Influence of Industrial Effluents on Protease Enzyme Activity in The Agricultural Soil of Anantapuramu District in Andhra Pradesh

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Abstract **- 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. 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 protease enzyme in both polluted and non-polluted soil samples of Saptagir Camphor industry was investigated. Discharge of effluents from camphor industry altered the physico-chemical properties of soil. Protease 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 enzyme activity was stimulated at 50% treated effluent concentrations in non-polluted soil samples and the activity subsided on further increasing the concentration.**

Index Terms **- Saptagir Camphor Industrial effluents, protease enzyme activity, Polluted and Non- polluted soils.**

I.INTRODUCTION

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 role 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 [3]. 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 [15].

Influence of effluents from Saptagir Camphor industry on activity of enzyme –protease in polluted and non-polluted soils should be examined under laboratory condition. Discharge of effluents from Saptagir Camphor industry to surrounding environment including agriculture, without neutralization has become general practice. Saptagir Camphor industrial effluents contain considerable amount of organic and inorganic pollutants. The impact of these pollutants on microbial activities in terrestrial eco system is scanty. 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. 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. Taking the above facts into consideration, a research was undertaken in Saptagir Camphor industry to explain the influence of Saptagir Camphor industrial effluents on the microbial diversity and soil microbial enzymes.

II. REVIEW OF LITERATURE

Protease activity

Proteases are widely distributed among soils and show a wide range of activities [7]. Proteases are involved in the initial hydrolysis of protein components of organic nitrogen to simple amino acids. Hydrolytic degradation of proteins is an important step in the nitrogen cycle. Proteases in soils hydrolyze not only added proteins but also native soil added proteins [8]. Proteases, detected in microorganisms, plants and animals, catalyze the hydrolysis of proteins to polypeptides and oligopeptides to amino acids [6] involved in the nitrogen cycle [12]. Treatment of soils with metal-contaminated sewage sludge [1], effluents from mango pulp waste [19], paper industry effluents [5 and 21] and pig slurry [16] increased protease activity. In contrast to this, decreased protease activity was observed in soils treated with organic matter [9] and crude oils [22].

Protease in soils plays a significant role in nitrogen mineralization [10], an important process regulating the amount of nitrogen availability for plant growth. This enzyme in the soil is generally associated with inorganic and organic colloids. Protease activities have been reported to occur partially in soil as a humopeptide complex [2], from arable soil, from municipal waste compost [17] and from forest or permanent grassland soils. The amount of this extracellular enzyme activity may be indicative not only of the biological capacity of soil for the enzymatic conversion of the substrate, which is independent of the extent of microbial activity, but might also have an important role in the ecology of microorganisms in the ecosystem [3].

III. 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 homogenesity 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 activity

To study the effect of industrial pollution on soil protease, 5 gms of polluted and non-polluted soil samples were placed in test tubes (25 × 200) mm. After the estimation of protease 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 ± 4ºC) 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 protease enzyme activity of Saptagir Camphor industry was estimated. Protease activity is measured from tyrosine equivalents formed from casein. The method employed for determining

protease activity is essentially the same followed by [20] adopted by [19].

Assay of protease

Five grams of soil samples incubated were treated with 10 ml of 0.1 M Tris (2-amino-2-(hydroxymethyl)-propane-1,3-diol) at pH 7.5 containing sodium caseinate (2% w/v) and incubated for 24 hours at 30ºC. Another set of soil samples treated in the same manner with modification of casein was replaced by acetate buffer, served as without substrate. All the tubes were incubated for desired incubation periods (0, 10, 20, 30 and 40 days). To this, 4 ml of aqueous trichloro acetic acid (17.5% w/v) was added and the contents were centrifuged. Suitable aliquots of the supernatant were further treated with 1.4 M Na2CO3, followed by 1 ml of Folin-Ciocalteu reagent (33.33% v/v) with rapid swirling. After 30 minutes blue colour was formed and it is read at 700 nm in a U.V Visible Spectrophotometer (Thermo Scientific) Evolution 201. Finally, the activity of protease was expressed in terms of mg of tyrosine equivalents per gram of soil. Silmilarly, another three sets of non-polluted soils samples of Camphor industry were treated with 10, 50 and 100 percentage effluents respectively and protease activity was assessed.

IV. RESULTS AND DISCUSSION

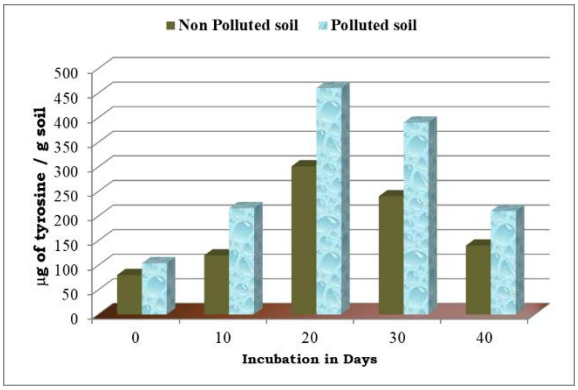
Protease activity

Proteases are involved in the initial hydrolysis of protein components of organic nitrogen to simple amino acids. Hydrolytic degradation of proteins is an important step in the nitrogen cycle. Proteases in soils hydrolyze not only added proteins but also native soil added proteins [8]. The activity of protease in polluted and non-polluted soil samples of Saptagir Camphor industry was measured by incubating the samples in the presence of substrate (2% casienate), with 60%water holding capacity and activity was measured in terms of tyrosine equivalents formed in trichloroaceticacid soluble fraction during 24 hrs, as described above and results were listed (Table 1). 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 activity at 20 days interval and then activity was reduced on further incubation (30 and 40 days). For instance, at 0 day interval, polluted sample exhibited 104 µg tyrosine g-1 soil protease activity against 80 µg tyrosine g-1 of non-polluted soil, later it was increased by 460 µg tyrosine g-1 soil at 20 days and declined by 390 and 210 µg tyrosine g-1 soil at 30 and 40 day intervals respectively in polluted samples and it was increased by 301 µg tyrosine g-1 soil at 20 days and declined by 240 and 140 µg tyrosine g-1 soil at 30 and 40 day intervals respectively in non-polluted soil samples. The increased protease activity in polluted soil over non-polluted soil may be due to availability of substrate (casein) and increased proteolytic microflora in polluted soil. 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.

[18] reported that the protease activity increased up to 14th day and then declined at 21st day probably due to exhaustion of the readily available substrates ammended with leather industrial effluents. Similarly, soil protease activity in soils treated with dairy shed effluents [23] and dairy factory effluents [14] increased at first and then decreased with the time. Similar results were reported by others, treatment of soils with metal-contaminated sewage sludge [1], effluents from sugarcane industry [13], effluents from dairy waste [4], paper industry effluents [21 and 5] and pig slurry [16] increased protease activity. In contrast to this, decreased protease activity was observed in soils treated with organic matter [9], crude oils [22] and waste water treatment plant discharge [11]. Hydrolases (Protease) recorded a steady increase in activity throughout the incubation period irrespective of the dose (palm oil mill effluent) applied. At high dose (POME) application there was no significant differences in Protease activity in all assayed samples [15].

Table 1: Protease 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	80(100)	104(130)
10	120(100)	216(180)
20	301(100)	460(153)
30	240(100)	390(162)
40	140(100)	210(150)



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

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

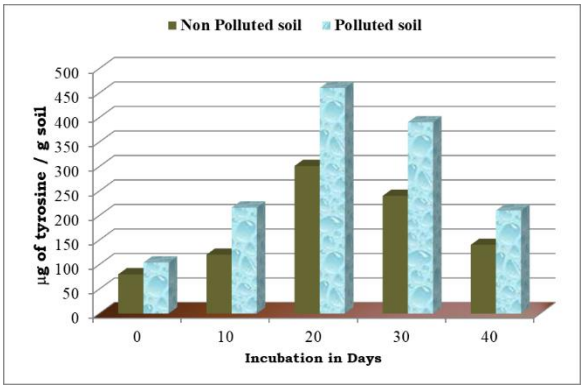
Figures, in parenthesis, indicate relative production percentages.

Values in the table and figure are means of triplicates.

The soil protease activity in polluted and non-polluted soil samples was measured by incubating without supplimentation of substrate, as described above and results were depicted (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 have shown higher activity 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 µg tyrosine g-1 soil protease activity against 43 µg tyrosine g-1 of non-polluted soil, later it was increased by 286 µg tyrosine g-1 soil at 20 days and declined by 183 and 116 µg tyrosine g-1 soil at 30 and 40 day intervals respectively in polluted samples and it was increased by 203 µg tyrosine g-1 soil at 20 days and declined by 146 and 79 µg tyrosine g-1 soil at 30 and 40 day intervals respectively in non-polluted samples. Comparitively at all incubations (0, 10, 20, 30 and 40 days) the protease activity without substrate in both polluted and non-polluted samples was less over protease activity with substrate.

Table 2: Protease 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	43(100)	60(139)
10	84(100)	104(124)
20	203(100)	286(141)
30	146(100)	183(125)
40	79(100)	116(147)



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

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

Figures, in parenthesis, indicate relative production percentages.

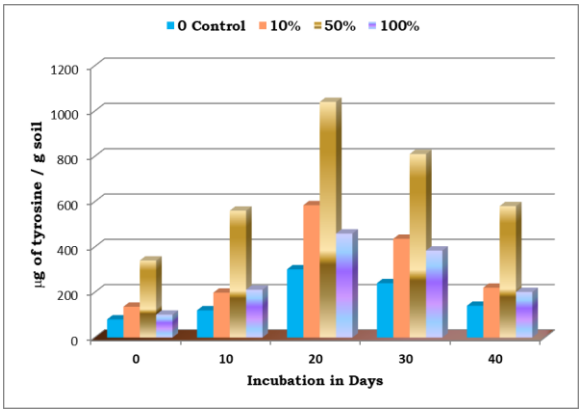
Values in the table and figure are means of triplicates.

Protease activity in soils treated with different concentrations of effluents like 10, 50 and 100 percentage was measured by incubating the samples with the addition of substrate (2% casienate) as described above and results were reported (Table 3). By increasing the concentration of effluents, the protease activity was increased upto 50% effluent, there after it was decreased (100%) at all incubations (0, 10, 20, 30 and 40 days). For instance, at 0 day, non-polluted soil exhibited 80 µg tyrosine g-1 soil, 10% and 50% effluent treated soil exhibited 136 µg tyrosine g-1 soil and 340 µg tyrosine g-1 soil respectively, whereas 100% samples showed 101 µg tyrosine g-1 soil and 100% samples have shown higher activity than non-polluted soils at all incubations. Similarly, by increasing the incubation period, protease activity was also increased, with maximum at 20 days, later it was reduced on further incubation (30 and 40 days). For instance, 50% (stimulatoery concentration) sample showed 340 µg tyrosine g-1 soil activity at 0 day, it was increased by 1040 µg tyrosine g-1 soil at 20 days and then reduced by 810 and 580 µg tyrosine g-1 soil at 30- and 40-day intervals respectively. Similar trend was observed in 10% and 100% concentration of effluents treated samples at all incubations. The overall protease activity without substrate was comparitively less at all incubations and concentrations over protease activity with substrate.

Table 3: Protease 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	80a (100)	136b (170)	340c (425)	101d (126)
10	120a (100)	198b (165)	560c (467)	211d (176)
20	301a (100)	584b (194)	1040c (345)	459d (152)
30	240a (100)	436b (182)	810c (337)	384d (160)
40	140a (100)	220b (157)	580c (414)	201d (143)



*Protease activity measured in terms of µg of tyrosine liberated per gram soil after 24 hours incubation

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

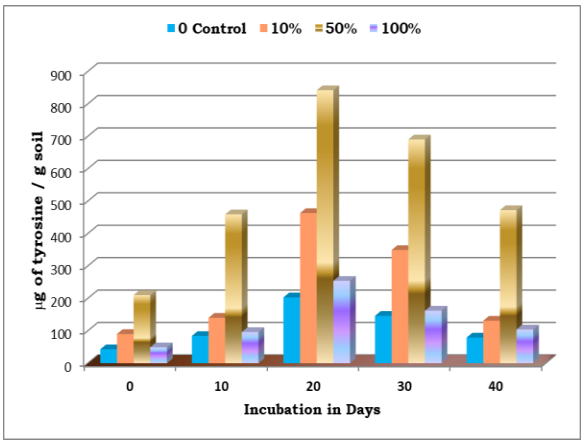
Figures, in parenthesis, indicate relative production percentages.

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.

Protease activity in soils treated with different concentrations of effluents like 10, 50 and 100 percentage was measured by incubating the samples without amendment of substrate, as described above and results were reported (Table 4). By increasing the concentration of effluents, the protease activity was increased upto 50% effluent, there after it was decreased (100%) at all incubations (0, 10, 20, 30 and 40 days). For instance, at 0 day, non-polluted soil exhibited 43 µg tyrosine g-1 soil, 10% and 50% effluent treated soil exhibited 90 µg tyrosine g-1 soil and 210 µg tyrosine g-1 soil respectively, whereas 100% samples showed 49 µg tyrosine g-1 soil and 100% samples have shown higher activity than non-polluted soils at all incubations. Similarly, by increasing the incubation period, protease activity was also increased, with maximum at 20 days, later it was declined on further incubation (30 and 40 days). For instance, 50% (stimulatory concentration) sample showed 210 µg tyrosine g-1 soil activity at 0 day, it was increased by 842 µg tyrosine g-1 soil at 20 days and then reduced by 690 and 472µg tyrosine g-1 soil at 30 and 40 day intervals respectively. Similar trend was observed in 10% and 100% concentration of effluents treated samples at all incubations. The overall protease activity without substrate was comparitively less at all incubations and concentrations over protease activity with substrate.

Table 4: Protease 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	43a (100)	90b (209)	210c (488)	49a (114)
10	84a (100)	140b (167)	459c (546)	96a (114)
20	203a (100)	463b (228)	842c (415)	254d (125)
30	146a (100)	349b (239)	690c (473)	162a (111)
40	79a (100)	131b (166)	472c (597)	104d (132)



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

**Incubation, in hours, of soil without casein (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.

V. CONCLUSIONS

Thus, the results revealed that the microbial density is high in polluted soil as compared to non-polluted soil. The current study of Saptagir Camphor effluent discharge suggested that the high inorganic as well as organic pollution load, serves as nutrients for microbial growth hence increasing their population in polluted soil around Saptagir Camphor industrial area as compared to the non-polluted soil taken in this study. The chemical nature of effluent was complex and contained large amounts of organic and inorganic compounds. The soil around the industry receives plenty of treated and non-treated effluent since decade serves as a broth for an enormous diversity of microbes. The microbial population able to use organic load like Camphor residues of effluent are reported in this study. These microbes are forced to live in the medium of high organic and toxic load in the soil thereby acclimatized to process them. The values of physico-chemical characteristics are slightly higher in polluted soil than in non-polluted soil. These effluents have deleterious effects on the soil sample collected from the site of effluent discharge. The effluents doesnot have a major impact on soil for cultivation purpose but changes the diversity of soil microflora and soil enzyme activities. Results suggest that the microbial population in the polluted site increased, indicating that the microbes have developed resistence towards the effluents discharged from the industry. The alkaline environment may have encouraged the activity of the enzymes. In fact, studies have shown that the ability of microbes to tolerate a definite level of pollutants under natural conditions might be owing to the complex nature of the soil environment.

Discharge of effluents from Saptagir Camphor industry altered the physico-chemical properties of soil. Protease 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. The enzyme activity was increased upto 20 days and on further increasing the days of incubation the activity was declined. This may be due to the depletion of nutrients in soil. By increasing the effluents concentration, the protease activity was stimulated at 50% treated effluent concentrations in non-polluted soil samples and the activity subsided on further increasing the concentration.

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REFERENCES

[1] Achberger, K. and R. Ohlinger. 1988. Effect of sewage sludge and waste compost on some enzymatic activities tested in a field experiments. Poster presentation at EC. EWPCA symposium, Amsterdam Google Scholar.

[2] Bastistic, L., J.M. Sarkar and J. Mayaudon. 1980. Extraction, purification and properties of soil hydrolases. Soil Boil. Biochem., 12: 59-63.

[3] Burns, R.G. 1982. Enzyme activity in soil: location and a possible role in m.6icrobial ecology. Soil Biol. Biochem., 14: 423-427.

[4] David Shyam Babu, S. 2010. Protease and phosphatase activity of soil contaminated with dairy waste water. Asian Jr. of Microbio. Biotech. Env. Sc., 12(2): 323-327.

[5] Gouthami, P., M. Sahana, L. Saida Naik and K. Venkateswar Reddy. 2014. Effect of paper industry effluents on soil protease and urease activity. DOI: 10.15680/IJIRSET. 2014.0310019.V(3):2319-8753.

[6] Handa, S.K., M.P. Agnihothri and G. Kulshresta. 2000. Effect of pesticides on soil fertility. In: Pesticide residue analysis and significance. Research Periodicals and Publishing House, New Delhi, pp: 184-198.Google Scholar

[7] Hayano, K. 1986. Cellulase complex in tomato field soil; introduction localization and some properties. Soil Biol Biochem., 18: 215-219 CrossRefGoogle Scholar

[8] Kiss, S., G. Stetanio and M. Diagnam-Vulandra. 1975. Soil enzymology in Romania. (Part) ii Contributi. Botanica Cluj., p;197-207.
[9] Ladd, J.N., J.H.A. Butler. 1969. Inhibitory effect of soil humic compounds on the proteolytic enzyme, pronase. Aust J Soil Res., 7: 241-251Google Scholar
[10] Ladd, J.N and R.B. Jackson. 1982. In: Stevenson FJ (Ed.). Nitrogen in Agricultural Soils.
[11] Montuelle, B. and B. Volat. 1998. Impact of wastewater treatment plant discharge on enzyme activity in fresh water sediments. Ecotoxicol Environ Saf., 40 (1-2): 154-159.
[12] Moreno, J.L., C. Garcia and T. Hernandez. 2003. Toxic effect of cadmium and nickel on soil enzymes and the influence of adding sewage sludge. Eur J Soil Sci., 54: 377-386. CrossRefGoogle Scholar
[13] 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.
[14] Nizamuddin, S., A. Sridevi and G. Narasimha. 2008. Impact of dairy factory effluents on soil enzyme activities. Eco.Env.Cons., 14 (1): 89-94.
[15] 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.
[16] Plaza, C., J. Garcia-Gil, C. Soler, P. Revira and A. Polo. 2002. Effect of agricultural application of Pig slurry on soil enzyme activities. Poster presentation. Centro de Ciencias Medioambientales (CSIC), Madrid, Espana.
[17] Rad, J.C., M. Navarro-Gonzalez and S. Gonzalez-Carcedo. 1995. Characterization of proteases extracted from a compost of municipal solid wastes. Geomicrobiol, J. 13: 45-56.
[18] Reddi pradeep, M. and G. Narasimha. 2012. Effect of leather industry effluents on soil microbial and protease activity. Journal of environmental biology Vol. 33, Pages: 39-42.
[19] 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.
[20] Speir, T.W and D.J. Ross. 1975. Effects of storage on the activities of protease, urease, phosphatase and sulphatase in three soils under pasture. New Zealand Journal Science., 18: 231-237.
[21] 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
[22] Walker, J., H. Austin and R. Colwell. 1975. J Gen Appl Microbiol, 21: 27 Google Scholar
[23] Zaman, M., H.J. Di and K.C. Cameron. 1999. A field study of gross rates of N mineralization and nitrification and their relationships to microbial biomass and enzyme activities in soils treated with dairy effluents and ammonium fertilizer. Soil Use and Management., 15 (3): 188-194.