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Comparative assessment of microbial enzyme activity with compost and sewage sludge amendment

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Abstract: Changes in soil microbial activities were investigated to examine the effect of aerobically digested sewage sludge (SS) and compared with compost under incubation conditions over 63 days. Sandy soil was amended with 0.25, 0.5, 1.0 and 1.5 % w/w of compost and sewage sludge. Enzyme activity (dehydrogenase, alkaline phosphatase, acid phosphatase, phytase and urease) were examined at an interval of 3, 7, 14, 21, 28, 42 and 63 days. At the end of the experiment the change in organic carbon, nitrogen, potassium and phosphorus was also recorded. Results indicated that enzyme activities were substantially enhanced in presence of both amendments for first few days and the higher increases were measured at 1.5% of compost and sewage sludge amendment. Then an overall decrease in enzyme activity was recorded. Both the amendments also significantly increased the organic carbon, nitrogen and potassium of the soil while increase in available phosphorus was only recorded in treatment receiving compost. The present experiment indicated that addition of compost and sewage sludge have positive effect on soil microbial activity and can be safely used as soil amendment without having any adverse effect. Though, a previous examination of sewage sludge to be used must be made for heavy metals and pathogens.

Keywords: Compost, Microbial enzyme, Sandy soil, Sewage sludge

INTRODUCTION

There is an increasing interest in the use of organic amendments in soil management and crop production fostered by the advantage of organics to improve or maintain soil biological, chemical and physical properties (Ferreras *et al.*, 2006; Guerrero *et al.*, 2000; Bouajila and Sanaa, 2011). Compost and sewage sludge are common soil amendments used to improve soil tilth, increasing water holding capacity, porosity, surface area (Cogger, 2005), soil structure (Thomas *et al.*, 1996) and decrease soil bulk density (Curtis and Claassen, 2009), thereby providing an environment that will allow for the growth of healthy root systems. Organic amendments also supply nutrients to growing plants and increase the concentrations of plant-available nutrients in soils.

Compost is considered to be an environmentally safe, agronomically advantageous, and relatively cheap organic amendment which stimulates soil microbial activity and crop growth (Garcia *et al.*, 1994; Pascual *et al.*, 1997; Van-Camp *et al.*, 2004). Decomposition and humification of biodegradable organic waste materials is predominantly carried out by a variety of mesophilic/thermotolerant and thermophilic microorganisms, predominately of soil origin (de Bertoldi *et al.*, 1983). The application of urban industrial sludge mixed with sewage sludge to agricultural soil is generally most economic outlet for waste disposal; in this way it is possible to re-cycle the plant nutrients such as N, P and organic matter and many more macro and micro nutrients (Bose and Bhattacharyya, 2012). As a soil conditioner, sludge reduces bulk density and increases porosity, improves structural stability, and enriches soil with organic carbon (Pagliai *et al.*, 1981; Metzger and Yaron, 1987; Tester, 1990; Sort and Alcaniz, 1999; Marinari *et al.*, 2000). Addition of sewage sludge increases basal respiration, microbial biomass, metabolic quotient and ezyme activity in soil (Fernandes *et al.*, 2005). However, due to the physical-chemical processes involved in the treatment, the sludge tends to concentrate heavy metals and poorly biodegradable trace organic compounds as well as potentially pathogenic organisms (viruses, bacteria etc) present in waste waters.

It is essential to determine the effect of compost and sewage sludge on microorganisms and their activity as the microorganisms play key role in nutrient cycling as well as in sustaining soil health. Changes in microbial population and activity precede detectable changes in soil physico-chemical properties as microorganisms have intimate relations with their surroundings due to their high surface to volume ratio (Pankhurst *et al.*, 1995). Soil enzymes derived primarily from soil microbes are considered as indicative measures of soil fertility (Zahir *et al.*, 2001) as they participate in elemental cycling and decomposition of organic residues and are considered fundamentally best indicators for

soil quality (Caldwell, 2005;Venkatesan and Senthurpandian, 2006; Kizilkaya *et al.,* 2007; Abdalla and Langer, 2009). Therefore, their activity is considered an indicator of the oxidative metabolism in soil and thus also of microbial activity (Quilchano and Maranon, 2002). Dehydrogenase being exclusively intracellular, is linked to viable cells. Dehydrogenase is considered an indicator of the oxidative metabolism in soils and thus of the microbiological activity (Skujins, 1973), because. Urease is one of the enzymes involved in environmental nitrogen transformations and can be used to indicate part of the N cycling in soil (Park and Hausinger, 1995). It catalyzes the hydrolysis of urea and amides to carbon dioxide and ammonia. It acts on carbon-nitrogen (C-N) bounds other than peptide linkage (Bremner and Mulvaney, 1978; Karaca *et al.,* 1999). Phosphatase is group of enzymes which cleave the phosphate groups from substrates such as nucleic acid and represents the P-cycle in the sludge-amended soil (Wang *et al.,* 1990). Numerous studies have been conducted to observe effect of compost and sewage sludge on soil physical and chemical properties but very few studies have been undertaken to determine the effect on sewage sludge soil's biological property. The objective of this incubation study was to determine the effect of soil amendments, compost and sewage sludge, on microbial activity and physico-chemical properties of sandy soil.

MATERIALS AND METHODS

Soil, compost and sludge description: Dewatered anaerobically digested sewage sludge was collected from the sewage treatment plant at Bhattian and stored at 4˚C until use. Sewage sludge was stabilized at 52˚C for 24 hr to kill off any pathogens and dried at room temperature for one week. The compost was prepared at Research farm of Punjab Agricultural University, Ludhiana (30º 56' North, 75º 52' East), Punjab, India, using paddy straw and cow dung. Sewage sludge and compost characteristics are summarized in table 1. The elemental analysis of compost and sewage sludge is presented in table 2. The plasma atomic emission spectrometer (ICAP-AES) was used to carry out the elemental analysis. Some of these metals, at low concentrations, are essential micronutrients and their limit in sludge, for use in agriculture is Cd (20), Cu (1200), Ni (200), Pb (1200), Zn (3000), Hg (25) and Cr (1200) ppm (Goyal *et al.,* 2008). Thus the sewage sludge used in present experiment contained heavy metals within safer limits for agricultural application. The sandy soil was air-dried at room temperature and sieved to pass through a 2mm sieve. The initial soil characteristics of experimental soil were: pH 7.53, EC 0.197dS^{-1}, organic carbon 0.29 %, $KMnO_4$-oxidizable N 37.6 kg ha^{-1}, Olsen P 27.3 kg ha^{-1} and 160.0 kg ha^{-1} of ammonium acetate exchangeable K.

Soil treatment and incubation: Stabilized sewage sludge and compost were thoroughly mixed with soil at the rate equivalent to 0.25, 0.5, 1.0 and 1.5% (w/w dry weight basis) and filled in polypropylene pots. The amended soil was then moistened to its field capacity and incubated for 63 days at 30˚C. The amended soils were moistened to its field capacity that was kept constant throughout incubation time and were turned daily to provide sufficient oxygen for the system. Sub samples were removed at time intervals of 3, 7, 14, 21, 28, 42 and 63 days to determine the changes in enzyme activities.

Experimental details: Soil without amendment was used as control. The experiment was performed with the following nine treatments (Table 3). The data was subjected to standard analysis of variance (ANOVA) of complete randomized design (Gomez and Gomez, 1984) and the means of the treatments were tested using least significant difference at 5% probability level by using IRRISTAT data analysis package (IRRI, 2000).

Measurement of soil enzymatic activities: Dehydrogenase (EC 1.1) activity was determined by measuring the amount of an artificial electron acceptor reduced by microbial activity (Camina *et al.,* 1998). To 1g fresh soil sample, 0.2ml of 3% triphenylterazolium chloride (TTC) and 0.5 ml of 1% glucose solution was added and incubated for 24 hours at 30˚C. After that 10ml of methanol was added and kept in refrigerator for 3 hrs. The contents were then filtered through Whatman No. 42 filter paper. The samples were washed thoroughly with methanol and the final volume made to 25ml using methanol. The red colour developed was read at 485nm. The concentration of dehydrogenase in the sample was obtained from the standard graph using triphenyl foramazan (TPF) (Casida *et al.,* 1964).

The activities of acid (AcdP; EC 3.1.3.2) and alkaline (AlkP; EC 3.1.3.1) phosphatases were assayed on the basis of p-nitrophenol (pNP) release after cleavage of enzyme-specific synthetic substrates at natural soil average pH. 1g of fresh soil sample was placed in 50ml Erlenmeyer flask, and to it 0.2ml toluene, 4ml of modified universal buffer (pH 6.5 for assay of acid phosphatase or pH 11 for assay of alkaline phosphatises), 1ml of p-nitrophenyl phosphate solution made in the same buffer were added. The flasks were stoppered and placed in incubator at 37˚C for 1 hour. After 1hour the stopper was removed and 1ml of 0.5M $CaCl_2$ and 4ml of 0.5M NaOH was added to the flasks. The soil suspension was then filtered and the yellow colour obtained was spectrophotometrically analyzed at 420nm. The p-nitrophenol content of the filtrate was calculated by reference to a calibration graph (Tabatabai and Bremner, 1969).

Phytase (EC 3. 1. 3. 8) activity in soil was measured by Ames (1966) method. One g of fresh soil was taken in 15ml screw capped tubes. To this 4ml sodium-acetate buffer solution (pH 4.5) and 1ml of 1µM sodium phytate solution was added. The contents were mixed thoroughly and incubated at 37˚C for 1 hour. After an hour, the caps were removed and 0.5ml of 10% tri-

Table 1. Physico-chemical properties of sewage sludge and compost.

Parameters	Sewage sludge	Compost
pH	6.66	8.99
Electrical conductivity (dS m^{-1})	4.82	8.52
Available Nitrogen (%)	0.64	0.88
Olsen Phosphorus (%)	0.68	1.18
Ammonium acetate exchangeable potassium (%)	0.30	2.70

Table 2. Available metal contents of the sewage sludge and compost.

	Concentration (µg g^{-1})	
Metal	**Sewage sludge**	**Compost**
Arsenic	4	1.6
Boron	17	7.5
Calcium	8029	2611
Cadmium	3.3	0.2
Chromium	10.6	2.2
Copper	62.2	6.6
Iron	7926	1085
Potassium	15372	15412
Magnesium	3592	1200
Manganese	64.7	57.7
Sodium	991	2560.6
Nickel	41	8.4
Phosphorus	4106.4	7180
Lead	87	3.8
Sulphur	2620	2260
Zinc	707	103

Table 3. Different treatments used during the study are enlisted below.

S. N.	Acronym	Treatment
1	Control	Sandy soil without amendment
2	COM0.25	Sandy soil with compost amendment @ 0.25%
3	COM0.50	Sandy soil with compost amendment @ 0.5%
4	COM1.0	Sandy soil with compost amendment @ 1.0%
5	COM1.5	Sandy soil with compost amendment @ 1.5%
6	SS0.25	Sandy soil with sewage sludge amendment @ 0.25%
7	SS0.5	Sandy soil with sewage sludge amendment @ 0.50%
8	SS1.0	Sandy soil with sewage sludge amendment @ 1.0%
9	SS1.5	Sandy soil with sewage sludge amendment @ 1.5%

chloroacetic acid was added to stop the reaction. The contents were then filtered through Whatman filter paper no.1. 2ml of the filtrate was taken in 25ml volumetric flasks and the test for phosphorus hydrolysed was done using the Olsen method. The inorganic phosphorous was calculated by reference to a calibration graph prepared from standard solution. Phytase activity was expressed as µg inorganic P released/g soil/hr.

Urease (EC 3.5.1.5) activity was assayed by method of Douglas and Bremner (1970). Five g of soil was thoroughly mixed with 5 ml urea solution (2000 µg ml^{-1}), and then incubated at 37±1ºC for 5 hrs. After incubation, the residual urea was extracted with 50 mL of 2M KCl-phenyl mercury acetate solution for 1 h on a rotary shaker, followed by filtration (Tabatabai, 1994). To 1ml of filtrate 5 ml of extracting reagent (H_3PO_4 and H_2SO_4) and 15 ml of coloring reagent (diacetylmonoxime and thiosemicarbazide) were added and boiled in water bath for 30 mins. The intensity of red colour obtained after cooling the samples was measured at 527nm. Soil urease activity was expressed as µg of hydrolyzed urea-N /g soil/h.

Soil physico-chemical analysis: The soil samples after completion of incubation period were analyzed for physico-chemical changes. The samples were air-dried and passed through 2mm sieve and was analyzed for basic soil parameters (pH, electrical conductivity, organic carbon, mineral nitrogen, Olsen P and ammonium acetate extractable potassium). Soil pH in 1:2 soil: water suspension, after 30-min equilibrium time (McLean, 1982), was measured using pH meter, electrical conductivity (EC) was determined in 1:2 soil: water supernatant solutions with the help of Conductivity Bridge. Organic carbon (OC) was determined by Walkley and Black's (1934) rapid titration method using diphenylamine indicator. Available P was extracted by Olsen's $NaHCO_3$ method (Olsen *et al.*, 1954). Phosphorus in all extracts was determined calorimetrically by the molybdenum blue colour method of Murphy and Riley (1962). Total Kjeldahl nitrogen (TKN) was deter-mined by Kjeldahl digestion followed by ammonia distillation. Ammonium acetate exchangeable K was determined using flame photometer.

RESULTS AND DISCUSSION

Considerable variations in dehydrogenase, urease and phosphatase activities were found for the different doses of compost and sewage sludge application under incubation conditions. A rapid and significant increase in enzymatic activities was observed after the addition of compost and sewage sludge followed by a progressive decrease.

Soil dehydrogenase that represents *in situ* metabolic oxidative activity of soil organisms was significantly influenced by both the organic amendments. The dehydrogenase activity of amended soil increased with increasing rate of application of compost and sewage sludge. The maximum dehydrogenase activity was obtained in treatment COM1.5 (59.44µg TPF g^{-1} h^{-1}) followed by SS1.5 (52.72µg TPF g^{-1} h^{-1}) during 14^{th} day of incubation (Table 5). In all the treatments the dehydrogenase increased upto 14^{th} day of incubation and then a decline was obtained with increasing period

Table 4. Changes in physico-chemical properties of amended soil samples after 63 days of incubation.

Treatments	pH	EC (dS m^{-1})	OC (%)	Potassium (kg /ha)	Available Nitrogen (kg/ha)	Phosphorus (kg/ha)
C	7.71	0.195	0.28	149.33	39.72	28.80
COM0.25	7.64	0.199	0.35	255.73	46.00	36.90
COM0.50	7.69	0.201	0.39	334.13	43.90	44.13
COM1.0	7.69	0.202	0.42	621.60	66.90	57.27
COM1.5	7.66	0.213	0.46	767.20	62.72	77.63
SS0.25	7.68	0.199	0.32	207.20	63.39	25.43
SS0.5	7.56	0.0205	0.33	250.13	48.09	22.63
SS1.0	7.51	0.209	0.41	276.27	43.90	25.00
SS1.5	7.33	0.202	0.44	268.80	50.17	27.00
LSD (p=0.05)	0.105	NS	0.038	53.53	3.15	4.06

Table 5. Dehydrogenase activity (µg TPF g^{-1} h^{-1}) of amended soil samples as influenced by incubation period.

Treatment	0 day	3rd Day	7th day	14th day	21st day	28th day	42nd day	63rd day
C	9.75	11.58	17.58	29.42	20.67	19.41	18.13	20.53
COM0.25	12.66	15.45	19.39	42.22	24.66	21.21	19.15	23.18
COM0.5	13.72	18.39	17.83	48.19	24.97	22.03	21.96	24.47
COM1.0	19.56	19.97	23.08	45.67	38.28	35.59	24.67	25.87
COM1.5	29.11	34.95	45.72	59.44	41.06	38.54	26.93	27.69
SS0.25	9.19	14.39	22.39	42.67	30.47	26.74	19.97	21.56
SS0.5	18.47	16.89	16.36	47.83	34.37	32.41	24.83	23.00
SS1.0	21.22	22.77	23.64	37.33	33.67	31.82	30.83	20.55
SS1.5	30.53	41.64	45.28	52.72	33.67	30.20	27.23	26.53
LSD (p=0.05)	2.68	2.39	2.66	5.11	1.97	1.38	3.31	1.77

Table 6. Acid phosphatase activity (µg PNP g^{-1} h^{-1}) of amended soil samples as influenced by incubation period.

Treatments	0 day	3rd Day	7th day	14th day	21st day	28th day	42nd day	63rd day
C	1.27	2.22	3.56	4.49	2.33	2.22	1.69	1.65
COM0.25	3.5	4.58	4.51	6.15	4.69	4.11	3.42	3.20
COM0.50	4.17	6.42	5.41	7.08	6.33	4.45	3.28	3.11
COM1.0	5.25	6.03	6.51	9.64	12.11	11.95	12.80	12.23
COM1.5	5.69	8.42	11.79	12.02	16.03	14.67	15.28	13.70
SS0.25	5.94	6.06	7.33	5.46	8.19	8.75	7.39	7.10
SS0.5	3.03	16.89	8.36	9.95	7.61	9.08	9.22	8.34
SS1.0	5.47	13.14	6.31	9.80	6.14	6.58	6.50	6.66
SS1.5	10.31	13.64	9.26	9.59	4.97	6.44	5.89	5.51
LSD (p=0.05)	1.20	1.23	2.03	1.21	1.76	1.27	2.36	2.18

Table 7. Alkaline phosphatase activity (µg PNP g^{-1} h^{-1}) of amended soil samples as influenced by incubation period.

Treatments	0 day	3rd Day	7th day	14th day	21st day	28th day	42nd day	63rd day
C	11.50	19.19	14.10	13.36	12.31	10.67	8.47	8.18
COM0.25	18.61	25.11	27.84	24.15	21.83	19.97	17.70	16.89
COM0.50	21.00	28.92	30.36	28.67	24.25	23.33	21.80	20.35
COM1.0	25.83	31.33	32.13	29.46	31.36	29.31	24.87	23.51
COM1.5	28.22	35.70	33.84	36.62	33.22	31.03	29.10	23.76
SS0.25	12.89	21.33	21.77	20.46	20.75	17.58	9.53	9.69
SS0.5	14.39	23.28	24.44	22.08	16.08	14.31	10.03	9.70
SS1.0	16.47	26.94	21.95	18.03	15.19	13.31	9.90	9.54
SS1.5	23.33	29.08	19.80	16.44	13.58	11.33	6.93	6.93
LSD (p=0.05)	1.88	1.51	1.66	2.11	1.63	1.27	2.91	3.26

of incubation. Soil dehydrogenase activity has been used as a parameter to study biological activity of soil (Wlodarczyk *et al.,* 2002). Since dehydrogenase activity is only present in viable cells, it is thought to reflect the total oxidative activity of soil microflora (Nannipieri *et al.,* 1990; Zhao *et al.,* 2010; Yuan and Yue, 2012) and therefore, is considered as a suitable indicator of soil quality and microbial activity (Salazar *et al.,* 2011; Paz-Ferreiro *et al.,* 2012). Dehydrogenase activity is usually enhanced by labile organic matter addition such as sewage sludge (Serra-Wittling et al., 1996) and compost. Higher dehydrogenase activity in compost applied plots may be due to higher organic matter content and relatively higher microbial quotient (Cmic) (Wlodarczyk *et al.,* 2002). Sludge is composed of highly oxidizable organic substrates and a large biomass which explains the high intracellular dehydrogenase activity in soil receiving sludge amendment (Garcia *et al.,* 1994). The decline in dehydrogenase activity after 14th day of incubation may be likely due to the depletion of organic substrates, accumulation of metabolic toxins and the decrease in pH after the peak

Table 8. Phytase activity (µg P hydrolyzed g^{-1} h^{-1}) of amended soil samples as influenced by incubation period.

Treatments	0 day	3rd Day	7th day	14th day	21st day	28th day	42nd day	63rd day
C	0.279	0.569	0.565	1.033	0.887	0.931	0.62	0.369
COM0.25	0.349	0.517	0.660	1.288	1.029	0.972	0.66	0.459
COM0.50	0.534	0.733	0.707	1.284	1.110	1.138	0.73	0.555
COM1.0	0.802	0.753	0.935	1.984	1.744	1.558	0.88	0.681
COM1.5	1.304	1.221	1.246	1.897	1.920	1.885	1.03	0.806
SS0.25	0.397	0.423	0.375	1.086	0.718	0.758	0.42	0.788
SS0.5	0.239	0.320	0.263	0.756	0.872	0.688	0.43	0.309
SS1.0	0.391	0.323	0.298	0.565	0.640	0.485	0.29	0.297
SS1.5	0.299	0.272	0.287	0.569	0.625	0.576	0.30	0.346
LSD (p=0.05)	0.511	0.174	0.064	0.225	0.237	0.139	0.059	0.065

Table 9. Urease activity (µg urea N hydrolyzed g^{-1} h^{-1}) of amended soil samples as influenced by incubation period.

Treatments	0 day	3rd Day	7th day	14th day	21st day	28th day	42nd day	63rd day
C	81.80	190.43	111.67	104.33	76.23	77.33	49.43	60.00
COM0.25	87.77	206.20	134.00	130.00	96.23	94.67	61.93	70.33
COM0.50	95.97	222.67	167.50	157.33	111.10	112.23	53.90	84.33
COM1.0	106.20	243.33	185.17	160.83	120.43	125.57	107.20	98.33
COM1.5	123.77	251.13	191.67	163.33	139.33	143.97	88.33	133.67
SS0.25	89.10	177.10	128.50	118.83	120.23	122.00	49.43	49.67
SS0.5	98.67	190.43	149.83	128.00	124.20	124.90	68.03	93.67
SS1.0	118.67	189.33	154.83	108.00	116.00	114.23	106.63	78.00
SS1.5	121.10	198.43	157.50	109.00	103.53	97.77	71.40	41.67
LSD (p=0.05)	6.42	8.27	8.35	14.31	9.3	11.76	25.76	16.83

of mineralization (Obbard *et al.,* 1994).

Unamended soil had a lower acid phosphatase activity than soil amended with sewage sludge and compost (Table 6). The acid phosphatase activity of all the treatment groups showed the same trend of change with an initial high and then a decline to a relatively constant level over incubation period. With increasing dose of compost application an increase in phosphatase activity was obtained. In COM0.25 and COM0.5 treatment increase in acid phosphatase activity was recorded till 14th day while in treatment COM1.0 and COM1.5, the increase in activity was observed till 21st day of incubation. This may be attributed to more availability of organic matter for microbial proliferation. In compost treatments maximum activity was obtained in treatment COM1.5 on 21st day of incubation. Crecchio *et al.* (2004) found a positive correlation between compost addition to soil and phosphatase activity.

In sewage treated soils, an overall high acid phosphatase activity was obtained in SS0.5 as compared to other treatments. Maximum activity was obtained in SS0.5 (16.89 µg PNP g^{-1} h^{-1}) on 3rd day of incubation. An alternate increasing and decreasing trend of acid phosphatase activity was obtained in sewage sludge treated soils. Maximum increase in activity was obtained on 3rd day of incubation.

Significantly detectable increases also occurred for alkaline phosphatase activity in the presence of compost. Increase in rate of compost application resulted in an increase in enzyme activity. Maximum acid phosphatase activity was obtained in COM1.5 (36.62 µg PNP g^{-1} h^{-1}) on 14th day of incubation. While in case of COM0.25, COM0.5 and COM1.0 maximum activity was obtained on 7th day of incubation. As compared to sludge treated soil, compost treated soil gave higher acid phosphatase activity. An increasing trend of acid phosphatase activity was obtained in SS0.25 (21.77 µg PNP g^{-1} h^{-1}) and SS0.5 (24.44 µg PNP g^{-1} h^{-1}) until 7th day of incubation followed by decline till steady activity was obtained while in SS1.0 and SS1.5, alkaline phosphatase activity increased only until 3rd day followed by decrease in activity with increase in incubation period. Maximum alkaline phosphatase activity in case of sludge treated soil was obtained in treatment SS1.5 (29.08 µg PNP g^{-1} h^{-1}) on 3rd day of incubation (Table 7).

The increase in phosphatase activities of amended soils was partly due to the direct contribution of phosphatase activities from compost and sewage sludge, and partly due to the stimulation of phosphatase production by microflora (Criquet *et al.,* 2007). According to Kizilkaya and Bayrakli (2005), sewage sludge amendment increases the content of readily available substrates in soils thereby promoting the growth of microorganisms. Among these available substrates, organic matter in sludge usually contains high amounts of substrates of acid and alkaline phosphomonoesterases (Garcia *et al.,* 1993) and of phosphodiesterases (Turner and Haygarth, 2005). The subsequent decrease in phosphatase activity might be due to the fact that sludge adds quite substantial amounts of C and N to the soil, thereby increasing microbial activity and thus P demand and release of phosphatases. Over time, C is depleted, resulting in lower microbial activity, lower P demand and thus less phosphatase activity (Allison and Vitousek, 2005). Pascual *et al.* (2002) attributed this decrease to depletion of biodegradable substrates by microbial activity, including the substrates of different phosphatises.

Overall alkaline phosphatase activity was two to three folds more as compared to acid phosphatase activity. Alkaline phosphates originate from soil bacteria, fungi and fauna (Nakas *et al.,* 1987; Tarafdar and Claassen, 1988). Microbes can produce and release large amounts of extracellular phosphatase due to their combined biomass, high metabolic activity and short life cycles (Speir and Ross, 1978). The activity of alkaline phosphatase is linked not only to the synthesis of animal and microbial cells (Juma and Tabatabai, 1988), but also to the transformation of organic to inorganic phosphorus (Yang *et al.,* 2008). The acid phosphatase activity on the other hand was found to be lower in both the organic amendments. It might be due to that plant roots are major producers of acid phosphatase (Speir and Cowling, 1991; Dinkelaker and Marschner, 1992). Moreover, soil acid phosphomonoesterase activity was higher at low inorganic P content of soil than at high content, and the enzyme activity was significantly correlated with herbage yield, probably due to the importance of organic P mineralization for plant nutrition (Speir and Cowling, 1991). Acid and alkaline phosphatase activities were significantly higher in sewage sludge and compost than unamended soil. The increase in activity of hydrolase enzymes might be due to higher levels of intracellular and/or extracellular enzymes, immobilized by recalcitrant humic moieties (Nannipieri, 1994).

Phytase was the only enzyme negatively affected by sludge treatment. The phytase activity of all the sludge treated soil samples on 0 day was at par with the control while with increase in incubation period and with an in increase in rate of sludge amendment, the phytase activity was significantly reduced (Table 8). The reduced activity of phytase may be attributed to high content of Iron (Fe) in sewage sludge. Soni et al. (2010) reported Fe has negative effect on phytase activity. Acuña *et al.* (2011) reported inhibition of phytase activity by 30-100% in presences of 10mM of Fe^{+3} in *Paenibacillus* sp. In case of compost amended soil samples significantly higher phytase activity was obtained in COM1.0 and COM1.5 throughout incubation study. Maximum phytase activity was obtained in COM1.0 (1.984 µg P hydrolyzed g^{-1} h^{-1}) followed by COM1.5 (1.920 µg P hydrolyzed g^{-1} h^{-1}) on 14^{th} and 21^{st} day of incubation respectively.

Unamended soil had lowest urease activity compared to that of soil amended with compost and sewage sludge. The maximum urease activity was obtained on 3^{rd} day of incubation in both sewage sludge and compost treated soil (Table 9). In comparison to sludge treated soil, the compost amendment gave higher urease activity. Maximum urease activity was obtained in COM1.5 (251.13 µg urea N hydrolyzed g^{-1} h^{-1}) on 3^{rd} day of incubation. Lai *et al.* (1999) showed the same trend of change with an initial high and then a decline to a relatively constant level. The higher urease activity at the beginning of incubation is likely due to the high substrate concentration in the amended soil. Moreover, wetting of dry soil causes the lysis of microbial cells which released the intracellular urease to degrade urea and its derivatives (Ladd and Jackson, 1982; Tabatabai, 1982; Singh and Nye, 1984). In addition, some protected urease bound to microbial cellular components and organic matters would also be released (Singh and Nye, 1984). The reason for the subsequent decline in urease activity after the peak activity may be due to the depletion of substrates and accumulation of toxic metabolites. In addition, during the incubation, free urease would be attacked by soil protease as well as re-bound into the microbial cellular components which would potentially reduce the activity and amount of urease in the soil (Singh and Nye, 1984).

Compost addition did not significantly affected soil pH and EC while increasing rate of sludge application significantly decreased soil pH as compared to control (Table 10). The decrease in pH was caused by the generation of organic acids from the mineralization of organic substrates. Moreover, the nitrification process taking place at this later period might also be responsible for the decrease in pH (Lai *et al.,* 1999). Soil amended with sewage sludge $\geq$ 0.5 % showed significant pH decrease, suggesting that these treatment groups had a higher decomposition rate. All treatment showed an increase in organic carbon content at the end of incubation period in comparison to control. With increasing dose of both the amendments an increase in OC was obtained. Marschner *et al.* (2003) reported an increase in organic carbon on addition of organic material to soil. They reported 0.82 % organic carbon in organic matter amended soil as compared to 0.67 % in control. The addition of compost and sludge resulted in significant increase in ammonium acetate extractable potassium. There was significant increase in potassium level with increasing rate of soil amendments. Extractable potassium was significantly more in compost amended soil when compared with sludge treated soil with same dose of organic amendment. Maximum potassium was obtained in treatment COM1.5 (767.20 kg/ha). Similarly, a significant increase in Olsen P was observed in case of soil amended with compost. Increasing the dose of compost lead to a significant increase in Olsen P. Kabirinejad and Hoodaji (2012) also obtained similar results. An increase in phosphorus from 44.5 mg kg^{-1} to 73 mg kg^{-1} was recorded by them and attributed the increase in Olsen P content due to the increase in microbial enzyme activity.

Conclusion

The present work revealed no apparent detrimental influence of both organic amendments on the studied soil quality indicators. Infact, positive effect on soil microbial enzymes has been observed. Moreover an increase in nutrient availability has also been recorded. It was concluded that soil enzymatic activities

(dehydrogenase, phosphatase, phytase and urease) of the soil were the most important parameters for assessing soil quality in the environment resulting from use of sewage sludge and compost as amendments .Therefore, compost and sewage sludge can be used as soil amendments after examining their heavy metal and pathogen load which must be within safe limits.

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