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Effect of leather industry effluents on soil microbial and protease activity

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Abstract

Release of leather industry effluents into the agricultural fields causes indicative changes in nutrient cycling and organic matter processing. In the present study, leather industry effluent discharged soil (test) and undischarged soil (control) were collected from the surrounding areas of industry. The physico-chemical, biological properties and soil protease activity were examined. The study reflected the average mean value of pH, electrical conductivity and water holding capacity of the test soil was found to be 7.94, 0.89 μMhos cm^{-1} and 0.51 ml g^{-1}, respectively. In chemical parameters, organic matter, total nitrogen, phosphorus and potassium has the mean of 6.73% , 0.23 g kg^{-1}, 4.28 mg g^{-1} and 28 μg g^{-1}, respectively. In all the respects, the test soil showed higher values than the control. The soil protease enzyme activity was determined by using substrate casein and the activity was found to be higher (180 μg TE g^{-1} 24 hr^{-1}) in test soil than the control soil (63 μg TE g^{-1} 24 hr^{-1}).

Key words

Leather industry effluents, Soil microbial activity, Biological parameters, Protease activity

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 (Nagaraju *et al.*, 2007). 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 (Narasimha *et al.*,1999), sugar industry (Nagaraju *et al.,* 2009), paper mill (Nilima and Madhuri, 2005), dairy industry (Nizamuddin *et al.,*2008), dairy wastewater (David shyam babu *et al.*, 2010). Effluents from leather processing, a major industry that produces huge volume of waste water normally discharged to irrigate agricultural lands. This tannery waste water contains some proteins released during enzymatic processing of leather but also contaminants, such as salts and chromium (Cr), that might affect soil processes and crop production (Alvarez-Bernal *et al.*, 2006).

Soil enzymes occupy a vital role in catalyzing reactions associated with organic matter decomposition and nutrient cycling (Sinsabaugh, 1994). Proteases participating in the protein catabolism either by degradative or biosynthetic pathways release hormones and pharmacologically active peptides from precursor proteins (Vivian hook *et al.*, 2008). Proteases are actively involved in carbon recycling and biological transformations of soil fertility (Bolon *et al.*, 2008). In the present study, an attempt was made to find out impact of effluents of leather industry on soil physical (pH, EC, water holding capacity), chemical (organic matter, total nitrogen, phosphorus and potassium), biological (bacteria and fungal populations) properties and soil protease activity.

Materials and Methods

Soil collection: Soil samples were collected from the surrounding areas (1/4 km) of “Avanthi Leather Industries”, Varadaiah pallem, Chittoor district, Andhra Pradesh, India. Soil sample without effluent discharges served as control was collected from adjacent site (1 km away) of leather industry. Soil samples both with and without effluents were used for determination of physico-chemical, biological and protease 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 physical and chemical properties of test and control soils were determined in accordance with standard analytical methods (APHA, 2000).

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 spreaded with a sterile spreader on nutrient agar medium (pH 7.2) and Czapeck-Dox agar medium for the growth of bacteria and fungi respectively. Nutrient agar plates were incubated at 37ºC for 24 hr, where as Czapeck-Dox plates were at room temperature for 7 days. After incubation period, colonies formed on the surface of the medium were counted by colony counter (Narasimha *et al*, 1999).

Assay of soil protease: Protease activities of both soil samples were determined by placing 5 gm of soil sample in each boiling test tube with 60% water holding capacity at 28 ± 4ºC. Triplicates of both test and control soil samples were drawn after 0, 7, 14 and 21 days of incubation to determine protease activity by the method of Speir and Ross (1975). Samples of 5 gm of soil were placed in 25 ml of boiling test tubes; 10 ml of 2% casein in 0.1 M tris buffer at pH 7.5 was added and were incubated for 24 hr. After incubation, to these, 4 ml of 17.5% trichloroacetic acid was added and the suspension was filtered by Whattman No. 1 filter paper. The amount of protein was determined following the method of Lowry *et al.,* (1951) by Elico digital spectrophotometer. Finally, protease activity was expressed in terms of microgram (µg) of tyrosine released per gram of soil per 24 hr.

Statistical analysis: Value of protease activity only was statistically calculated. Values at $p<0.05$ was considered significant.

Table - 1: Physico chemical properties of control and test soil of leather industrial area

Properties	Test soil	Control
Color	Black	Brown
Odor	Foul	Normal
pH	7.94	7.41
Electric conductivity (µ Mhos cm^{-1})	0.89	1.24
Water holding capacity (ml g^{-1})of soil	0.51	0.2
Texture:		
Sand (g)	59	71
Silt (g)	23	19
Clay (g)	18	10
Organic matter (%)	6.73	3.52
Total nitrogen (g kg^{-1})	0.23	0.12
Phosphorus (mg g^{-1})	4.28	2.52
Potassium (µg g^{-1})	28	12

Table - 2: Biological properties of control and test soil of leather industrial area

Properties	Test	Control
Bacteria (cfu g^{-1})	107 x 10^4	52 x 10^4
Fungi (cfu g^{-1})	5 x 10^4	2 x 10^4

Results and Discussion

Physico-chemical properties: The pH of the test soil increased from 7.41 to 7.94. Soil texture in terms of percentage of sand, silt, clay was 59, 23, 18 in test and 71, 19, 10 in the control soils respectively. Higher water holding capacity was observed in test soil than control, values were found to be 0.51 and 0.2 ml g^{-1}, respectively. The electrical conductivity of both test and control soils were 0.89 and 1.24 µ Mhos cm^{-1}, respectively. Increased water holding capacity and decreased electrical conductivity in contaminated soil may be due to the accumulation of organic waste such as amino acid residues, acids and alkalis in the leather industry effluents (Alvarez-Bernal *et al.*, 2006). 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 6.73%, 0.23 g kg^{-1}, 4.28 (mg g^{-1}), 28 (µg g^{-1}) and control soil were 3.52%, 0.12 g kg^{-1}, 2.52 (mg g^{-1}), 12 (µg g^{-1}), respectively (Table 1). Polluted soil caused two fold increases in bacterial and fungal population compared to control soil (Table 2). For instance, the bacterial and fungal population in control and polluted soil was 52 X 10^4, 2 X 10^4 g^{-1} and 107 X10^4, 5 X 10^4 cfu g^{-1} of soil, respectively.

Protease activity: With increasing the soil incubation period, the protease activity increased up to 14 days interval and was declined in both samples. For instance protease activity with 2% casein as substrate of the test sample at 0 day was 86 µg TE g^{-1} 24 hr^{-1}, it increased to 180 µg TE g^{-1} 24 hr^{-1} after 14 days and later declined to 82 µg TE g^{-1} 24 hr^{-1} after 21 days. Same trend was also seen in the test soil . The protease activity without amendment of substrate

Table - 3: Protease activity in soil without substrate and with substrate after 24hrs incubation.

Days	With substrate		Without substrate	
	Control	Test	Control	Test
0	41.04 ± 0.27	86.0 ± 0.70*	19 ± 0.79	41.1 ± 0.65*
7	54.08 ± 0.19	125.44 ± 0.27*	41.4± 0.89	87.0 ± 0.70*
14	63.42 ± 0.29	180.51 ± 0.45*	55.2± 0.25	91.2 ± 0.27*
21	47.31 ± 0.43	82 .20 ± 0.35*	32.1± 0.74	48 .1± 0.41*

Values are mean of three replicates ± S.D, Significant at $p<0.05$

of the test sample at 0 day was 41 µg TE g^{-1} 24 hr^{-1}, it increased to 91 µg TE g^{-1} 24 hr^{-1} after 14 days and later declined to 48 µg TE g^{-1} 24 hr^{-1} after 21 days. Same trend was also seen in the control soil. Higher activity was recorded in test sample than control at all incubations (Table.3).

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 (Narasimha *et al.*, 2011). Discharge of effluents from various industries like sugar industry effluents (Nagaraju *et al.,* 2007*)*, dairy factory effluents (Nizamuddin *et al.,* 2008*)* and petrochemical industry (Andrade, 2002) influences 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).

In the present study, leather industry effluents generated from preliminary processing of leather were discharged into the soil had relatively higher clay and silt contents than that of control soil. Similar results reported by Narasimha *et al*., (1999), revealed that application of long term cotton ginning mill effluents to the soil led to increase in clay and silt contents respectively. In the present study, decreased electrical conductivity was observed in the test soil than the control. Sridevi *et al.,* (2007) reported a low EC in soil exposed to long term application of ground nut oil mill effluents. However, increased water holding capacity may be due to the accumulation of organic wastes in the test soil (Table.1). Similarly, soil discharged with effluents from paper mills (Medhi *et al.,* 2005), cotton ginning mills (Jyoshna Devi and Narasimha*,* 2007) increased the water holding capacity. Slight increase in pH of the test soil is explained in terms of release of effluents with basic in nature containing some alkalis released from leather industry. Similarly, Nanda kumar (1990) reported the discharge of effluents from tannery increased the soil pH slightly. Higher organic matter content in the test soil may be due to the discharge of effluents in an organic nature (amino acid residues). Dodar and Tabatabai, (2003) reported that the discharge of effluents from dairy increased the soil organic matter. Two fold higher microbial populations were observed in the test soil. It may be due to the presence of high organic matter contents in basic effluents. Similarly, Narasimha *et al.,* (1999) reported that microbial populations were increased in soils polluted with cotton ginning mill effluents.

Soil enzyme protease is excreted by the soil microorganisms, plants and animals by means of their metabolic activities. This is an extracellular enzyme secreted by soil microorganisms, including bacteria and fungi widely available, where the protein rich effluents dislodge into the soil increases proteolytic activity in the test soil is due to the presence of high organic wastes (amino acids) discharged in the effluents (Nagaraju *et al.,* 2007). The protease activity increased up to 14^{th} day and then declined at 21^{st} day probably due to exhaustion of the readily available substrates. Similarly, soil protease activity in soils treated with dairy shed effluents (Zaman *et al.,* 1999), dairy factory effluents (Nizamuddin *et al.,* 2008) increased at first and then decreased with the time. In contrast, soils polluted with herbicides (Abd-Alla *et al.*, 2000), chlorothionil (Singh *et al.,* 2002), decreased soil protease. Finally, we conclude that the discharge of effluents from leather industry altered the physico-chemical properties of soil, affected the microflora and enhanced the soil protease activity.

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