &Pulp Industry 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 P, and K 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 paper and pulp Industry effluent contaminated soil by Cellulose Congo Red Agar Medium.

Key Words: Paper and Pulp Industry-Effluents-Physico-chemical-Biological Properties-Cellulolytic bacteria.

INTRODUCTION

Paper industry is an agro based industry, effluents generating from this industry contain considerable amounts of organic and inorganic chemical components such as fibers, cellulosic wastes, wood dust, chlorine compounds, carbonates and bicarbonates [1]. Direct discharge of effluents from this industry may have profound influence of soil physico-chemical and biological properties. Though wealth of information on occurrence of changes in properties of soils due to discharge of effluents from other industries is available [2-9]. However, few researchers [10-14] emphasized in the area of screening of cellulose degrading microorganisms of polluted soil, and efficiency of their cellulase, since cellulase have significant role in different industries. Cellulose, a complex carbohydrate or polysaccharide, consisting of 3000 or more glucose units. The basic structural component of plant cell walls, cellulose comprises about 33 Percent of all vegetable matter (90 percent of cotton and 50 percent of wood are cellulose) and is the most abundant of all naturally occurring organic compounds. Alarmingly, cellulose containing materials (Table I) can be used as one of the renewable energy sources, if microbial cellulase are properly used.

The paper and pulp industry is one of the major consumers of water. Pulp and paper industrial wastewaters usually contain halogenated organic materials, because general use is made of chlorine containing compounds as bleaching agents during pulp and paper manufacture. Chlorination is generally the first stage in Kraft pulping and during this treatment phase chlorinated organic compounds are produced. Several methods have been attempted for decolorization and detoxification of bleached Kraft effluents. These include physicochemical and biotechnological methods. The problems underlying the physicochemical treatments are those associated with cost and reliability. Biotechnological methods have the potential to eliminate/reduce the problems associated with physicochemical methods. [15]

In this direction, an attempt has therefore been made to find out the effect of Paper &Pulp industry on physico-chemical and biological properties of soil. Cellulose degrading bacteria from soils contaminated with effluents from paper industry were also isolated.

Table 1: The Percentage of Cellulose in Different Vegetations and Bacteria

Material	% of Cellulose
Cotton	95-99
Ramie	80-90
Bamboo	40-50
Wood	40-50
Woodbark	20-30
Mossess	25-30
Bacteria	20-30

MATERIALS AND METHODS

Collection of soil sample

Soil samples were collected from Andhra Pradesh Paper Mills, Rajahmundry, East Godavari, 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 and biological Properties. These two soil samples were air dried and mixed thoroughly to increase homogeneity and shifted to <2 mm sieves for determination of soil texture.

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Physico-Chemical Properties of 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 [16]. Whereas water holding capacity, organic carbon, total nitrogen, and soluble phosphorous of soil samples were determined by the methods of a Johnson & Ulrich [17], Walkey-black [18], Microkjeldhal [19] and Kuprevich and Shcherbakova [20], respectively. Electrical conductivity and pH were determined by Elico conductivity meter and pH meters, respectively.

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 37°C for 24 h, whereas Czapeck-Dox plates were at room temperature for 7d. After incubation period, colonies formed on the surface of the medium were counted by colony counter [21].

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 plated on the modified cellulose Congo red agar (CCRA) medium [22] and incubated for 5 days at 28±2°C. For bacterial isolates candid antibiotic (100µg/ml medium) was added to the CCRA medium after autoclaving to prevent the fungal growth.

Screening of cellulolytic bacteria

The Colonies grown on CCRA 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-Congo red 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 [23]. In this method, the bacterial colonies grown on cellulosic medium without Congo red for two days at 28±2°C, then the medium was flooded with an aqueous solution of Congo red (1mg/ml) for 15 min to allow to reach to cellulose. The excess Congo red solution was then poured off, and plates were further flooded with 1M NaCl for 15 min. 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 red into blue 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 [24] and Cullimore [25], which was based on morphological and biochemical tests.

RESULTS AND DISCUSSION

The analytical results of Physico-chemical and biological parameters of both the test and control soils were presented in table II. 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 were 50, 36, 14 and 36, 25 and 40 in test and control soil, respectively as shown in Table II. 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 [26] 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 effected with increasing the quantity of effluent in soil. Also, Nizamuddin *et al.,* [27] reported that discharge of dairy factory effluents decreased the soil sand content. Numerous results reported that the soils treated with long term sewage effluents [28], effluents of cotton ginning mills [29] and sugar industry [30] 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 II) may be due to accumulation of organic wastes and salts in paper industry effluents. Likewise, soil discharged with effluents from cotton ginning mills [29], paper mills [31], and sugar industry [30] increased the water holding capacity and electrical conductivity. In contrast, soils polluted with cement industries had low water holding capacity and higher electrical conductivity [32, 33]. 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 II). Kannan and Oblisami [34] 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 [35]. Similarly, discharge of effluents from cotton ginning mills [29] and sugar industry [30] 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 III. One fold higher bacterial and fungal population noticed in test soil over control sample. Same was reported by Kannan and Oblisami [34] 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.,* [35] reported that rhizosphere micro flora increased in amended plots receiving paper mill effluent irrigation. Similarly, Monanmani *et al.,* [3], Narasimha *et al.,* [29], and Nagaraju *et al.,* [30]observed higher microbial population in soil polluted with effluents from alcohol, cotton ginning mills, and sugar industries, respectively. In another study, Pourcher *et al.,*[12] 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 IV), while another one (Isolate 2) was gram positive, 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 V). 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 strains are resemble to *Bacillus sps.*

Table 2: Physico-Chemical Characteristics of Test & Control soil samples:

Character	Control	Test
Color	Black	Thick black
Odor	Normal	Normal
pH [1:1.25 soil–water slurry]	8.9	9.02
Texture:		
Clay [%]	36	50
Silt [%]	24	36
Sand [%]	40	14
Electrical conductivity [µmhos/cm]	0.34	1.91
60%Water-holding capacity [mL g^{-1}]	0.6	1.4
Organic matter [%]	3.82	6.92
Total nitrogen [g kg^{-1} soil]	0.18	0.29
Available phosphorus [P_2O_5] in [kg/ha]	201	399
Available potassium [K_2O] in [kg/ha]	1201	1699

Table 3: Biological Parameters of Test & Control soil samples:

Micro flora	Control *	Test *
Bacteria	20×10^6	40×10^8
Fungi	14×10^4	20×10^5

*Colony forming units per gram of soil.

Table 4: Morphological Characteristics of Bacteria

S. No	Characters Tested	Isolate-1	Isolate-2
1	Colony Morphology	Whitish round non slime	Whitish round non slime
2	Cell Morphology	Cocco Bacilli	Rod Bacilli
3	Gram's Stain	Positive	Positive
4	KOH Test	Positive	Positive
5	Spore Stain	Positive	Negative
6	Sporulation	Positive	Negative
7	Motility Test	Positive	Positive

Table 5: Biochemical Characteristics of Bacteria.

S.No	Name Of The Test	Isolate-1	Isolate-2
1	Amylase Production	+	+
2	Gelatin Hydrolysis	+	+
3	Catalase hydrolysis	+	+
4	Nitrate Reduction Test	+	+
5	Indole Test	-	-
6	Methyl Red Test	-	-
7	V-P Test	+	+
8	Simmons citrate utilization Test	+	+
9	Carbohydrate fermentation Test	-	-
10	Urease Test	-	-
11	Starch Hydrolysis	+	+
12	Casein Hydrolysis	+	+
13	H-L Test	+	+
14	Lipase Activity	+	+

CONCLUSION

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

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