

Influence of industrial effluents on amylase activity in the agricultural soils of Anantapuramu district in Andhra Pradesh

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Abstract

Introduction: Industrial activity has been the biggest contributor to the soil pollution in the last century, especially since the amount of mining and manufacturing has increased. Industrial effluents would have adverse impact on agriculture and would cause environmental degradation.

Materials and methods: Taking the above facts into consideration, a research was undertaken in Saptagir Camphor industry to explain the influence of industrial effluents on the microbial diversity and soil microbial enzymes. In this study the influence of industrial effluents on soil amylase activity in both polluted and non-polluted soil samples of Saptagir Camphor industry was investigated.

Result and Discussion: Discharge of effluents from camphor industry altered the physico-chemical properties of soil. Amylase activity was enhanced in polluted soil than in non-polluted soil and it was maximised in soil amended with suitable substrates than in non amended soil.

Conclusion: By increasing the effluents concentration, the amylase activity was stimulated at 10% treated effluent concentrations in non-polluted soil samples and the activity subsided on further increasing the concentration.

Keywords: Soil microorganisms, Industrial effluents, amylase enzyme activity, Polluted and Non-polluted soils

INTRODUCTION

Effluent is a liquid waste discharged from a sewage system, factory, nuclear power station or other industrial plant. One of the most serious environmental problems is the existence of hazardous and toxic pollutants in industrial wastewaters. Since most of these wastewaters end up being discharged to the environment, they pose a whole lot of threat to air, water and soil quality which invariably affects the environment as a whole. It is for this reason that made it a

necessity that these contaminants needs to be effectively treated. An effluent is an inevitable production of industrial process. It is defined by the United States Environmental Protection Agency as “wastewater (treated or untreated) that flows out of a treatment plant, sewer or industrial outfall. Increased number of industries has enlarged the disposal of effluent to open land or to natural water resources. Effluent of different industries may vary in composition depending upon the source of

production. Effluent may contain essential nutrients and some toxic substances. The available macronutrients and micronutrients of effluents can increase soil fertility. On the other hand, the heavy metals and toxic components can accumulate in soil. The various effects depend on the period of effluent application. The wastes of industrial activities are finally discharged into the soil and natural water courses resulted in the undesired influence on environment particularly on soil micro flora and soil enzymes. In recent years microbes have been drawing tremendous attention due to their ability to degrade waste materials and thereby improving soil quality. Enzymes respond to soil management changes long before other soil quality indicator changes are detectable. Some enzymes only facilitate the breakdown of organic matter, while others are involved in nutrient mineralization.

Amylase catalyses the hydrolytic depolymerisation of polysaccharides in soil (Tu and Miles, 1976). Amylases are widely distributed among soil with a wide range of activities (Ladd and Butler, 1972) and properties (Mayourdon *et al.*, 1975). Amylase activity was correlated significantly with fungal and bacterial numbers and moisture content and the pH of the litter. Assays are based on the hydrolysis of soluble starch and subsequent analysis of the reducing sugar content (Tu, 1982). The practice of disposal of pulp and paper mill effluent onto the land adds large quantities of organic carbon and major essential elements to soils. There was an increase in amylase, phosphatase and dehydrogenase activities in these soils as the period of effluent irrigation increased (Kannan and Oblisami, 1990b). Production of these extracellular enzymes from microbes during litter degradation may be influenced by temperature, moisture, pH and substrate involvement (Sinsabaugh and Linkins, 1987). Increased amylase activity was

observed when soil treated with effluents released from cotton ginning mill (Narasimha *et al.*, 2012), sugarcane industry (Nagaraju *et al.*, 2007), paper industry (Venkateswar Reddy *et al.*, 2013), brewery industry (Pattlola Aishwarya *et al.*, 2014) and pressmud plus paper mill effluents (Chinnaiah *et al.*, 2002).

MATERIALS AND METHODS

Soils

Soil samples were collected from the surrounding areas (1/4th km) of Saptagir Camphor industry, Anantapuramu, Andhra Pradesh, India. Soil samples without effluent discharges served as control were collected from adjacent site (1 km) away from the same camphor industry. Soil samples were air dried and mixed thoroughly to increase homogeneity and shifted to < 2 mm sieves for estimation of physico-chemical properties by standard methods. Both polluted and non-polluted soil samples of camphor industry were used for studying various microbial populations and soil enzyme activities.

Saptagir Camphor Limited: Manufacture and supply of terpene chemical and associated products. Camphor is used in traditional pooja rituals as well as pharmaceuticals. Synthetic camphor is white crystalline solid with pharma grade 95% purity.

Soil incubation for estimation of enzyme activities

To study the effect of industrial pollution on soil amylase, 5 gms of polluted and non polluted soil samples were placed in test tubes (25 × 200) mm. After the estimation of amylase activity in both polluted and non-polluted soils, the non-polluted soils were treated with different concentrations of effluents (polluted soil) in percent which were equivalent to 0, 10, 50 and 100. Soil samples without effluents were served as controls. Triplicate soil samples were maintained for each treatment at room

temperature ($28 \pm 4^\circ\text{C}$) and moisture content was adjusted to 60% water holding capacity (WHC) throughout the experiment. After determining the stimulatory concentration in soil samples treated with different concentrations (0, 10, 50 and 100) of effluents in percent, at days interval, the rate of amylase enzyme activity of Saptagir Camphor industry was estimated.

Amylase activity

Soil samples of Camphor industry, in test tubes (25×200) mm with/without effluent discharges were incubated in the manner as specified under section 3.5. Samples were withdrawn at (0, 10, 20, 30 and 40 days) of incubation at room temperature ($28 \pm 4^\circ\text{C}$) and the enzyme amylase activity was determined as detailed earlier by Narasimha *et al.*, (2012).

Assay of amylase enzyme

Five grams of soil samples with/without effluent discharges were transferred to 250 ml Erlenmeyer flasks and 0.5 ml of toluene was added. After 15 minutes, 6 ml of 0.2 M acetate Phosphate buffer pH (5.5) containing 2% starch was added to the soil samples and respective flasks were closed with cotton

plugs. In other set, soil samples were treated in the same manner by replacing starch with acetate buffer served as without substrate and held for 24 and 72 hours at 30°C . Soil extracts were passed through Whatman No.1 filter paper and glucose content in the filtrate was assayed in the same manner as detailed earlier Nelson (1944) and Narasimha *et al.*, (2012). Suitable aliquots of filtrate were transferred to test tubes and one ml of alkaline copper reagent was added and covered with glass marbles and placed in boiling water bath for 20 minutes. The tubes were then cooled under running tap water and then one ml of arsenomolybdate reagent was added and the final volume in tube was made upto 50 ml with distilled water and bluish green colour was read at 500 nm in a U.V Visible Spectrophotometer (Thermo Scientific) Evolution 201. Silmilarly, another three sets of non-polluted soils samples of camphor industry were treated with 10, 50 and 100 percentage effluents respectively and amylase activities were assessed. The amount of glucose was calculated by referring to a calibration curve.

RESULTS AND DISCUSSION

Physico-chemical characteristics of polluted and non-polluted soil samples of Saptagir Camphor industry

Table 1: Physico chemical properties of polluted and non-polluted soil of Saptagir Camphor industry

S. No	Physico-Chemical Properties of soil	Saptagir Camphor polluted soil	Saptagir Camphor non-polluted soil
1	Color	Red	Red
2	Water holding capacity (ml g^{-1} soil)	42.39	37.97
3	Texture		
	Sand (%)	54.3	52.2
	Silt (%)	32.4	34.5
	Clay (%)	13.3	13.3
4	pH ^a	8.58	8.5
5	Electrical conductivity (m.mhos)	223	202
6	Organic carbon (%)	1.61	0.48
7	Organic matter (%) ^b	2.76	0.82
8	Total nitrogen (%) ^c	0.079	0.050

9	$\text{NH}_4^+ \text{-N}$ ($\mu\text{g g}^{-1}$ soil) ^d	7.10	6.22
10	$\text{NO}_2^- \text{-N}$ ($\mu\text{g g}^{-1}$ soil) ^e	0.64	0.41
11	$\text{NO}_3^- \text{-N}$ ($\mu\text{g g}^{-1}$ soil) ^f	0.99	0.80
12	Sulphur (%)	1.62	2.13

^a1:1.25 (soil:water) ;

^bWalkey-Black method (Jackson, 1971) ; ^cMicro-Kjeldhal method (Jackson, 1971) ;

^dNesslerization method (Jackson, 1971) ; ^eDiazotization method (Barnes and Folkard, 1951) ;

^fBrucine method (Ranney and Bartler, 1972)

Effects of Saptagir Camphor industrial effluents on soil enzyme activities

The pollution of the soil by effluents is one of the worst legacies of our intensive agricultural-industrial activities and it negatively affects various characteristics of the soil, including soil enzyme activities. Soil enzymes are natural molecules that catalyze soil microbial reactions and mainly originate from microorganisms and plants. Since enzyme activities play fundamental roles in soil chemical and biological reactions, their inhibition by industrial effluents has received considerable attention and has been well documented by many researchers over the last few decades. The activities of soil enzymes have often been proposed as sensitive indicators of important microbial reactions involved in nutrient cycles and they respond to changes in the soil caused by natural or anthropogenic factors. In this regard, soil enzyme activities are often used to evaluate the impact of human activity on soil life (Ayten Karaca *et al.*, 2009). Soil POME (palm oil mill effluent) amendment improved the activities of some enzymes during the incubation period. Compared to control soil, the addition of POME promoted a significant increase in the enzymatic activities throughout the incubation period irrespective of the dose applied (Nwoko and Salo, 2010).

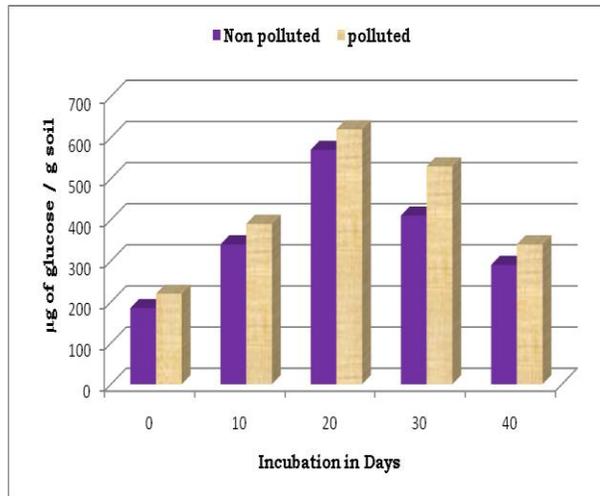
Amylase activity

Amylase is the enzyme which plays an important role in catalyzing the hydrolysis and solubilisation of starch. These enzymes are usually extracellular and inducible. The activity of amylase in polluted and non-polluted soil samples of Saptagir Camphor industry was measured by incubating the samples for 24 hours in the presence of substrate (2% starch), as described above and results were listed (Table 2). Polluted samples showed higher activity over non-polluted samples at all incubations (0, 10, 20, 30 and 40 days). Both (polluted and non-polluted) samples showed higher activities at 20 days interval and then activity was declined on further incubation (30 and 40 days). For instance, at 0 day interval, polluted sample exhibited $220 \mu\text{g glucose g}^{-1}$ soil amylase activity against $185 \mu\text{g glucose g}^{-1}$ of non-polluted soil, later it was increased by $620 \mu\text{g glucose g}^{-1}$ soil at 20 days and declined by 530 and $340 \mu\text{g glucose g}^{-1}$ soil at 30 and 40 days intervals, respectively in polluted samples and it was increased by $570 \mu\text{g glucose g}^{-1}$ soil at 20 days and declined by (410 and 290) $\mu\text{g glucose g}^{-1}$ soil at 30 and 40 days intervals respectively in non-polluted soil samples. The increased amylase activity in polluted soil over non-polluted soil may be due to availability of substrate and or amyolytic microflora in polluted soil.

Table 2: Amylase activity* in soil (with substrate) after 24 hours incubation as**

influenced by Saptagir Camphor industrial effluents

Incubation in days	Non Polluted soil	Polluted soil
0	185(100)	220(119)
10	340(100)	390(115)
20	570(100)	620(109)
30	410(100)	530(129)
40	290(100)	340(117)



*Amylase activity measured in terms of μg of glucose liberated per gram soil after 24 hours incubation.

**Incubation, in hours, of soil with starch (2% W/W) (substrate).

Figures, in parenthesis, indicate relative production percentages.

Values in the table and figure are means of triplicates.

Similar results were reported by others, in addition of pressmud and paper mill effluent irrigation (Chinnaiah *et al.*, 2002) effluents from mango waste (Reddi pradeep *et al.*, 2012), paper industry (Venkateswar Reddy *et al.*, 2013) and sugarcane industrial effluents (Nagaraju *et al.*, 2007) improved soil amylase activity was observed. In contrast, soil polluted with cement dust in cement industry ceased the soil amylase activity (Shanthi, 1993). The disposal of effluents from brewery industry alters the

activities of enzymes such as amylase. Amylase activity was stimulated in polluted soil over control. Nonetheless, prolonged incubation causes adverse effects (Pattlola Aishwarya *et al.*, 2014).

The soil amylase activity in polluted and non-polluted soil samples was measured by incubating for 24 hours without supplementation of substrate, as described above and results were depicted (Table 3). Polluted samples showed higher activity over non-polluted samples at all incubations (0, 10, 20, 30 and 40 days). Both (polluted and non-polluted) samples have shown higher activities at 20 days interval, later activity was lowered on further incubation (30 and 40 days). For instance, at 0 day interval, the polluted sample exhibited $60 \mu\text{g glucose g}^{-1}$ soil amylase activity against $40 \mu\text{g glucose g}^{-1}$ of non-polluted soil, later it was increased by $325 \mu\text{g glucose g}^{-1}$ soil at 20 days and declined by 240 and $85 \mu\text{g glucose g}^{-1}$ soil at 30 and 40 days intervals respectively in polluted samples and it was increased by $285 \mu\text{g glucose g}^{-1}$ soil at 20 days and declined by 190 and $75 \mu\text{g glucose g}^{-1}$ soil at 30 and 40 days intervals respectively in non-polluted samples. Comparatively at all incubations (10, 20, 30 and 40 days) the amylase activity without substrate in both polluted and non-polluted samples was less over amylase activity with substrate.

Amylase activity in soils treated with different concentrations of effluents like 10, 50 and 100 percentage was measured by incubating the samples for 24 hours with the addition of substrate (2% starch) as described above and results were reported (Table 4). By increasing the concentration of effluents, the amylase activity was increased upto 10% effluent, there after it was decreased (50% and 100%) at all incubations (0, 10, 20, 30 and 40 days). For instance, at 0 day, the non polluted soil

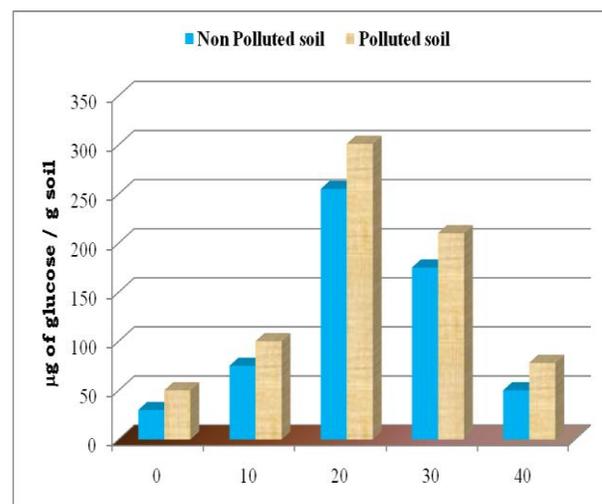
exhibited 185 μg glucose g^{-1} soil, the 10% effluent treated soil exhibited 320 μg glucose g^{-1} soil, whereas, 50% and 100% samples showed 160 μg glucose g^{-1} soil and 80 μg glucose g^{-1} soil amylase activity respectively. More over 100% samples have shown lesser amylase activities than non-polluted soils at all incubations. By increasing the incubation days, amylase activity also increased, with maximum at 20 days, later it was subsided on further incubation (30 and 40 days). For instance, 10% (stimulatory concentration) sample showed 320 μg glucose g^{-1} soil activity at 0 day, it was increased by 990 μg glucose g^{-1} soil at 20 days and then reduced by 860 and 440 μg glucose g^{-1} soil at 30 and 40 days intervals, respectively. Same trend was observed in 50% and 100% concentration of effluents treated samples at all incubations.

Amylase activity in soils treated with different concentrations of effluents like 10, 50 and 100 percentage was measured by incubating the samples for 24 hours without amendment of substrate, as described above and results were reported (Table 5). By increasing the concentration of effluents, the amylase activity was increased upto 10% effluent, there after it was decreased (50% and 100%) at all incubations (0, 10, 20, 30 and 40 days). For instance, at 0 day, non-polluted soil sample exhibited 40 μg glucose g^{-1} soil, 10% effluent treated soil exhibited 88 μg glucose g^{-1} soil, whereas 50% and 100% samples showed 62 μg glucose g^{-1} soil and 35 μg glucose g^{-1} soil respectively. More over 100% samples have shown less activity than non-polluted soils at all incubations. By increasing the incubation period, amylase activity was also increased, with maximum at 20 days, later it was declined on further incubation (30 and 40 days). For instance, 10% (stimulatory concentration) sample showed 88 μg glucose g^{-1} soil activity at 0 day, it was

increased by 520 μg glucose g^{-1} soil at 20 days and then reduced by 320 and 130 μg glucose g^{-1} soil at 30 and 40 days intervals, respectively. Same trend was observed in 50% and 100% concentration of effluents treated samples at all incubations. The overall amylase activity without substrate was comparatively less at all incubations and concentrations over amylase activity with substrate.

Table 3: Amylase activity* in soil (without substrate) after 24 hours incubation as influenced by Saptagir Camphor industrial effluents**

Incubation in days	Non Polluted soil	Polluted soil
0	40(100)	60(150)
10	95(100)	115(121)
20	285(100)	325(114)
30	190(100)	240(126)
40	75(100)	85(113)



*Amylase activity measured in terms of μg of glucose liberated per gram soil after 24 hours incubation.

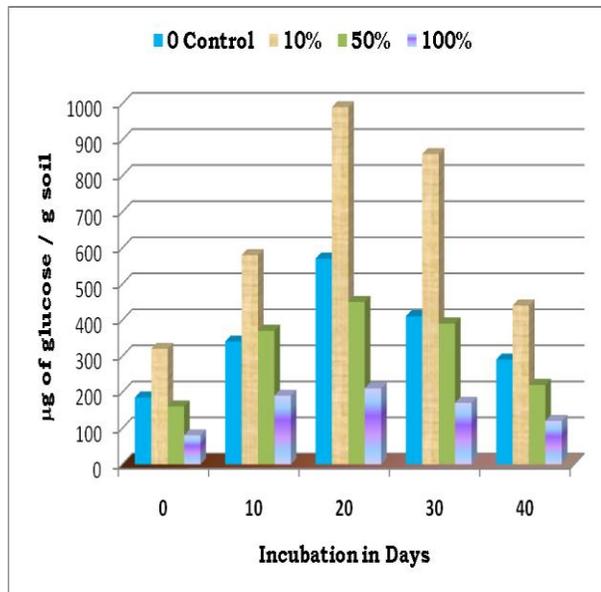
**Incubation, in hours, of soil without starch (2% W/W) (substrate).

Figures, in parenthesis, indicate relative production percentages.

Values in the table and figure are means of triplicates.

Table 4: Amylase activity* in soil (non polluted) (with substrate) after 24 hours incubation as influenced by different concentrations of Saptagir Camphor industrial effluents**

Incubation in days	Different concentration of effluents, in percent			
	0	10	50	100
0	185a(100)	320b(173)	160a(86)	80c(43)
10	340a (100)	580b(170)	370a(109)	190c(90)
20	570a(100)	990b(174)	450c(79)	210d(210)
30	410a(100)	860b(210)	390a(95)	170c(170)
40	290a(100)	440b(152)	220c(76)	120d(41)



*Amylase activity measured in terms of μg of glucose liberated per gram soil after 24 hours incubation

**Incubation, in hours, of soil with starch (2% W/W) (substrate).

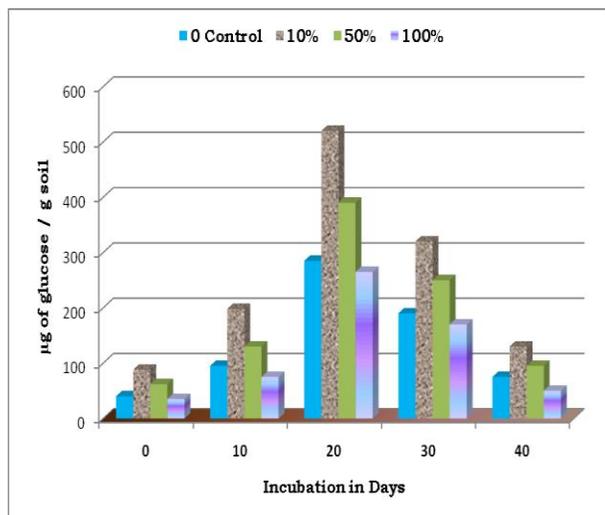
Figures, in parenthesis, indicate relative production percentages.

Values in the table and figure are means of triplicates.

Means, of each row, obtained for each samples, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to Duncan's multiple range (DMR) test.

Table5: Amylase activity* in soil (non polluted) (without substrate) after 24 hours incubation as influenced by different concentrations of Saptagir Camphor industrial effluents**

Incubation in days	Different concentration of effluents, in percent			
	0	10	50	100
0	40a (100)	88b (220)	62c (155)	35a (87)
10	95a (100)	198b (208)	130c (137)	75a (79)
20	285a (100)	520b (182)	390c (137)	265a (93)
30	190a (100)	320b (168)	250c (131)	170a (89)
40	75a (100)	130b (173)	95c (126)	50a (66)



*Amylase activity measured in terms of µg of glucose liberated per gram soil after 24 hours incubation.

**Incubation, in hours, of soil without starch (2% W/W) (substrate).

Figures, in parenthesis, indicate relative production percentages.

Values in the table and figure are means of triplicates.

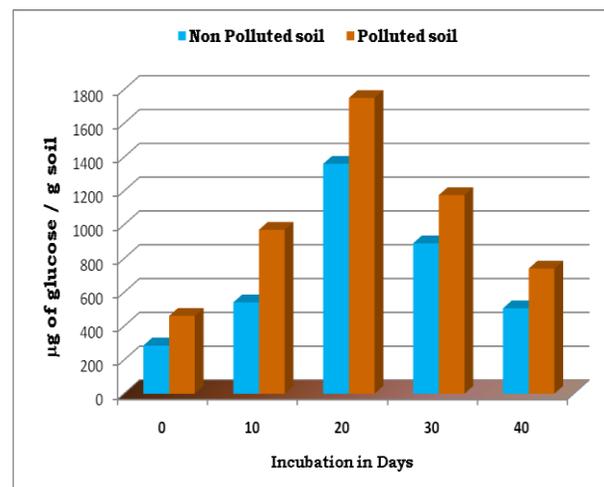
Means, of each row, obtained for each samples, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to Duncan's multiple range (DMR) test.

The activity of amylase in polluted and non-polluted soil samples of Saptagir Camphor industry was measured by incubating the samples for 72 hours in the presence of substrate (2% starch), description above and results were listed (Table 6). Polluted samples showed higher activity over non-polluted samples at all incubations (0, 10, 20, 30 and 40 days). Both (polluted and non-polluted) samples showed higher activities at 20 days interval and then activity was subsided on further incubation (30 and 40 days). For instance, polluted sample exhibited 460 µg glucose g⁻¹ soil amylase activity against 285 µg glucose g⁻¹ of non-polluted soil at 0 day interval, later it was increased by 1750 µg glucose g⁻¹ soil at 20 days and declined by 1175 and 740 µg

glucose g⁻¹ soil at 30 and 40 days intervals respectively in polluted samples and it was increased by 1360 µg glucose g⁻¹ soil at 20 days and declined by 890 and 505 µg glucose g⁻¹ soil at 30 and 40 days intervals respectively in non-polluted samples.

Table 6: Amylase activity* in soil (with substrate) after 72 hours incubation as influenced by Saptagir Camphor industrial effluents**

Incubation in days	Non Polluted soil	Polluted soil
0	285(100)	460 (161)
10	540(100)	970 (180)
20	1360 (100)	1750 (129)
30	890(100)	1175 (132)
40	505 (100)	740 (146)



*Amylase activity measured in terms of µg of glucose liberated per gram soil after 72 hours incubation.

**Incubation, in hours, of soil with starch (2% W/W) (substrate).

Figures, in parenthesis, indicate relative production percentages.

Values in the table and figure are means of triplicates.

The soil amylase activity in polluted and non-polluted soil samples was measured by incubating for 72 hours without supplementation of substrate, as described

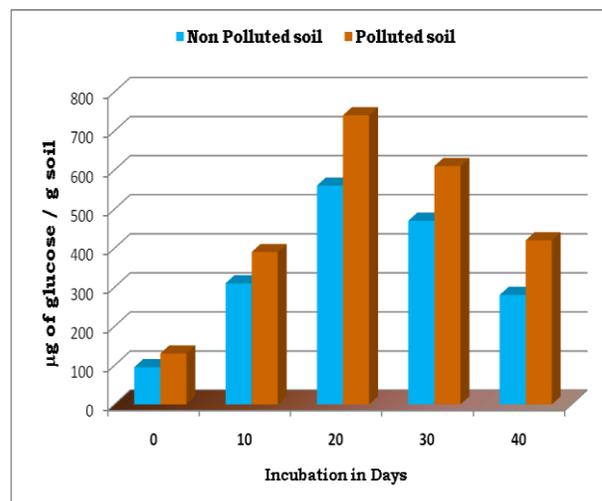
above and results were depicted (Table 7). Polluted samples showed higher activity over non-polluted samples at all incubations (0, 10, 20, 30 and 40 days). Both (polluted and non-polluted) samples showed higher activities at 20 days interval and then activity was subsided on further incubation (30 and 40 days). For instance, the polluted sample exhibited 130 μg glucose g^{-1} soil amylase activity against 95 μg glucose g^{-1} of non-polluted soil at 0 day interval, later it was increased by 740 μg glucose g^{-1} soil at 20 days and declined by 610 and 420 μg glucose g^{-1} soil at 30 and 40 days intervals respectively in polluted soil samples and it was increased by 560 μg glucose g^{-1} soil at 20 days and declined by 470 and 280 μg glucose g^{-1} soil at 30 and 40 days intervals respectively in non-polluted samples. Comparatively at all incubations (0, 10, 20, 30 and 40 days) the amylase activity without substrate in both polluted and non-polluted samples was less over amylase activity with substrate.

Amylase activity in soils treated with different concentrations of effluents like 10, 50 and 100 percentage was measured by incubating the samples for 72 hours with the addition of substrate (2% starch) as described above and results were reported (Table 8). By increasing the concentration of effluents, the amylase activity was increased upto 10% effluent, thereafter it was decreased (50% and 100%) at all incubation intervals (0, 10, 20, 30 and 40 days). For instance, at 0 day, the non polluted soil exhibited 285 μg glucose g^{-1} soil, the 10% effluent treated soil exhibited 690 μg glucose g^{-1} soil, whereas, 50 and 100% samples showed 450 μg glucose g^{-1} soil and 260 μg glucose g^{-1} soil respectively. More over 100% samples have shown less activities than non-polluted soils at all incubations. By increasing the incubation period, amylase activity also increased, with maximum at 20 days, later it was declined

on further incubation (30 and 40 days). For instance, at 0 day, 10% (stimulatory concentration) sample showed 690 μg glucose g^{-1} soil activity, it was increased by 2690 μg glucose g^{-1} soil at 20 days and then reduced by 1654 and 1170 μg glucose g^{-1} soil at 30 and 40 days intervals respectively. Same trend was observed in 50% and 100% concentration of effluents treated samples at all incubations.

Table 7: Amylase activity* in soil (without substrate) after 72 hours incubation as influenced by Saptagir Camphor Industrial effluents**

Incubatio n in days	Non Polluted soil	Polluted soil
0	95 (100)	130 (137)
10	310 (100)	390 (126)
20	560 (100)	740 (132)
30	470 (100)	610 (130)
40	280 (100)	420 (150)



*Amylase activity measured in terms of μg of glucose liberated per gram soil after 72 hours incubation.

**Incubation, in hours, of soil without starch (2% W/W) (substrate).

Figures, in parenthesis, indicate relative production percentages.

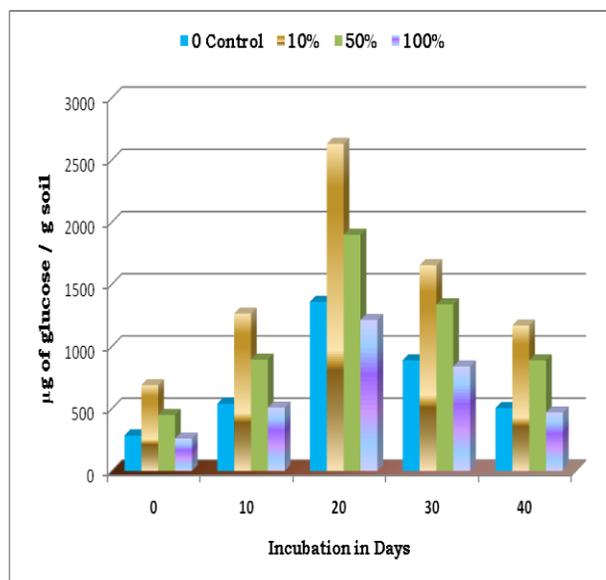
Values in the table and figure are means of triplicates.

Amylase activity in soils treated with different concentrations of effluents like 10, 50 and 100 percentage was measured by incubating the samples for 72 hours without amendment of substrate, as described above and results were reported (Table 9). By increasing the concentration of effluents, the amylase activity was increased upto 10% effluent, there after it was decreased (50% and 100%) at all incubation intervals (0, 10, 20, 30 and 40 days). For instance, at 0 day, non-polluted soil sample exhibited 95 $\mu\text{g glucose g}^{-1}$ soil, 10% effluent treated soil exhibited 340 $\mu\text{g glucose g}^{-1}$ soil, whereas 50% and 100% samples showed 165 $\mu\text{g glucose g}^{-1}$ soil and 80 $\mu\text{g glucose g}^{-1}$ soil respectively. More over 100% samples have shown less activities than non-polluted soils

at all incubations. By increasing the incubation period, amylase activity was also increased, with maximum at 20 days, later it was declined on further incubation (30 and 40 days). For instance, at 0 day, 10% (stimulatory concentration) sample showed 340 $\mu\text{g glucose g}^{-1}$ soil activity, it was increased by 1120 $\mu\text{g glucose g}^{-1}$ soil at 20 days and then reduced by 860 and 640 $\mu\text{g glucose g}^{-1}$ soil at 30 and 40 days intervals respectively. Similar trend was observed in 50% and 100% concentration of effluents treated samples at all incubations. The overall amylase activity without substrate was comparatively less at all incubations and concentrations over amylase activity with substrate.

Table 8: Amylase activity* in soil (non polluted) (with substrate) after 72 hoursincubation as influenced by different concentrations of Saptagir Camphor industrial effluents**

Incubation in days	Different concentration of effluents, in percent			
	0	10	50	100
0	285a(100)	690b (242)	450c (158)	260a(91)
10	540a (100)	1265b (234)	895c (166)	509a (94)
20	1360a (100)	2690b (198)	1900c (140)	1215a (89)
30	890a (100)	1654b (186)	1339c (150)	840a (94)
40	505a (100)	1170b (232)	890c(176)	470a (93)



*Amylase activity measured in terms of μg of glucose liberated per gram soil after 72 hours incubation

**Incubation, in hours, of soil with starch (2% W/W) (substrate).

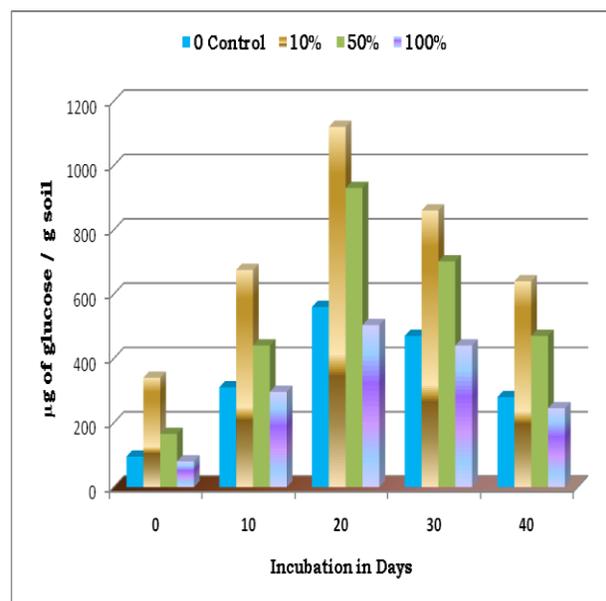
Figures, in parenthesis, indicate relative production percentages.

Values in the table and figure are means of triplicates.

Means, of each row, obtained for each samples, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to Duncan's multiple range (DMR) test.

Table 9: Amylase activity* in soil (non polluted) (without substrate) after 72 hours incubation as influenced by different concentrations of Saptagir Camphor industrial effluents**

Incubation in days	Different concentration of effluents, in percent			
	0	10	50	100
0	95a (100)	340b (358)	165c (174)	80a (84)
10	310a (100)	675b (218)	440c (142)	296a (95)
20	560a (100)	1120b (200)	930c (166)	504a(90)
30	470a (100)	860b (183)	702c (149)	440a (94)
40	280a (100)	640b (228)	470c (168)	245a (87)



*Amylase activity measured in terms of μg of glucose liberated per gram soil after 72 hours incubation.

**Incubation, in hours, of soil without starch (2% W/W) (substrate).

Figures, in parenthesis, indicate relative production percentages.

Values in the table and figure are means of triplicates.

Means, of each row, obtained for each samples, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to Duncan's multiple range (DMR) test.

Conclusions

Discharge of effluents from this industry to surrounding environment including

agriculture, without neutralization has become general practice. Camphor effluents contain considerable amount of organic and

inorganic pollutants. There is also considerable interest in the study of soil enzymes because such effect reflects the potential capacity of a soil to perform certain biological transformation of soil fertility. These effluents also have an impact on the nitrogen transformations in soil. These industrial effluents have an impact on soil physico-chemical, biological properties and also on enzyme activities. Soil contamination or soil pollution as a part of land degradation is caused by the presence of xenobionis (human-made) chemicals or other alternations in the natural soil environment. It is typically caused by industrial activity, agricultural chemicals or improper disposal of waste. The most common chemicals involved are petroleum, hydro carbons poly nuclear aromatic hydrocarbons such as (naphthalene and benzo (a) pyrene), solvents and pesticides, lead and other heavy metals. Contamination is correlated by degree of industrialization and intensity of chemical usage. Amylase enzyme activity was enhanced in polluted soil than in non-polluted soil and it was maximised in soil amended with suitable substrates than in non amended soil. By increasing the effluents concentration, the amylase activity was stimulated at 10% treated effluent concentrations in non-polluted soil samples and the activity subsided on further increasing the concentration.

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References

Ayten Karaca, S., C. Cetin, O. C. Turgay and R. Kizilkaya. 2009. Effects of Heavy Metals on Soil Enzyme Activities Soil Heavy Metals, pp: 237-262.

- Barnes, H. and B.R. Folkard. 1951. The determination of nitrite. *Analyst*, 76: 599-603.
- Chinnaiah, T. and V. Trelo-ges. 2002. An important of yasothon soil fertility (toxic pollutants) using municipal fermented organic compost and Panicum maximum TD 58 grass. *Pakistan Journal of Biological Sciences*, 4(8): 968-972.
- Jackson, M.L. 1971. *Soil Chemical Analysis*. Prentice-Hall of India, New Delhi.
- Kannan, K. and G. Oblisami. 1990b. Influence of paper mill effluent irrigation on soil enzyme activities, *Soil Biology and Biochemistry*, Volume 22, Issue 7, Pages: 923-926.
- Ladd, J.N., J.H.A. Butler. 1972. Short-term assays of soil proteolytic enzyme activities using proteins and dipeptide derivatives as substrates. *Soil Biol Biochem.*, 4:19-30 Cross Ref Google Scholar
- Mayourdon, J, L. Batistic and J.M. Sarkar. 1975. Properties des activities proteolytiques extraites des sols frais. *Soil Biol Biochem.*, 7s: 281-286.
- Nagaraju, M., G. Narasimha and V. Rangaswami. 2007. Impact of effluents of sugarcane industry on soil physico-chemical and biological properties. *J. Ind. Pollut. Cont.*, 23: 73-76.
- Narasimhaa, G., A. Sridevi, G.V. Subba Reddy, M. Tahaseen Banu and B. Rajasekhar Reddy. 2012. Effect of cotton ginning mill industrial effluents on soil dehydrogenase, phosphatase, amylase and invertase enzyme activities. *International Journal of Agricultural and Food Science*, 2(1): 1-6.
- Nelson, N. 1944. A Photomeric adaptation of Somogyi method for determination of glucose. *J. Biol. Chem.*, 153: 375-380.
- Nwoko, Chris.O. and Sola Ogunyemi. 2010. Effect of Palm Oil Mill Effluent (POME) on Microbial Characteristics in a Humid Tropical Soil under Laboratory

- Conditions. International Journal of Environmental Science and Development, Vol. 1, No. 4, October 2010. ISSN: 2010-0264. pg: 307-314.
- Pattlola Aishwarya, L. Saida, K. Venkateswar Reddy and P. Ranjit. 2014. Impact of Brewery industry Effluents on soil enzyme activities. Int.J.Curr.Microbiol.App.Sci., 3(10): 686-692.
- Ranney, T.A and R.J. Bartlett. 1972. Rapid field determination of nitrate in natural waters. Communications in Soil Science and Plant Analysis, 3: 183-186.
- Reddi pradeep, M., Vimal doss, A. Praveen, A. Janardhan and G. Narasimha. 2012. Effect of mango pulp waste on soil physicochemical, microbial and enzyme activities. Asian Jr. of Microbiol. Biotech. Env. Sc., Vol. 14, No. (3): 391-397.
- Shanthi, M. 1993. Soil biochemical process in industrially polluted area of cement industry. M.Phil dissertation, Sri Krishnadevaraya University, Anantapur.
- Sinsabaugh, R.L and A.E. Linkins. 1987. Inhibition of the Trichoderma vlrde cellulas complex by leaf litter extracts. Soil Biol. Biochem., 19: 719-725.
- Tu, C.M. 1982. Influence of pesticides on activities of amylase, invertase and level of adenosine tryphosphate inorganic soil. Chemosphere, 2: 909-914.
- Tu, C.M and J.R.W. Miles. 1976. Interaction between insecticides and soil microbes. Residue Rev., 64: 17-66.
- Venkateswar Reddy, K., T. Vijayalakshmi, M, Lakshmi Narasu and L. Saida. 2013. Soil microbial response to Paper industry effluents. Science Journal, Volume 2, Issue 1. ISSN: 2320-0421, DAMA International
- Walkley, A and I.A. Black. 1934. An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci., 37: 29-38.