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EFFECT OF UNTREATED SEWAGE EFFLUENT IRRIGATION ON HEAVY METALCONTENT, MICROBIAL POPULATION AND ENZYMATIC ACTIVITIESOF SOILS IN ALIGARH

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ABSTRACT

The study pertains to the impact of domestic and industrial sewage water irrigation on the chemical, biological and enzymatic activities in alluvial soils of Aligarh District. Results showed that soil enzymatic [dehydogenase (DHA), acid and alkaline phosphatase, urease and catalase] activities in the soils increased up to 14 days of incubation and thereafter inhibited significantly. The enzymatic activity were in the order sewage effluent> partial sewage effluent> ground water irrigated soils. Increase in soil enzymatic activities up to 2^nd^ week of incubation was due to decomposition of organic matter. Maximum inhibition of enzymatic activities, after 14 days of incubation were found in sewage effluent irrigated soils and minimum in ground water irrigated soils. Similar trend was also seen for microbial population. Soil enzymatic activities and microbial population were significantly and positively correlated with soil organic matter. Results also indicated that the microbial population and enzymatic activities in sewage irrigated soils decreased continually with irrigation period. The average concentration of total heavy metals in sewage irrigated soils and partial sewage irrigated soils increased and was 3and 2 times higher for Zn; 4.5and 1.7 times higher for Cu; 3.8 and 2.4 times higher for Cr; 5.7and 3.5 times higher for Pb; 3.5and 2.2 times higher for Cd and 2.7and 2.0 times higher for Ni respectively than that of ground water irrigated soils. Results also showed that though total heavy metals concentration increased with period of sewage irrigation but the concentration of diethylenetriaminepentaacetic acid (DTPA) extractable heavy metals in partial sewage irrigated and sewage irrigated soils remained almost same, which might be due to deposition of heavy metals in crops grown on the soils.

Keywords :Sewage irrigation, Soil enzyme activities, Microbial population, Heavy metals

I. INTRODUCTION

Assessment of microbial biomass and various enzyme systems have widely been used to diagnose the soil state, to describe the effect of different influences of pollutants, agricultural management and land use. Soil enzyme activity is involved in nutrient cycling and availability of nutrients and can be used as index of soil functioning (Nanniperi, 2003). They are not only essential for plant growth but also important equally for soil fertility (Gonzalez *et al.*, 2007). Anthropogenic activities leading to intentional or unintentional deposition of contamination maybe harmful to soil environment, affect the amount and activities of soil enzyme at different functional levels, and reduce the growth andyield of plants (Gianfreda and Bollage, 1996). Enzyme activity in soil is regulated by soil pH and microbial biomass which is co-related to soil organic matter and soil moisture content as well as to soil compaction (Karaca*et al.*, 2000). Soil enzyme activity varies with time and is limited by available substrate supply andmay provide useful linkage between microbial community composition and carbon processing (Waldrop *et al.*, 2000).

In India sewage and industrial wastewater is commonly used for agricultural purposes (Pandey*et al*., 2008; Nath et *al*., 2009: Nagjyoti*et al*., 2009). Continuous use of wastewater leads to enrichment of soil with essential micro and macro nutrients (Kanan*et al* ., 2005). At lower concentration these micronutrientsare beneficial but become toxic when their concentration exceeds the requirement limit**.** These heavy metals may accumulate to a toxic concentration level in humans via crops which can lead to impairment in the quality of human life (Sipter*et al.*, 2008; Wang *et al.*, 2005).. Several metals, such as Pb, Cd, Cr and Co areconsidered hazardous and may accumulate with in . human body, with a relatively long half-life (Onder and Dursun, 2006).In light of the above, the present study aimed to investigate the effect of sewage effluent irrigation, on the heavy metal accumulation; population of soil microorganisms and enzymatic (DHA, acid phosphatase, alkaline phosphatase, urease and catalase) activities in soils of Aligarh district.

II. MATERIALS AND METHODS

Soil samples (0-25 cm) from ten sewage irrigated (samples 1-10), eight partially sewage irrigated (samples 11-18) and three ground water irrigated (samples 19-21) were collected bimonthly from different agricultural lands of Aligarh districts (27°53′N 78°35′E) (from each site 5 samples were collected and were bulked) from the month of June 2011 to May, 2012. The collected soil samples were brought to the laboratory in sterile polythene bags and stored at 4^oC for further analysis.The collected soil samples were air-dried, grounded and sieved through 2 mm sieve. The soil characteristics like soil moisture, pH, electric conductivity, organic matter, nitrogen and phosphorous were determined by usual methods (Bansal, 1982). Heavy metal (Cu, Cd, Zn, Cr, Pb and Ni) concentration in the soil samples were estimated after tri-acid digestion (70% HNO_3, 65% $HClO_4$ and 70% H_2SO_4 in 5:1:1 ratio), plant available heavy metals were estimated by treating soil with solution of diethylenetriaminepentaacetic acid (DTPA). The concentration of heavy metals was analyzed by atomic absorption spectrophotometer (Lindsay and Norvell, 1978). Bacteria, fungus and Actinomycetes were isolated from each soil sample after incubation at 25± 2ºC for 56 days. The soil samples were drawn at different time intervals (0 (4h), 7, 14, 21, 28, 42 and 56 d) after incubation. Bacteria, fungus and actinomycetes population were counted using dilution plate techniques; dilution was made up to 10^{-7} for bacteria, 10^{-3} for fungus and 10^{-3} for actinomycetes (Cuppucino and Sherman, 1983).

Table 1 :Physico-chemical properties of collected soil samples (June, 2011 to May, 2012)

Property	Sewage irrigated soils (60)	Partial irrigated soils (48)	Ground water irrigated soils(18)
pHLSD	7.89 (7.45-8.52)0.14	7.97 (7.42-8.32)0.14	8.34 (8.25-8.64)0.12
Organic matter %LSD	4.42 (3.03-5.82)0.08	2.93 (2.0-3.14)0.10	1.98 (1.78-2.18)0.04
CEC cmole kg^{-1}LSD	15.4 (13-16.8)0.42	12.9 (11.8-14)0.32	10 (9.8-10.2)0.05
Total Nitrogen %LSD	0.161 (0.15-0.174)0.008	0.135 (0.12-0.14)0.004	0.075 (0.07-0.08)0.002
Total phosphorous %LSD	0.054 (0.049-0.062)0.002	0.050 (0.046-0.057)0.002	0.031 (0.027-0.035)0.002

LSD= least significant difference; (Values in parentheses are the range of samples collected)

III. ESTIMATION OF ENZYMATIC ACTIVITIES

Enzymatic activitiesstudies were studied at 50% of water retention capacity maintained on the basis of initial soil analysis. Moisture was regularly maintained based on the difference between two consecutive days. Sample bottles were incubated at 25± 2 ºC for 56 days. The soil samples were drawn at different time intervals (0 (4hr), 7, 14, 21, 28, 42 and 56 days) after incubation and stored in plastic vials at 4ºC to evaluate the enzyme activities. Dehydrogenase, acid and alkaline phosphatase, urease and catalase activities in soil samples were estimated following the method of Casida*et al* (1964), Tabatabai and Bremner (1969,1972) and Johnson and Temple (1964), respectively.All the chemicals used were of analytical grade and all the experiments were done in triplicate.

Table 2 :Average Concentration (mg kg^{-1}) of heavy metals in collected soil samples of Aligarh district (June11-May, 12)

Metal	Sewage irrigated soils (60)		Partial irrigated soil(48)		Ground water irrigated soils(18)	
	T	E	T	E	T	E
ZnLSD	99.3 (90-126) 1.2	50.6 (44-64) 0.7	64.6(54-78) 0.7	39.7 (33-48) 0.8	39(34-45) 0.6	23 (21-25) 0.5
Cu LSD	40 (32-45) 0.9	24.3 (20-30) 0.6	25.9 (23-32) 0.9	14.6 (11-21) 0.8	16.7 (15-19) 0.5	9.7 (9-11)0.4

CrLSD	64.9 (55-74) 1.6	38. (30-58) 9 1.5	46 (42-59) 1.2	29. 3 (26-37) 0.8	19.7(17-22) 0.5	11. 7 (10-14) 0.4
PbLSD	58.8(52-69) 1.7	27. (22-32) 2 0.8	38 (33-45) 0.8	21. 1 (19-26)0.7	12.2 (10-15) 0.5	11. 7 (9-15) 0.8
CdLSD	0.456(0.38-0.56) 0.052	0.264(0.21-0.38) 0.038	0.323(0.28-0.40) 0.034	0.198 (0.18-0.23) 0.032	0.143 (0.13-0.17) 0.022	0.093 (0.07-0.13) 0.024
NiLSD	23.2(19-29) 1.0	12.5(10-16) 0.7	16.5 (13-22) 0.8	10.1(8-14) 0.7	10 (9-13) 0.4	6.2 (5-7.5) 0.3

T= Total; E= DTPA- extractable; LSD= least significant difference; (Values in parentheses are the range of samples collected in a year

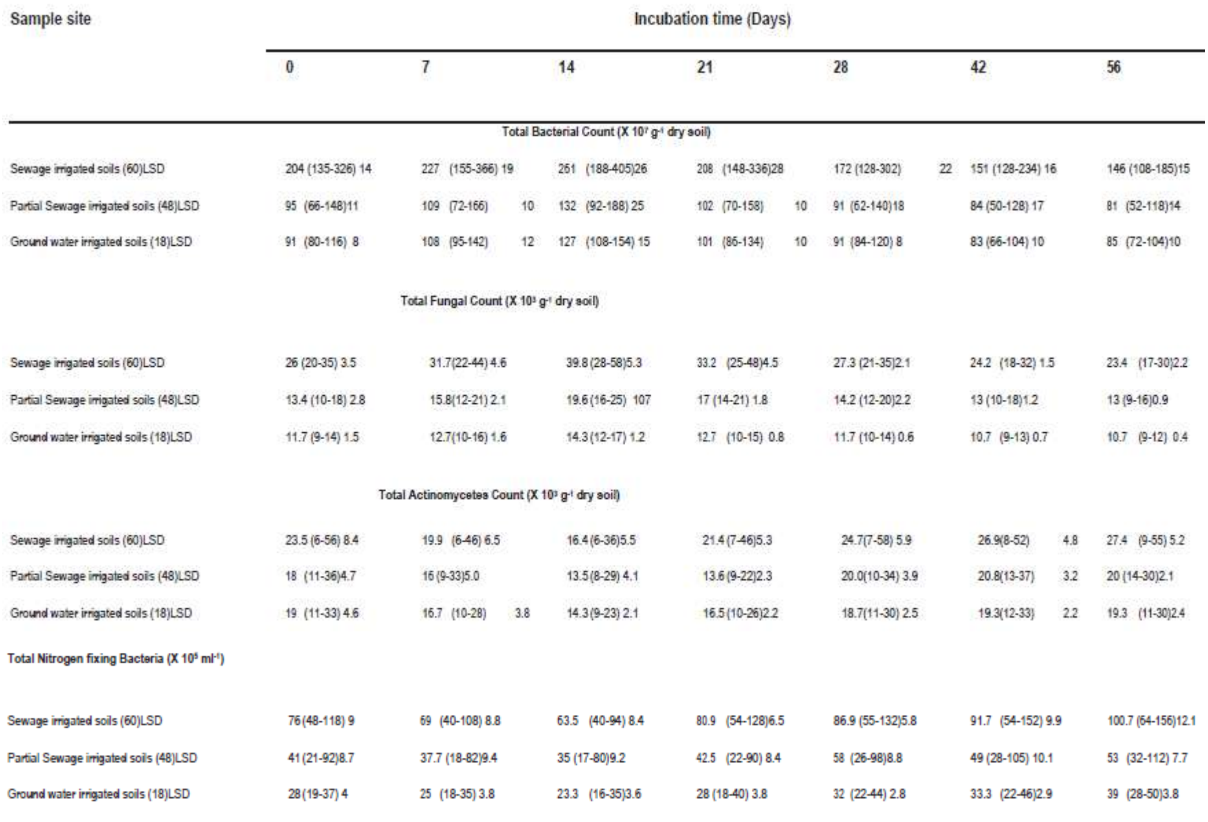
IV. RESULTS AND DISCUSSION

The results showed no significant change in soil pH values in sewage irrigation, partial sewage irrigation and ground water irrigation (Table 1). Soil organic matter was much higher in sewage irrigated soils than partial sewage irrigated soils or ground water irrigated soils. The cation exchange capacity (CEC), an important index of soil holding cation nutrients capacity, increased significantly in sewage irrigated soils than partial or ground water irrigated soils (Table 1). Total nitrogen content was 0.16% in sewage irrigated soils and 0.13± 0.03% in partial sewage irrigated soils, which were much higher than 0.07 in ground water irrigated soils. There was no distinct difference observed in the total phosphorous content in sewage irrigated soils and partial sewage irrigated soils, but was much higher in sewage irrigated soils than ground water irrigated soils.

The average concentration of total heavy metals and DTPA extractable heavy metals *viz.*, Cu, Zn, Cr, Cd, Pb and Ni are tabulated in Table 2. The results showed that the concentration of total heavy metals in sewage irrigated soils were 3 times higher for Zn; 4.5 times higher for Cu; 3.8 times higher for Cr; 5.7 times higher for Pb; 3.5 times higher for Cd and 2.7 times higher for Ni than ground water irrigated soils, while in partial sewage irrigated soils it was 2 times higher for Zn; 1.7 times higher for Cu; 2.4 times higher for Cr; 3.5 times higher for Pb; 2.2 times higher for Cd and 2.0 times higher for Ni than groundwater irrigated soils.Resultsalso showed that concentration of total heavy metals increased.with time in sewage irrigated and partial sewage irrigated soils. The lowest concentration of heavy metals in the top layer was during September- October which might be due to leaching of metals from top layer to lower depths. The concentration of DTPA extractable heavy metals in partial sewage irrigated and sewage irrigated soils were almost same but higher than ground water irrigated soils. Almost the same concentration of heavy metals in partial sewage irrigated and sewage irrigated soils might be due to deposition of heavy metals in crops grown on the soils. Results also denoted that both total heavy metal concentration and DTPA-extractable metal concentration was significantly and positively correlated with soil organic matter (Malla and Totawat, 2006).

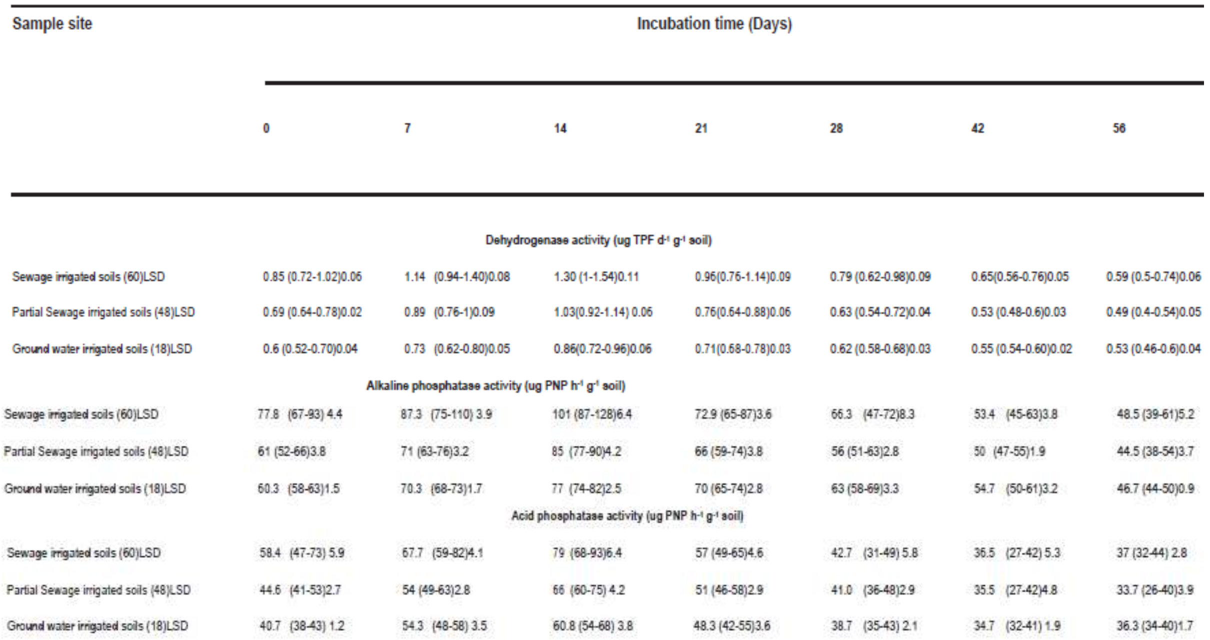
Data on bacterial, fungal and actinomycetes populations of the soils (Table 3) showed that number of bacterial and fungal colonies increased significantly with sewage water irrigation, maximum being in sewage irrigated soils followed by partial sewage irrigated soils and least in ground water irrigated soils. Results also showed that microbial population (bacterial, fungal and actinomycetes) increased up to 14 days of incubation and decreased thereafter up to 56 days of incubation. The fungal and bacterial population slightly increased with time (Table 3). Increase in bacterial or fungal population might be due to mineralization of organic matter (Stepniewska*et al.*, 2007). Minimum population of actinomycetes was observed in sewage water irrigated soils (Table 3). Soil organic matter was significantly and positively correlated with soil bacterial and fungal population, and negatively correlated with actinomycetes. Results also showed (Table 3) that number of asymbiotic nitrogen fixing bacteria decreased with increase in bacterial population, denoting that mineralization of nitrogen decreased in presence of sewage.Soil dehydrogenase (DHA), representing metabolic oxidative activity of soil organisms is considered as a soil quality indicator, because it is involved in electron transport system of oxygen metabolism and requires an intracellular environment (viable cells) to express its activity (Kandler and Dick, 2007) was found to be influenced significantly by applications of sewage effluents. Dehydrogenase activity increased significantly up to 14.days of applications and decreased thereafter (Table 4). Increase in DHA activity was 1.32 from 0.76 for sewage irrigated soils, 1.04 from 0.66 for partial sewage irrigated soils and 0.78 from 0.54 for ground water irrigated soils. Table 4 shows that dehydrogenase activity depends on the amount of sewage sludge by which the soil was amended. Results also showed that dehydrogenase activity was significantly correlated with soil organic matter content, as organic matter is the seat of microbial population and activity. Similar results were reported earlier by Min *et al.* (2001) and Stepniewska*et al.* (2007). Results of the present studies also demonstrated that the bacterial count and dehydrogenase activity were significantly and positively correlated.Phosphorus is an essential nutrient for plant growth and crop yield; however a large portion remains immobilized due to intrinsic characteristics of soil, such as pH that affects the availability of nutrients and the activity of enzymes, altering the equilibrium of soil solid phase (Dick et *al.*, 2000). Soil microorganisms play a key role on phosphate solubilisation (Sundra and Hari, 2002) and production of extracellular enzymes such as phosphatases. The activity of acid and alkaline phosphatase initially increased significantly up to 14 days of application (Table 4), and thereafter the activity of acid and alkaline phosphatase decreased till the end of the experiments. Increase in phosphatase activity up to14 days of application in the presence of sewage sludge may be due to increase in the amount of organic matter and rich microbial population in soil samples via sewage sludge (Punita*et al.*, 2012). Decrease in the activities of both phosphatase after 14 days of application might be due to the release of orthophosphates from lysed cells, which may act as competitive inhibitor of phosphates in soil (Tabatabai, 1994).Urease enzyme play an important role in the efficient use of urea fertilizer and some environmental risk assessments. Urease has been widely used to evaluate changes in the quality of soil related to management, since its activity increases with organic fertilization and decreases with soil tillage (Saviozzi*et al.*, 2001). It has been shown that its activity depends on microbial community, physical, and chemical properties of soil (Yang *et al*., 2006;Corstanje*et al.*, 2007), and its stability is affected by several

Table 3 :Average total microbial count in collected soil samples of Aligarh district (June11-May, 12)

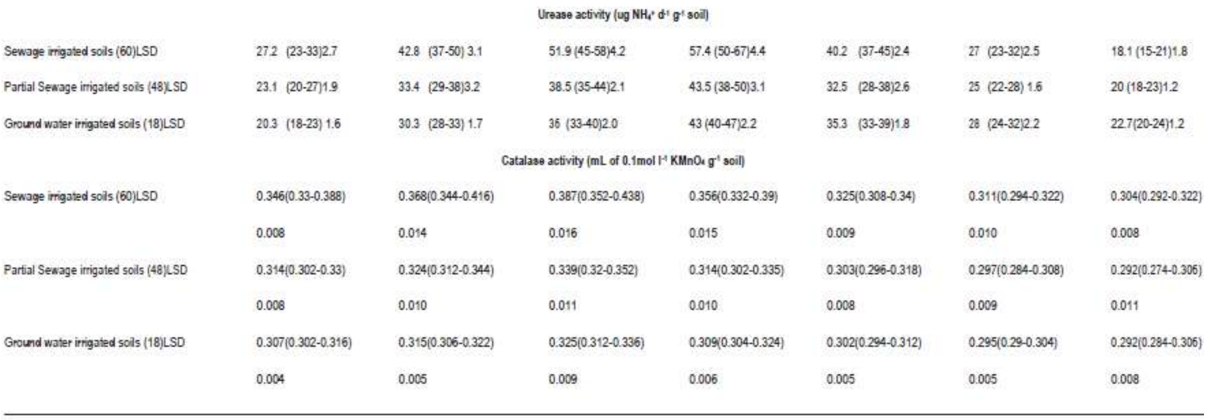


Sample site	Incubation time (Days)						
	0	7	14	21	28	42	56
Total Bacterial Count (X 10^7 g^{-1} dry soil)							
Sewage irrigated soils (60)LSD	204 (135-326) 14	227 (155-366) 19	261 (188-405)26	208 (148-336)28	172 (128-302) 22	151 (128-234) 16	146 (108-185)15
Partial Sewage irrigated soils (48)LSD	95 (66-148)11	109 (72-166) 10	132 (92-188) 25	102 (70-158) 10	91 (62-140)18	84 (50-128) 17	81 (52-118)14
Ground water irrigated soils (18)LSD	91 (80-116) 8	108 (95-142) 12	127 (108-154) 15	101 (86-134) 10	91 (84-120) 8	83 (66-104) 10	85 (72-104)10
Total Fungal Count (X 10^3 g^{-1} dry soil)							
Sewage irrigated soils (60)LSD	26 (20-35) 3.5	31.7(22-44) 4.6	39.8 (28-58)5.3	33.2 (25-48)4.5	27.3 (21-35)2.1	24.2 (18-32) 1.5	23.4 (17-30)2.2
Partial Sewage irrigated soils (48)LSD	13.4 (10-18) 2.8	15.8(12-21) 2.1	19.6 (16-25) 107	17 (14-21) 1.8	14.2 (12-20)2.2	13 (10-18)1.2	13 (9-16)0.9
Ground water irrigated soils (18)LSD	11.7 (9-14) 1.5	12.7(10-16) 1.6	14.3 (12-17) 1.2	12.7 (10-15) 0.8	11.7 (10-14) 0.6	10.7 (9-13) 0.7	10.7 (9-12) 0.4
Total Actinomycetes Count (X 10^3 g^{-1} dry soil)							
Sewage irrigated soils (60)LSD	23.5 (6-56) 8.4	19.9 (6-46) 6.5	16.4 (6-36)5.5	21.4 (7-46)5.3	24.7(7-58) 5.9	26.9(8-52) 4.8	27.4 (9-55) 5.2
Partial Sewage irrigated soils (48)LSD	18 (11-36)4.7	16 (9-33)5.0	13.5 (8-29) 4.1	13.6 (9-22)2.3	20.0(10-34) 3.9	20.8(13-37) 3.2	20 (14-30)2.1
Ground water irrigated soils (18)LSD	19 (11-33) 4.6	16.7 (10-28) 3.8	14.3 (9-23) 2.1	16.5 (10-26)2.2	18.7(11-30) 2.5	19.3(12-33) 2.2	19.3 (11-30)2.4
Total Nitrogen fixing Bacteria (X 10^5 ml^{-1})							
Sewage irrigated soils (60)LSD	76 (48-118) 9	69 (40-108) 8.8	63.5 (40-94) 8.4	80.9 (54-128)6.5	86.9 (55-132)5.8	91.7 (54-152) 9.9	100.7 (64-156)12.1
Partial Sewage irrigated soils (48)LSD	41 (21-92)8.7	37.7 (18-82)9.4	35 (17-80)9.2	42.5 (22-90) 8.4	58 (26-98)8.8	49 (28-105) 10.1	53 (32-112) 7.7
Ground water irrigated soils (18)LSD	28 (19-37) 4	25 (18-35) 3.8	23.3 (16-35)3.6	28 (18-40) 3.8	32 (22-44) 2.8	33.3 (22-46)2.9	39 (28-50)3.8

Table 4 : Average enzymatic activities in collected soil samples of Aligarh district (June11-May, 12)



Sample site	Incubation time (Days)						
	0	7	14	21	28	42	56
Dehydrogenase activity (ug TPF d^{-1} g^{-1} soil)							
Sewage irrigated soils (60)LSD	0.85 (0.72-1.02)0.06	1.14 (0.94-1.40)0.08	1.30 (1-1.54)0.11	0.96(0.76-1.14)0.09	0.79 (0.62-0.98)0.09	0.65(0.56-0.76)0.05	0.59 (0.5-0.74)0.06
Partial Sewage irrigated soils (48)LSD	0.69 (0.64-0.78)0.02	0.89 (0.76-1)0.09	1.03(0.92-1.14) 0.06	0.76(0.64-0.88)0.06	0.63 (0.54-0.72)0.04	0.53 (0.48-0.6)0.03	0.49 (0.4-0.54)0.05
Ground water irrigated soils (18)LSD	0.6 (0.52-0.70)0.04	0.73 (0.62-0.80)0.05	0.86(0.72-0.96)0.06	0.71(0.68-0.78)0.03	0.62 (0.58-0.68)0.03	0.55 (0.54-0.60)0.02	0.53 (0.46-0.6)0.04
Alkaline phosphatase activity (ug PNP h^{-1} g^{-1} soil)							
Sewage irrigated soils (60)LSD	77.8 (67-93) 4.4	87.3 (75-110) 3.9	101 (87-128)6.4	72.9 (65-87)3.6	66.3 (47-72)8.3	53.4 (45-63)3.8	48.5 (39-61)5.2
Partial Sewage irrigated soils (48)LSD	61 (52-66)3.8	71 (63-76)3.2	85 (77-90)4.2	66 (59-74)3.8	56 (51-63)2.8	50 (47-55)1.9	44.5 (38-54)3.7
Ground water irrigated soils (18)LSD	60.3 (58-63)1.5	70.3 (68-73)1.7	77 (74-82)2.5	70 (65-74)2.8	63 (58-69)3.3	54.7 (50-61)3.2	46.7 (44-50)0.9
Acid phosphatase activity (ug PNP h^{-1} g^{-1} soil)							
Sewage irrigated soils (60)LSD	58.4 (47-73) 5.9	67.7 (59-82)4.1	79 (68-93)6.4	57 (49-65)4.6	42.7 (31-49) 5.8	36.5 (27-42) 5.3	37 (32-44) 2.8
Partial Sewage irrigated soils (48)LSD	44.6 (41-53)2.7	54 (49-63)2.8	66 (60-75) 4.2	51 (46-58)2.9	41.0 (36-48)2.9	35.5 (27-42)4.8	33.7 (26-40)3.9
Ground water irrigated soils (18)LSD	40.7 (38-43) 1.2	54.3 (48-58) 3.5	60.8 (54-68) 3.8	48.3 (42-55)3.6	38.7 (35-43) 2.1	34.7 (32-41) 1.9	36.3 (34-40)1.7



			Urease activity (ug NH_4^+ d^{-1} g^{-1} soil)				
Sewage irrigated soils (60)LSD	27.2 (23-33)2.7	42.8 (37-50) 3.1	51.9 (45-58)4.2	57.4 (50-67)4.4	40.2 (37-45)2.4	27 (23-32)2.5	18.1 (15-21)1.8
Partial Sewage irrigated soils (48)LSD	23.1 (20-27)1.9	33.4 (29-38)3.2	38.5 (35-44)2.1	43.5 (38-50)3.1	32.5 (28-38)2.6	25 (22-28) 1.6	20 (18-23)1.2
Ground water irrigated soils (18)LSD	20.3 (18-23) 1.6	30.3 (28-33) 1.7	36 (33-40)2.0	43 (40-47)2.2	35.3 (33-39)1.8	28 (24-32)2.2	22.7(20-24)1.2
			Catalase activity (mL of 0.1mol l^{-1} $KMnO_4$ g^{-1} soil)				
Sewage irrigated soils (60)LSD	0.346(0.33-0.388)	0.368(0.344-0.416)	0.387(0.352-0.438)	0.356(0.332-0.39)	0.325(0.308-0.34)	0.311(0.294-0.322)	0.304(0.292-0.322)
	0.008	0.014	0.016	0.015	0.009	0.010	0.008
Partial Sewage irrigated soils (48)LSD	0.314(0.302-0.33)	0.324(0.312-0.344)	0.339(0.32-0.352)	0.314(0.302-0.335)	0.303(0.296-0.318)	0.297(0.284-0.308)	0.292(0.274-0.306)
	0.008	0.010	0.011	0.010	0.008	0.009	0.011
Ground water irrigated soils (18)LSD	0.307(0.302-0.316)	0.315(0.306-0.322)	0.325(0.312-0.336)	0.309(0.304-0.324)	0.302(0.294-0.312)	0.295(0.29-0.304)	0.292(0.284-0.306)
	0.004	0.005	0.009	0.006	0.005	0.005	0.008

(Values in parentheses are the range of samples collected)

factors: organo-mineral complexes and humic substances make them resistant to denaturing agents such as heat and proteolytic attack (Makoi and Ndakidemi,2008). Understanding urease activity may provide better ways to manage urea fertilizer, especially in warm high rainfall areas, flooded soils and irrigated conditions (Makoi and Ndakidemi, 2008)· Urease activity in presence of different amount of sewage effluent increased up to 14 days of incubation and thereafter decreased (Table 4). Urease activity was significantly correlated with amount of sewage effluent and soil organic matter while negatively correlated with nitrogen fixing bacteria. Decrease in urease activity after 14 days of incubation might be due to decomposition of organic matter and increase in soil nitrogen fixing bacteria. Similar results were also reported by Kakhki*et al.* (2008) and Noorbakhsh*et al.* (2001).Enzyme catalase splits hydrogen peroxide into molecular oxygen and water, thus preventing cells from damage by reactive oxygen species (Yao *et al*., 2006). This enzyme can be found in all the aerobic microorganisms, plant and animal cells. Response of sewage effluent irrigation to soil on catalase activity in Aligarh soils is given in Table 4. Catalase activity of increased up to 14 days of incubation, and the increase in catalase activity was more in sewage irrigated soils than in partial sewage irrigated soils, suggesting thereby that the presence of a minimum amount of fresh organic matter is needed to activate soil catalase activity (Kizilkaya*et al.*, 2004).

The present study showed that total concentration of heavy metals were found maximum in sewage irrigated soils followed by partial sewage irrigated soils. The value of DTPA extractable metal concentrations denote that deposition of heavy metals in crops grown on the soils were positively correlated with total metal concentrations. Microbial population and soil enzymatic activity increased up to 14 days of incubation and thereafter was significantly inhibited and were positively correlated with soil organic matter.

REFERENCES

1. Bansal, O.P.: Adsorption and interaction of oxamyl and dimecron with some soils of India. *J. Ind. Soc. Soil Sci.*,
2. **30**, 459-467 (1982).
3. Cassida, L.E., D.A. Klein and T. Santaro: Soil dehydrogenase activity.

4. *Soil Sci.*, **98**, 371-376 (1964).
5. Corstanje, R., R. Schulin and R. Lark: Scale–dependent relationships between soil organic matter and urease activity. *Europ. J. Soil Sci.*,**58**, 1087-1095 (2007).
6. Cuppucino, J. and N. Sherman: Microbiology. A Laboratory Manual.
7. Addison-Wesley, London (1983).
8. Dick, W., L. Cheng and P. Wang: Soil acid and alkaline phosphatase activity as pH adjustment indicators. *Soil Biol. Biochem.*,**32**, 1915–1919 (2000).
9. Gianfreda, L. and J.M. Bollage: Influence of natural and anthropogenic factors on enzyme activity in soils. In: Soil Biochemistry (Eds.: G. Stotzky and J.M. Bollage). New York , Marcel Dekker, p. 123-193 (1996).
10. Gonzalez, M.G., J.F. Gallardo, E. Gomez, G. Masciandaro, B.Ceccanti and S. Pajares: Potential universal applicability of soil bioindicators: Evaluation in three temperate ecosystems. *Cr.Suelo (Argentina)*, **25**, 151-158 (2007).
11. Johnson,J.I. and K.L. Temple: Some variables affecting the measurement of catalase activities in soil. *Soil Sci. Soc. Amer. J.*, **28**, 207-216 (1964).
12. Kakhki, F.V. G. Haghnia and A.Lakzain: Effect of enriched sewage sludge on soil urease activity. *Soil Environ.*,**27**, 143-147 (2008).
13. Kandeler, E. and R. Dick: Soil Enzymes: Spatial Distribution andFunction inAgroecosystems. In: Biodiversity in Agricultural Production Systems (Eds.: G. Benckiser and S. Schnell). Boca Raton, Fl: Taylor & Francis Group, p. 263-79 (2007)
14. Kanan,V., R. Ramesh and C. Sasikumar: Study on ground water characteristics and the effects of discharged effluents from textile units at Karur district. *J. Environ. Biol.*,**26**, 269-272 (2005).
15. Karaca, A., A. Baran and K. Kaktanir: The effect of compacation on urease enzyme activity, carbon dioxide evaluation and nitrogen mineralization. *Turkey J. Agricul*., **24**, 437-441 (2000)
16. Kizilkaya, R., T. Askin, B. Bayrakli and M. Saglam: Microbiological characteristics of soils contaminated with heavy metals. *Europ. J.Soil Biol*.,**40**, 95-102 (2008).
17. Lindsay, W.L. and W.A. Norvell: Development of DTPA soil test for zinc, iron, manganese and copper. *Soil Sci. Soc. Ame. J.*, **42**, 421-428 (1978).
18. Makoi, J. and P. Ndakidemi: Selected soil enzymes: Examples of their potential roles in the ecosystem. *Afri. J. Biotech*., **7**,181-191 (2008).
19. Malla, R. and K.L.Totawat: Effect of municipal sewage on soil properties and chemical build-up in vegetables crops grown on Haplustepts of sub-humid southern plains of Rajsthan. *J. Indian Soc. Soil Sci.*,**54**, 226-231(2006).
20. Min, H., Y.F. Ye, Z.Y. Chen, W.X. Wu, and Y.F. Du: Effects of butachlor on microbial populations and enzyme activities in paddy soil. *J.Environ. Sci. Hlth.*,**36**, 581-595 (2001).

21. Nagajyothi, P.C., N. Dinakar, S. Suresh, Y. Udaykiran, C. Suresh and T. Damodharan: Effect of Industrial effluent on the morphological parameters and chlorophyll content of green gram (*Phaseoulsaureus*Roxb). *J. Environ. Biol.,* **30**, 385-388 (2009).
22. Nannipieri, P., J. Ascher, M.T. Ceccherni, L. Landi, G. Pietramellara and G. Rengella: Microbial diversity and soil functions. *Europ. J. SoilSci.*, **54,**655-670 (2003).
23. Nath, Kamlesh, Dharam Singh, ShilpaShyam and Y.K.Sharma: Phytoxic effects of chromium and tannery effluent on growth and metabolism of *Phaselousmungo*Roxb. *J. Environ. Biol*., **30,** 227-234 (2009).
24. Noorbakhsh, F., S. Hajrasuliha and G. Emtiazy: Factors affecting urease enzyme activity in some soils in Isfahan Province. *JWSS- IsfahanUniv. Technol*.,**5,**95-106 (2001).
25. Onder, S. and S. Dursun: Air borne heavy metal pollution of *Cedruslibani* (A. Rich.) in the city centre of Konya (Turkey). *AtmosphericEnviron.*,**40**, 1122-1133 (2006).
26. Pandey, S.N., B.D. Nautiyal and C.P. Sharma: Pollution level in distillery effluent and its phytotoxic effect on seed germination and early growth of maize and rice. *J. Environ. Biol*., **29,** 267-270