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Microbiological and enzymatic properties of soil contaminated with hydrocarbon industrial waste

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ABSTRACT

The impact of hydrocarbon industrial wastes on soil physico-chemical, biological and enzyme properties was assessed in the present study. Contamination of soil with hydrocarbon industrial wastes cause the changes in physico-chemical, biological and enzymatic properties but undetectable towards soil water holding capacity, sand, Potassium and organic carbon. Soil enzymes such as protease and cellulase enzyme activities were higher whereas dehydrogenase enzyme activity is lower in test than control. With increasing the soil incubation period, soil protease and cellulase activities were increased whereas dehydrogenase activity was ceased in polluted soil in comparisons to control. Higher bacterial and fungal population was observed in the contaminated soil than control.

KEYWORDS

Hydrocarbon industrial waste;
Microbiological properties;
Enzyme activities.

INTRODUCTION

There is increasing pressure to provide basic needs such as food, fiber and shelter to the growing population, in particular, developing countries in the world. In order to meet basic needs, many agro-industries are being developed with least concern towards environment. 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[1]. 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. Thus, advance in technology and industrialization bring with them unpleasant partners, pollution and degradation of the environment. The effects on the environment, connected with industrial activities are mainly related to the production of industrial wastes. Damage to the environment in particular, soil a natural resource through industrial effluents, adversely affects agricultural production and may lead to food crisis.

Soil is a dynamic, living, non-renewable resource that plays many key roles in terrestrial ecosystems[2,3]. Anthropogenic activities affect the quality of soil, which was defined by Doran and Parking, 1994[2] as ''the capacity of soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health". Soil enzymes play key biochemical functions in the overall process of organic matter decomposition in the soil system[4,5]. They are important in catalyzing several important reactions necessary for the life processes of microorganisms in soils[6]. Soil enzymes are highly involved in the decomposition of soil organic matter and nutrient cycling. These enzyme activities are biosensors of soil degradation since they integrate information about microbial status and also from physico-chemical conditions[7-9]. Hydrocarbon industry is one of the agro-based industries, which produces the High viscose carbon source ie.Carboxy methyl cellulose is main product of this industry and it is mainly using for the reduction of soil friction while making soil drilling. In this process cellulosic waste was treated with 0.1N NaOH (sodium hydroxide). In this process huge volume of waste was generated and discharged to surrounding fields. The waste contain considerable amount of organic substrates in the form of cellulosic waste[10]. Discharge of the effluents may alter the physicochemical and biological properties in terrestrial ecosystem including coastal marine and inland water bodies received more attention than inland terrestrial system[11,12]. Soil enzymes are highly involved in the degradation of soil organic matter and nutrient cycling. These enzyme activities are sensors of soil degradation and fertility[13]. Since they integrate information about microbial status, and also, from physico-chemical conditions[7-9]. They may correlate well with nutrient availability[14]. The main objective of this study was to determine soil physico-chemical, biological parameters and soil enzyme activities like protease, cellulase and dehydrogenase in waste contaminated soil.

MATERIALS AND METHODS

Collection of soil

Soil samples collected from different location, where organic waste is being discharged by hydrocarbon factory located Nagari village, Chittor District of Andhra Pradesh, India. Soil samples with effluent discharges were used in all experiments conducted in the present study. These soil samples were air dried and mixed thoroughly to increase homogeny and shifted to < 2 mm sieves for determination of soil texture and used for physico chemical and enzymatic activities.

Physico-chemical properties of hydrocarbon industrial waste

Mineral matter of soil sample such as sand, silt clay contents were analyzed with use of different sizes of sieves by following method[15]. Cent percent water holding capacity of soil sample was measured by finding amount of distilled water added to soil sample to get saturation point and then sixty percent water holding capacity of soil samples were calculated by the method[16]. Soil PH was measured at 1:1.25 soil to water in ratio in Elico digital PH meter with a calomel glass electrode assembly. Organic carbon content in soil samples was estimated method[17] and the organic matter was calculated by multiplying the values with 1.72[18]. Electrical conductivity of soil sample with effluent discharges after addition of 100 mL distilled water to one gram of soil sample was measured by Conductivity Bridge. Soluble phosphorous in soil sample was quantified by the method[19].

Microbial properties of soil polluted with hydrocarbon industrial waste

The microorganisms play a vital role in nutrient cycling and soil fertility. Microflora of both soil samples was enumerated and listed in TABLE 2. Bacterial and fungal populations were observed and compared with control soil. Higher bacterial and fungal populations are observed in the contaminated soil. Higher bacterial and fungal population in the contaminated soil may be due to higher PH in the soil. In contrast irrigation with lactose dairy factory effluent enhanced soil biological activity and nutrient cycling[20,21]. Similarly Narasimha *et al.*[10], reported that discharge of effluents from cotton ginning industry and sugar industry[22], improved soil microbial populations. For instance bacterial and fungal population in hydrocarbon industry waste soil was 110x103 CFU/g of soil, 3x103 CFU/g of soil respectively.

Soil protease Assay

At desired intervals, one set of triplicate soil samples received 10mL of 0.1 m Tris, (2– amino-2–hydrox–methyl propane 1:3 diol, PH 7.5) containing sodium

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caseinate (2% w/v) where as condition of 10 mL of 0.1 m Tris buffer without caseinate was made to another set of triplicate soil samples. Both sets were incubated for 24 hours at 30°C and four mL of (17.5% w/v) trichloro acetic acid was then added and the mixture was centrifuged. A suitable aliquots of the supernatant was treated with 3 mL of 1.4 M Na2Co3 (sodium carbonate) followed by the addition of folin-ciocalteu reagent (33.3% v/v). The formation of blue color was read after 30 minutes at 700 nm in a spectrophotometer (Baush and Lomb). Tyrosine equivalents formed in the supernatant was estimated by referring tyrosine standard curve.

Soil cellulase assay

Five gram samples of soil were placed in 50 ml Erlenmeyer flasks and 0.5 ml of toluene was added. The contents in the flasks were mixed thoroughly and after 15 minutes, 10 ml of 1% carboxy methyl cellulose (CMC). The flasks were then incubated for 30 minutes and approximately 50 ml of distilled water was added. The suspension was filtered and the volume of the filtrate was made up to 100 ml with distilled water. Reducing sugar content in the filtrate was determined in spectrophotometer (Bausch and Lomb).

Soil dehydrogenase assay

This method was based on reduction of 2,3,5 Triphenyl tetrzolium chloride (TTC) soil sample were treated with 0.1g CaCo3 and 1ml of 0.18M acqueous TTC incubated for 24hrs at 30^0C temperature The end product, triphenyl formazone formed was extracted with methanol from the reaction mixture and the end product was measured at 485nm in spectrophotometer (Bausch and Lomb).

RESULTS

Physicochemical properties of hydrocarbon industrial waste

Soil fertility mediated by microorganism is dependent on maintenance of physicochemical characteristics in soil. Soil sample contaminated with hydrocarbon waste underwent changes in all measured parameters of physical and chemical properties.

Disposal of industrial waste made the soil unpleasant odor and imports light red color. Electrical conductivity of contaminated soil was 0.35mhos/cm and water holding capacity was higher than the control soil. Higher water holding capacity and lower electrical conductivity in the polluted soil may be due to accumulation of organic wastes in form of cellulosic waste in the soil. Soil texture was measured in terms of percentages of sand; silt and clay. In this study these were 93.95 3.45 and 2.6 and 65.42%, 17.38 and 17.20 percentages in the control and polluted soils respectively (TABLE 1) These results indicated that wasted polluted soil had relatively higher sand, lower silt and clay contents than control soil (TABLE 1) The pH of the test soil was slightly declined from 8.6 to 8.5 in contaminated soil, whereas higher organic carbon, phosphorous and lower Potassium content were observed in contaminated soil.

TABLE 1: Physico- chemical operties of soil contaminated with/without industrial waste

Properties	Contaminated soil[1]	Control soil[2]
Colour	Gray	Red
Odor	Bad	Normal
Texture: (%)		
Sand	93.95	65.42
Silt	3.45	17.38
Clay	2.6	17.20
pH	8.5	8.6
Electrical conductivity (μMhos/cm)	0.35	0.93
Water holding capacity (ml/g of soil)	0.6	0.4
Organic carbon (%)	high	low
Phosphorus (kg/h)	56	50
Potassium (kg/h)	78	115

Contaminated1: Soil with hydrocarbon industrial waste. Control2: Soil without hydrocarbon industry waste.

Biological characteristics

The micro flora of both soil samples was enumerated and is listed in TABLE 2. Two fold higher bacterial and fungal populations were observed in the test soil over the control soil.

Cellulase activity

The cellulase activity in soils supplemented with and without substrates was measured in terms of release of glucose from externally added substrate carboxy methyl cellulose (Figure 1). There is increment in the for-

TABLE 2 : Microbial population soil with/without contaminated with industrial waste

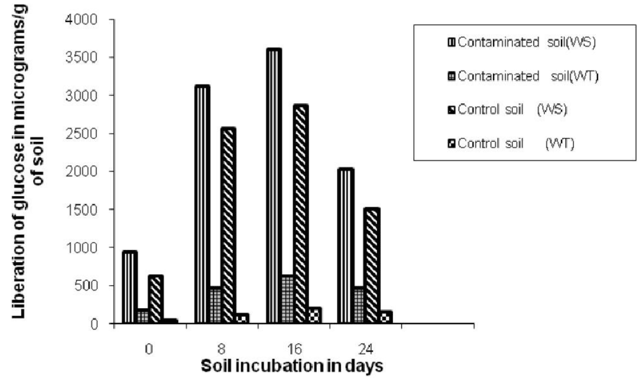
Parameter	Contaminated soil[1]	Control soil[2]
Bacteria	110x10^3	60x10^3
Fungi	3x10^3	2x10^3

* Microbial population were counted in the form of CFU/g soil
Contaminated1: Soil with hydrocarbon industry waste.
Control2: Soil without hydrocarbon industry waste.

mation of glucose as a end product with increasing the incubation days. For instance in polluted soil with increasing the soil incubation periods soil cellulase activity also increased up to 16th day interval and further the activity was ceased at 24 day of interval (Figure 1). Cellulase activity in polluted soil was 933ug-1GEg-124h-1 of glucose at 0 day and 3111 ug-1GEg-124h-1 at 16th day intervals. Cellulase activity increased by 2-3 folds at 16th day interval and declined by 1-2 folds at further incubation days. (Figure 1) Furthermore, higher cellulase activity was recorded in test soil than the control soil at all incubation periods. The cellulase activity was measured in native soil sample without supplementation of substrate, carboxy methyl cellulase. Same trend was observed in this case also cellulose activity increased up to 16th day of interval and was ceased in both soil samples at 24th day. For instance cellulase activity in polluted soil was 177ug-1GEg-124h-1 of glucose at 0 day and this was increased by 2-3 folds higher activity to 622ug-1GEg-124h-1 at 16th day and later it was declined to 1-2 folds (466ug-1GEg-124h-1 at 24th day interval). Similar trend was noticed in the control soil. Overall improved cellulase activity was observed in test sample than in control sample at all incubation periods. The present results clearly indicate that the activity of cellulase as greatly enhanced in test soil over the control (Figure 1).

Protease activity

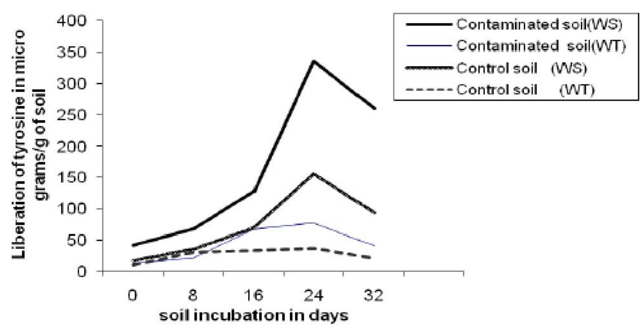
Protease activities of both the test and the control soil samples with and without substrates were determined with the amendment of substrate (1% casein) and the results shown in Figure 2. With increasing the soil samples at different days of intervals, the protease activity was raised up to 3 folds at 24th day of interval and further ceased in both control and test soil samples at 32nd day interval. For instance, protease activity of the test sample at 0 day was 42 ug TE g-1 24h-1, it



* Values represented in the Figure are mean of + SD (Standard Deviations).

Figure 1 : Cellulase activity of soil contaminated with/without industrial waste.

increased to 336u g TE g-124h-1at 24 days, and later declined to 260 ug TE g-124h-1at 32 days (Figure 2). The protease activity suited without supplementation of substrate and the results shown in Figure 2. Protease activity of the test sample at initial day was 14ug TE g-1 24h-1, it increased to 77 ug TE g-124h-1at 24 days, and later declined to 42 ug TE g-124h-1at 32 day of intervals.

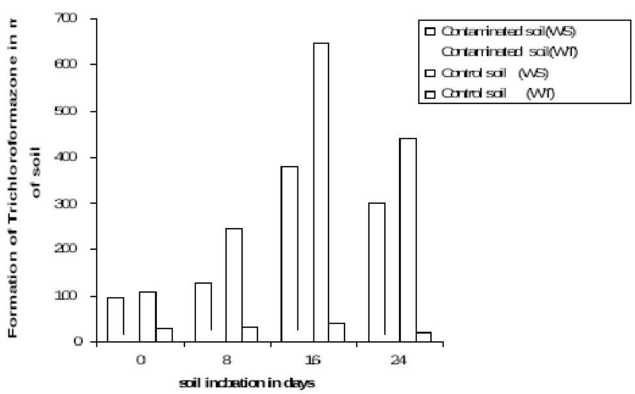


* Values represented in the Figure are mean of + SD (Standard Deviations).

Figure 2 : Protease activity of soil contaminated with/without industrial waste

Dehydrogenase activity

The Dehydrogenase activity was measured in terms of release of trichloroformazone from the externally added substrate 2, 3, 5 Triphenyl tetrzolium chloride and results shown in (Figure 3). While incubating the soil at different time intervals the dehydrogenase activity was increased up to 16th day of interval, and was declined at 24th day in both soil samples. For instance dehydrogenase activity of the control soil at 0 day was 108ug TF g-1 24h-1, and 647g TFg-1 24h-1at 16th day (6 folds improvement in dehydrogenase activity), and thereafter declined by 2 folds to 439 ug TF g-1 24h-1at 24 days. in polluted soil same trend observed. Furthermore, higher dehydrogenates activity was recorded in control soil sample than in polluted sample at all incubation periods. The polluted sample exhibited lowered dehydrogenase activity over the control at 0 day interval, it was 94 ug TF g-1 24h-1against 108 ug TF g-1 24h-1of the control soil and same trend was continued at the rest of the incubation periods. The present results clearly indicate that the Dehydrogenase activity was decreased in contaminated soil over the control (Figure 3) the experiment was conducted without supplementation of substrate and the results shown in Figure 3. By increasing the soil incubation period, the dehydrogenase activity was increased up to 16th day of interval and was declined in both samples at 24th day. For instantce dehydrogenase activity in industrial waste contaminated soil was 16 ug-1TFg-124h-1 and control soil was 27ug-1TFg-124h-1 of at 0 day and this was increased by 2 folds higher activity to 24ug-1TFg-124h-1 and 40 ug-1TFg-124h-1 at 16th day and later it was declined by 1-2 folds to 16 ug-1TFg-124h-1 and 19 ug-1TFg-124h-1 at 24th day interval.



*** Values represented in the Figure are mean of + SD (Standard Deviations).**

Figure 3 : Dehydrogenase activity of soil contaminated with/ without industrial waste.

DISCUSSION

In general, organic amendments such as crop residues, animal manures, logging and wood manufacturing residues, various industrial organic wastes, sewage wastes, food processing and fiber harvesting wastes, are naturally occurring compounds that are used as additives to improve soil physical conditions and/or plant nutrition. One of the possible reasons for improving the soil properties could be due to dumping of organic waste that may contribute increase the organic matter and nutrient content in the soil[23]. In this study, hydrocarbon industrial waste had relatively lower clay and silt contents than the control soil. Other studies have found the same, like long term application of sewage effluents and cotton ginning mill effluents[10], Dairy waste water[24]. Increasing in water holding capacity and decreasing electrical conductivity in the test soil may be due to accumulation of organic wastes and salts in the effluents waste of carbohydrate industry. Similar observations made by other workers like cotton ginning mills[10,25,26,1], Paper mills[27], Dairy industry[28]. Higher electrical conductivity also observed in soils treated with distillery effluents[29] and sodium based black liquor from fiber pulping for paper making[30]. In contrast, soil polluted with cement dust from cement industries had low water holding capacity and high electrical conductivity [31]. The slight drop in the pH of the test soil is explained in terms of release of effluents with acidic in nature, from hydrocarbon industry. Same was noticed in the discharges of sugar cane residues from sugar industry[32], sewage effluents[33] to sols decrease the soil pH. The higher organic matter of the test soil may be due to the discharge of effluents in an organic nature (cellulosic wastes). Similarly, municipal waste[34], effluents of cotton ginning mills[10,25] onto the soils, significantly increased the soil organic matter and total nitrogen content. Higher microbial population in the test soil possibly due to the presence of higher organic matter with acidic nature of effluents. Monanmani et al.[35] and Narasimha et al.[1,10,25] reported that microbial populations was profusely increase in soils polluted with alcohol and cotton ginning mills. Cellulase is a core enzyme; it consists of exo, endo and β-glucosidases. This enzyme synergistically acts on cellulose polymer substrate, are abundantly available on earth surface in wood, chips, rocks, municipal wastes. On the other hand, cellulose is the most abundant polysaccharide of plant cell walls and represents significant input to soils[36]. Cellulose hydrolysis into glucose is mainly achieved by complex enzyme cellulase, produced by fungi[37]. However, these enzymes are extensively studied in plant litter[38-40]. Furthermore, liberation of these enzymes by microbes during litter decomposition may be influenced by many factors like temperature, pH and substrate concentration[41]. The activity of cellulase was indicated by the presence of substrates like cellulose polymer of cellophane[42,43], cellulose powder[44] and carboxymethyl cellulose[45]. Nevertheless, cellulase activity was potentially correlated with fungal and bacterial populations in soil[46]. Little information is available on the effect of industrial effluents on soil cellulase activity. In this direction, cellulase activity was enhanced in soils treated with the effluents of textile and sugar industry[47], Cotton ginning mills[48], paper mill effluent and amendment addition[49], solid urban waste[50] and sodium based black liquor from fiber pulping for paper making[30] over untreated soils. Similarly, urban expansion into wild lands significantly increased the cellulase activity[51], Contrary to this, soil contaminated with cement dust, the cellulase activity was ceased[52]. In this assessment, results showed that the cellulase activity in the test sample was relatively higher than in the control sample at all incubations. The increased percentage of cellulase activity of the test sample range was in between 22 and 57 over the control. Thus, activity was increased gradually but not significantly. However, increase cellulase activity in soils with effluent discharges may be due to high availability of substrate, and abundant cellulolytic microorganisms. But the activity was declined with time intervals maximum at 30 days, it is probably because of the exhaustion of the readily available substrate. It has been very well established that the discharge of effluents from tomato processing unit[53], cotton ginning mill[48], paper mill and pressmud addition[49], and cotton ginning mill[48], paper mill and black liquor from straw pulping[30] increased the cellulase activity in the test over the control sample. Parama *et al*.[54] reported that the soil treated with urban wastes along with additives such as cow dung, rock phosphate, green leaves and coir dust increased the cellulase activity in the early incubations, later it was stabilized. Similarly, by increasing the incubation period, cellulase activity in soils treated with and without fungicide were increased upto 20 days, later were decreased[55]. According to Joshi *et al*.,[46], cellulase activity was greatly increased in soils treated with cellulose and increased cellulase activity was positively correlated with fungal, bacterial number and moisture content of litter. Nonetheless, high significant correlation between cellulase activity and soil respiration was observed[56] and microbial biomass by Kanazawa and Miyashita; Donnelly *et al*.[57,58]. Additionally, by increasing the effluents concentration in the control sample, the cellulose activity was increased, maximum at 50%, there after decreased. Decreased activity of cellulase at higher concentrations of effluents may be due to the exposure of cell free enzyme to highly concentrated effluents. Similar observation was made by Sreenivasulu,[55] that, at high concentration of fungicide in soil, the cellulase activity was inhibited. 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. It is distributed among soils exhibited a wide range of activities[59]. Proteases in soils hydrolyze not only added proteins, but also native soil added proteins[60]. In the present assessment, increased proteolytic activity in the test soil is due to the organic substrates, nutrients applied and increased proteolytic microorganisms in the test soil sample. Similar reports were made by other workers in different incidents, such as, soils treated with tomato processing waste[53], effluents of cotton ginning mills[10,25], dairy shed effluents[61] and pig slurry[62] improved the soil protease activity than the control soil. But the activity was declined with time, maximum at 30days; it is probably because of the exhaustion of the readily available substrates. Similarly, in soils treated with dairy shed effluents[61], the activity decreased with the time. In contrast, soils polluted with cement dust from cement industries[52], waste water treatment plant discharge[63,64], herbicides[65], insecticides[66], and chlorothionil[67] ceased the soil protease activity. On the other hand, ammonium fertilizer application[61] did not result in any significant increases in protease activities due to the lack of carbonaceous materials in the ammonium fertilizer. Increased proteolytic activity by increasing the concentration of effluent is also correlated with the results reported[62], treatment of soil with pig slurry, higher protease activity was observed at higher concentration of this residue. In contrast, Sreenivasulu[55] reported that the protease activity was decreased at higher concentrations of fungicides in soil. Soil dehydrogenase activity is a good indicator of overall microbial activity in soil, and it can serve as a good indicator of soil condition[68]. Reddy and Faza[69] compared dehydrogenase activity in soils amended with[1]. without sludge of industrial origin at different intervals, the activity was higher in soils without sludge than in soils amended with industrial sludge. According to Doelman and Haanstra[70]. dehydrogenase activity was inhibited by addition of trace elements to the soil. Reduction in dehydrogenate activity observed in soil polluted with cement dust[55]. The present report correlated with Karr and Emerich[71]. Decrease in the dehydrogenase activity was attributed due to higher pH and exchangeable Mg in the reaction mixture as the cofactor.

CONCLUSION

The present study clearly indicates that disposal of waste from hydrocarbon industry alters the soil physical -chemical and biological properties and improved the soil protease, cellulase, and declined the dehydrogenase enzyme activities in contaminated rather than control soil

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