Effect of cadmium-contamination with sewage sludge and phosphate fertiliser amendments on soil enzyme activities, microbial structure and available cadmium

Ayten Karaca[a], David, C. Naseby[b] and James, M. Lynch[c*].

[a]Soil Science Department, University of Ankara,Turkey.

[b]Bioscience Department University of Hertfordshire Hatfield Herts AL10 9AB, UK

[c]School of Biological Sci., University of Surrey, Guildford,Surrey GU2 5XH, UK.

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*Corresponding author

Tel + (0)1483 259721 - Fax + (0)1483 2597283 - e-mail: j.lynch@surrey.ac.uk

Abstract. The effect of Cd pollution (50 $mgkg^{-1}$), with and without sewage sludge (Sw) and phosphate fertiliser (P) addition on soil biochemical activity and available Cd was assessed in a 112 day soil incubation experiment. The availability of Cd decreased with incubation time and was reduced by the sludge and P additions resulting in the following order, Cd>P+Cd>Sw+Cd. With the exception urease and NAGase activities, all other enzyme activities were negatively correlated with available Cd.

The total culturable bacterial population was significantly higher with the addition of sewage sludge alone (Sw) than the control during the incubation period ($P<0.05$). The number of fluorescent pseudomonas decreased with time, but was significantly increased by the addition of sewage sludge. The total fungal populations decreased with time in all treatments, whilst the addition of sewage sludge and phosphate fertilisers increased the fungal population. Addition of sewage sludge in the presence of Cd increased the fungal populations in relation to the addition of Cd alone. The results support the view that Cd contamination has a large detrimental effect on nutrient cycling and microbial activity and the effects of Cd were reduced by P and sewage sludge additions

Key words: cadmium, pollution, sewage sludge, phosphate fertilizer, enzyme activity.

Introduction

In recent years several reports have documented the harmful effects of long-term heavy metal contamination of agricultural soils due to sewage sludge and phosphate fertiliser application on soil microorganisms and microbial activity at several sites. Of the heavy metals found in sewage sludge and phosphate fertilisers, Cd is one of the most toxic and has been recognised as an environmental contaminant of considerable interest in various human and animal diseases (Bramley, 1990; Loganathan *et al*. 1996). When present in sufficient concentrations this element could become toxic to living systems. Microbial growth and soil enzyme activities are affected by high concentrations of Cd (Reber 1992, McGrath *et al*. 1995, Dar 1996, Moreno *et al*. 1998 and 1999)

Large amounts of Cd are found in various phosphate fertilisers (Williams, 1974), for example the main rock phosphate source in Australia are from oceanic sedimentry deposits, containing between 42 and 99 mg kg^{-1} cadmium (McLaughlin 1991). Over 80 % of the Cd added in phosphate fertilisers may remain in the topsoil (Taylor, 1997). Ross *et al*. (1995) examined the influence of rock phosphate on invertase, phosphodiesterase and sulphatase activities and found that fertilisers increased extractable soil inorganic P, but no consistent, effect on soil biochemical properties were found. In a more fertile lowland pasture, invertase activity increased significantly under wet spring conditions, whereas fluctuations in sulphatase activity were small.

Taylor (1997) found that Cd levels increased in the top soil and was associated with the application of phosphate fertilisers. However, Richards *et al*. (1998) found no evidence of Cd enrichment of either soil or crop after 25 years of phosphate fertiliser applications.

Fliebbach *et al*. (1994) reported that low metal sludge had beneficial effects on the soil microbial activity. Furthermore, Dar (1996) found that Cd addition at 10µg g^{-1} in sewage sludge caused no significant changes in soil enzyme activities. However, the addition of 50µg g^{-1} Cd detrimentally decreased the soil enzyme activities; whilst the effect was greater in sandy loam than in loam or clay loam soil.

Moreno *et al*. (1999) reported that the effect of high Cd content (815 $mgkg^{-1}$) in sewage sludge varied with the enzyme studied. Cd negatively affected dehydrogenase activity whereas β-glucosidase activity was unaffected and urease and phosphatase activities were stimulated. However, Brendecke *et al*. (1993) found no effects on several microbial parameters (populations or activities) after four years of sewage sludge application.

The influence of toxicants on microorganisms has often been studied under controlled conditions. The heavy metal effects on the soil microbial community have been investigated quantitatively (plate count, ATP and direct observation) or with emphasis on specific microbial activities (soil enzymes, N_2 fixation and respiration) as well as by estimating heavy metal tolerance or microbial diversity (Doelman and Haanstra 1979; Brookes *et al*. 1986; Reber 1992).

Several investigations have shown that soil microorganisms are adversely affected by heavy metals at concentrations close to the maximum concentrations permitted under the European Community directive (Giller *et al*. 1989). However, other studies have found little effect at the same concentrations (Chander and Brookes 1991).

Chaudri *et al*. (1992) found that of the metals found in sewage sludge, Zn and Cd were the most toxic to *R. Leguminosarum bv. Trifolii* in soil. Other workers have also found reduced populations of *R. Leguminosarum bv. Trifolii* in metal contaminated soils (Obbard *et al*. 1992; Martensson and Witter 1990).

McGrath *et al*. (1995) found that microbial activity and populations of cyanobacteria and *Rhizobium leguminasorum bv. Trifolii* were adversely affected by

metal concentrations below the EC's maximum allowable concentration limits for metals in sludge-treated soils.

Recent interest in defining soil quality has focus on identifying soil properties that affect soil health and quality (Doran *et al*. 1994). It has been proposed that measurement of changes in soil enzyme activities may provide a useful index of changes in soil quality (Dick, 1992). It is important to obtain a complete assessment of soil enzyme activities that reflect the changes in soil metabolic processes by using different biochemical reactions involved in nutrient cycling in soils (Naseby and Lynch, 1998).

Although the effects of Cd on biochemical transformations and microbial populations have been studied, very little information is available on the relative effects of sewage sludge and phosphate fertilisers on Cd polluted soil. Since the application of organic wastes such as sewage sludge to agricultural soils is a widespread practice, further studies are needed to evaluate the effect of these materials on several biochemical processes in soils.

Therefore, the objective of this study was to assess the influence of cadmium contamination on the soil enzyme activities, microbial population structure and available Cd of sandy soil amended with sewage sludge and phosphate fertiliser.

Materials and methods

Soil and Sludge Description

The soil used was a sandy loam of the Holiday Hills Series taken from Merrist Wood Agricultural College 5 miles south east of Guildford, UK; it had been under permanent pasture at least 15 years. The pH of the soil was 5.36, particle ratio was 10:9:81 clay: silt: sand, respectively, and the organic content was 1.6 % by weight.

The sewage sludge used in this experiment came from on urban wastewater treatment plant in the city of Ankara. The properties of the sewage sludge, analysed in the faculty of agriculture, Ankara University, are shown in Table 1.

Experimental Design

Each pot consisted of 300g of coarsely sieved soil with various amendments. Three replicates of each treatment were assembled as follows:

1. The control pots were unamended.
2. Soils were supplemented with analytical reagent grade cadmium chloride ($CdCl_2\ H_2O$) to yield 50 mg Cd kg^{-1} soil (Cd)
3. Sewage sludge was added to soil at 20 ton ha^{-1} (Sw).
4. Sw amended soils were supplemented with analytical reagent grade cadmium chloride ($CdCl_2\ H_2O$) to yield 50 mg Cd kg^{-1} soil (Sw+Cd).
5. Phosphate fertiliser ($Na_4P_2O_7.10H_2O$) was added to soil at 250kg P ha^{-1} (P)
6. P amended soils were supplemented with analytical reagent grade cadmium chloride ($CdCl_2\ H_2O$) to yield 50 mg Cd/kg soil (P+Cd).

The water content of the soil was adjusted to 75% of field capacity. The pots were placed in an incubator at 21 °C and 70% relative humidity. Throughout the incubation period, water losses exceeding 10% of the initial values were compensated for by addition of distilled water.

Sampling and Analysis

Samples were taken at 7, 14, 28, 56 and 112 days for the following analyses. For each sample, the activities of key enzymes involved in the four major nutrient cycles were determined as described by Naseby and Lynch (1997a). They included β-

galactosidase under acid conditions (C cycle), urease (N cycle), N-acetylglucosaminidase (C and N cycle), acid and alkaline phosphatase (P cycle) and arylsulphatase under alkaline conditions (S cycle).

Soil was analysed for water-soluble cadmium contents by adding 9 ml water to 1g soil in a 10 ml centrifuge tube. The soil suspensions were mixed for 1h on a carousel rotor before being centrifuged at 4000xg for 15 min. The supernatant was decanted off into clean test tubes and kept at 4 ^{0}C until required on the same day. A Pyeunicam SP9 atomic absorption spectrophotometer was used to determine the concentrations of Cd in samples of the supernatant fluid.

1 g soil sample from each replicate was macerated in 9 ml sterile quarter-strength Ringers solution. Filamentous fungi populations were quantified by plating a 10-fold dilution series of each soil macerate onto 10% malt extract agar containing 50 mgkg^{-1} rose bengal. Plates were incubated at 20 C^{0} for 7 days before enumeration. P1 medium (Katoh and Itoh, 1983) was used for the enumeration of indigenous, fluorescent *Pseudomonas*. To enable quantification of introduced *Ps. fluorescens* strains, this media was amended with 50 mgkg^{-1} X-Gal, upon which recovered lac2Y modified *pseudomonas* could be identified as blue colonies. P1 plates were incubated at 25C^{0} and enumerated after 7 days of growth. Tryptone soya agar (10%) was used for the enumeration of total culturable bacterial populations. For this purpose, the plates were incubated at 25 C^{0} for 7 days and enumerated.

Statistical Analysis

Treatments were compared by analysis of variance and Least Square Difference (P<0.05). The relationships between variables were investigated using the Spearman Correlation Coefficient followed by a test of significance (significant results indicated in results section). All statistical analyses were conducted with SPSS for windows (SPSS Inc.).

Results and discussion

Available Cd

Available Cd decreased with incubation time in all treatments (Table 2). Available Cd was reduced by the sludge and phosphate additions resulting in the following significant differences in Cd availablity, Cd>P+Cd>Sw+Cd. The reduced availability of Cd with the addition of sewage sludge is not surprising, as Dar (1996) also found that sewage sludge reduced the availability of Cd. Dar (1996) also went on to demonstrate that organic complexion of the Cd with the organic matter was the primary cause of this effect.

The cause for the reduction in Cd availability with the addition of phosphate fertiliser is more deceptive. The mechanisms of this effect have recently been described by Bolan *et al* (1999), who also found a decrease in Cd availability with phosphate fertiliser application. They concluded that specific sorption of phosphate to soil particles leads to an increase in negative charge which in turn leads to an increase in Cd sorption to soil particles.

The initial large reduction in available Cd at T1 in the P+Cd treatment, which is followed by an increase in Cd availability at T2, can therefore by explained by this theory. The initial addition of P provides a large sink of negative charge on the soil particles, which makes a large amount of the added Cd unavailable. This sink is reduced over time by desorption of some of the P by the soil microbial biomass, causing an increase in available Cd, which subsequently falls over time as it is again rendered unavailable by complexion to the soil and biomass.

Soil enzymatic activities

Measurement of soil enzyme activities may be useful for gaining a greater understanding of the nature of perturbations caused to ecosystem function (Naseby and Lynch 1997b) and has been used as an indicator of the effect of microbial inoculation (Naseby and Lynch 1998) and impacts upon nutrient cycling (Naseby *et al* 1999).

Phosphatase enzymes are important agronomically as they play a key role in the P cycle. They catalyse the hydrolysis of organic P to inorganic P, which can be assimilated by plants.

Acid phosphatase activity increased with time and was significantly reduced by the addition of Cd at the beginning of the incubation (Table 3). Addition on Cd in the presence of Sw and P treatments reduced the acid phosphatase activity with respect to the Sw and P treatments. By the end of the incubation period, Cd caused significant reductions in acid phosphatase activities ($P<0.05$). Whilst, Sw and P amendments in the absence of Cd resulted in significantly greater acid phosphatase activity than all other treatments. This contradicts the results of Moreno *et al*. (1999), who reported an increase in acid phosphatase activity caused by amendment with sewage sludge containing high levels of Cd. Whereas, the correlation coefficient between the activity of acid phosphatase and available Cd in this study was -0.531 ($P<0.01$).

Alkaline Phosphathase activities significantly reduced by the adding of Cd during the incubation period (Table 4). At the end of the incubation period, Sw amendment in the absence of Cd resulted in significantly greater alkaline phosphatase activity than all other treatment ($P<0.05$). The activity of this enzyme was negatively correlated with available Cd (r: -0.438, $P<0.01$).

Aryl sulphatase is the enzyme involved in the hydrolysis of arylsulfate by fission of the O-S bond. This enzyme is believed to be involved in mineralization of ester sulfate in soils (Tabatabai, 1994). All the treatments with Cd had significantly greater aryl

sulphatase activities than the control at the beginning of the incubation ($P<0.05$). However, at the end of the incubation period all the treatments with Cd had significantly lower aryl sulphatase activities than the control (Table 4). The addition of P significantly increased aryl sulphatase activity and had significantly greater aryl sulphatase than the Sw treatment. Significant negative correlation was found between aryl sulphatase activity and available Cd (r: -0.430, $P<0.01$).

β-galactosidase is an important C cycle enzyme involved in the breakdown of complex carbohydrates by the hydrolysis of β-galactosidase bonds. This enzyme plays an important role in the degredation of organic carbon compounds (e.g., sewage sludge, crop residues, animal manure, biotechnology by-products) in soils (Martinez and Tabatabai, 1997). β-galactosidase activity decreased with time. At the beginning of the incubation, the addition of Sw+Cd significantly reduced the β-galactosidase activity with respect to all other treatments (Table 4). By the end of the incubation period, all the treatments amended with Cd had significantly lower β-galactosidase activity than the control ($P<0.05$). However, this decrease was much greater with the addition of Cd and P+Cd rather than the addition of Sw+Cd. The addition of P resulted in significantly greater β-galactosidase activity than all other treatments at the end of the incubation period ($P<0.05$). The correlation coefficient between the activity of β-galactosidase and available Cd was -0.433 ($P<0.01$).

Urease is an important N cycle enzyme as it catalyses the breakdown of urea to ammonia, which can be assimilated by microbes and plants. The sewage sludge amendment (Table 5) substantially increased the urease activity both with and without Cd addition ($P<0.05$). This effect continued to the end of the incubation. However, the Sw+Cd resulted in a significant greater urease activity than the Sw treatment at the end of the incubation. The Cd and P+Cd treatments also resulted in significant greater activities than the controls at the end of the incubation (Table 5). Urease enzyme activity was not correlated to the levels of available Cd.

The fact that urease activity was greater in soil amended with Cd indicates that Cd has a positive effect on this enzyme activity. This is supported by Moreno *et al*. (1999), who found that the soil enzymatic activities were stimulated by addition of sewage sludge with low heavy metal content. After incubation, urease activity increased in soil amended with the high dose of sludge.

NAGase releases N-acetyl glucosamine subunits from chitin polymers, which are abundant in soil in the form of fungal cell walls. NAGase activity has been correlated to fungal biomass (Miller *et al* 1998), however this is a new method, and in other work this correlation was overridden by gross changes in nutrient cycles (Naseby *et al* 1999). Sw+Cd addition significantly increased the NAGase activity at the beginning of the incubation, whilst the P+Cd significantly reduced the NAGase activity (Table 5). At the end of the incubation period, the Cd and P+Cd treatments had significantly lower NAGase activities than the control ($P<0.05$). Sw and P amendments in the absence of Cd resulted in significantly greater NAGase activities than all other treatments. NAGase activity was not correlated to the levels of available Cd.

Microbial Populations

The total culturable bacteria population, was significantly greater with the addition of sewage sludge alone (Sw) than all other treatments during the incubation period ($P<0.05$), (Table 6). The bacterial numbers were significantly lower in all treatments with Cd than their respective non Cd amended controls ($P<0.05$). The organic matter added in the form of sewage sludge therefore had a direct effect on the bacterial population, providing a nutrient source capable of supporting a greater bacterial population. This effect was suppressed by the addition of Cd to the soil, which indicates that the Cd had a detrimental effect on the bacterial population.

The supposition that the Cd addition had a detrimental effect on the bacterial community is supported by the fact that significant negative correlation was found

between the total culturable bacterial population and available cadmium (r=-0.604, P<0.001). It is therefore notable that the availability of the Cd in soil may be the overriding factor controlling the effect of Cd on microbial populations. This is supported by the cycling of Cd availability found in phosphate treated soil, where an initially large bacterial population day 1 is reduced dramatically by day 7 and rises again after 30 days incubation, mirroring the fluctuations in Cd availability.

Chaudri *et al*. (1992) found that in their Cd treatments, *rhizobium* populations were reduced at concentrations > 7 $mgkg^{-1}$ soil. Below this Cd concentration there was no difference in numbers of bacteria between the control and the Cd treated soils. Furthermore, Giller *et al.* (1989, 1993) and McGrath *et al.* (1988) found significant reductions in numbers of *R. Leguminosarum bv. Trifolii* in soils treated with metal contamination sewage sludge.

The total number of bacteria was significantly and positively correlated with the soil enzyme activities. The correlation coefficient was the greatest for acid phosphatase (r: 0.692, P<0.001), followed by alkaline phosphatase (r: 0.633, P<0.001), β-galactosidase (r:0.607, P<0.001), urease (r. 0.565, P<0.01) and NAGase (r:0.555, P<0.01).

The fluorescent pseudomonad populations significantly decreased with time, but were significantly increased by the addition of sewage sludge alone in comparison to the control (Table 6), ($P<0.05$). However, the soil pseudomonas populations were not significantly affected by P treatments with and without Cd. Therefore, sewage sludge alone increased the soil pseudomonas populations, whilst the addition of Cd alone decreased the pseudomonad population throughout the incubation period.

There were no significant correlations between the total soil pseudomonas populations and available cadmium. However, the total soil pseudomonas populations were significantly and positively correlated with the soil enzyme activities. The correlation coefficient was the greatest for NAGase (r: 0.720, $P<0.001$), followed by urease (r: 0.683, $P<0.001$), alkaline phosphatase (r:0.652, $P<0.001$), β-galactosidase (r. 0.644, $P<0.001$) and asit phosphatase (r:0.568, $P<0.01$).

The total fungal populations significantly decreased with time in all treatments. The addition of sewage sludge and phosphate fertilisers with and without Cd significantly increased the numbers of fungi at the beginning of the incubation (Table 6), ($P<0.05$). However, after 7 days of incubation, the addition of P with Cd had similar value with the control. Culturable fungal populations were not correlated to the levels of available Cd. However, significant positive correlations were found between the total fungi populations and the soil enzyme activities. The correlation coefficient was the greatest for NAGase (r: 0.797, $P<0.001$), followed by β-galactosidase (r: 0.642, $P<0.001$), urease (r:0.448, $P<0.01$), alkaline phosphatase (r. 0.446, $P<0.01$) and asit phosphatase (r:0.409, $P<0.01$). The correlation coefficients indicating that the enzyme activities correspond closely with the microbial structure in soil.

Cd addition had dramatic effects on most of the parameters studied. The negative correlation between the available Cd and β-galactosidase, aryl sulphatase, acid and alkaline phosphates enzymes and total bacterial populations indicates that Cd contamination has a large detrimental effect on nutrient cycling and microbial activity. In general, the organic matter added with the sewage sludge had positive effect on the

enzymatic activities, which, in some cases, counteracted the negative effect that a high Cd contamination might have had on them.

Many of the effects of Cd were reduced by the sewage sludge and phosphate fertiliser amendments. Therefore, reducing the input of phosphate fertilisers and sewage sludge to contaminated agricultural sites will result in an increase in the availability of Cd. A positive way of reducing the impact of Cd contamination is therefore to continue P and sewage sludge/organic matter amendments, which are low in pollutants, on a limited basis. For example, if the conclusion of Taylor (1997) that 80% of Cd added to soils remains in the top soil per year, is taken as a model then addition of P or organic matter with an overall content of Cd less 20% of the total Cd in soil per unit area may eventually result in reduced Cd in the soil. This will also reduce the availability of Cd resulting in the soil being less toxic and less Cd being sequestered into the crop biomass. However further long term studies will be needed to evaluate this hypothesis.

The results provide information on important biochemical reactions that have potential as early and sensitive indicators to soil stress or health and quality.

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Table 1. Physicochemical characteristics of sewage sludge

EC (25^0C)	2.10 dSm^{-1}
pH (1:2.5)	7.08
CEC	67 mEq $100g^{-1}$
Organic matter	25 (%)
Total N	1.54 (%)
Total P	4079 ($mgkg^{-1}$)
Total Cd	1.8 ($mgkg^{-1}$)
Total Pb	144 ($mgkg^{-1}$)
Total Zn	276.2 ($mgkg^{-1}$)
Total Cu	123.9 ($mgkg^{-1}$)

Table 2. Changes in water available Cd in a sandy soil as affected by sewage sludge and phosphate fertiliser amendments.

Cd †	Incubation Time (Days)	Cont	Cd	Sw	Sw+Cd	P	P+Cd
	7.	0.00^{e}	344.50^{a}	0.16^{e}	181.00^{b}	2.03^{d}	70.35^{c}
	14.	0.00^{e}	233.00^{a}	0.15^{e}	60.50^{c}	2.13^{d}	183.00^{b}
	28.	0.00^{d}	215.00^{a}	0.91^{d}	50.10^{c}	1.47^{d}	150.70^{b}
	56.	0.00^{d}	163.33^{a}	1.25^{d}	37.67^{c}	0.31^{d}	101.00^{b}
	112.	0.04^{d}	114.33^{a}	0.84^{d}	26.98^{c}	0.12^{d}	81.33^{b}
$LSD_{0.05}$	1.633						

† concentration expressed in $mgkg^{-1}$; Cont, unammended; Cd, soil supplemented with 50 $mgkg^{-1}$ $CdCl_2.H_2O$; Sw, sludge alone; Sw+Cd, sludge soil supplemented with 50 $mgkg^{-1}$ $CdCl_2.H_2O$; P, phosphate fertiliser alone; P+Cd, phosphate fertiliser soil supplemented with 50 $mgkg^{-1}$ $CdCl_2.H_2O$. Significant differences between treatments (columns) at $P<0.05$ level indicated by different letters

Table 3. Phosphorus cycle enzyme activities in a sandy soil as affected by sewage sludge and phosphate fertiliser amendments.

Enyzme†	Class/ EC number	Incubation Time (Days)	Cont	Cd	Sw	Sw+Cd	P	P+Cd
Acid phos	3.1.3.2.	7.	2.24^{d}	1.42^{f}	5.74^{a}	2.91^{c}	4.34^{b}	1.88^{e}
		14.	2.91^{d}	1.95^{f}	6.67^{a}	3.21^{c}	5.71^{b}	2.35^{e}
		28.	2.86^{d}	1.24^{f}	7.95^{a}	3.02^{c}	5.94^{b}	2.16^{e}
		56.	2.82^{d}	0.93^{f}	9.51^{a}	2.90^{c}	6.13^{b}	1.92^{e}
		112.	2.04^{d}	0.53^{f}	13.07^{a}	2.51^{c}	5.96^{b}	1.46^{e}
$LSD_{0.05}$		0.0516						
Alk Phos	3.1.3.1.	7.	0.76^{d}	0.51^{e}	1.80^{a}	1.02^{b}	0.96^{c}	0.76^{d}
		14.	0.76^{e}	0.48^{f}	6.83^{a}	2.75^{c}	3.90^{b}	1.41^{d}
		28.	0.93^{e}	0.34^{f}	5.41^{a}	1.97^{b}	1.83^{c}	1.24^{d}
		56.	0.81^{e}	0.40^{f}	6.07^{a}	2.15^{c}	2.75^{b}	1.36^{d}
		112.	0.72^{e}	0.12^{f}	4.12^{a}	1.77^{b}	1.16^{c}	0.76^{d}
$LSD_{0.05}$		0.0365						

† Activities expressed as mg pNP released $h^{-1}g^{-1}$ dry soil. Acid phos, acid phosphatase; Alk phos, alkaline phosphatase; Cont, unammended; Cd, soil supplemented with 50 $mgkg^{-1}$ $CdCl_2.H_2O$; Sw, sludge alone; Sw+Cd, sludge soil supplemented with 50 $mgkg^{-1}$ $CdCl_2.H_2O$; P, phosphate fertiliser alone; P+Cd, phosphate fertiliser soil supplemented with 50 $mgkg^{-1}$ $CdCl_2.H_2O$. Significant differences between treatments (columns) at $P<0.05$ level indicated by different letters

Table 4. Sulfur and carbon cycle enzyme activities in a sandy soil as affected by sewage sludge and phosphate fertiliser amendments.

Enyzme†	Class/ EC number	Incubation Time (Days)	Cont	Cd	Sw	Sw+Cd	P	P+Cd
Sulph	3.1.6.1.	7.	0.16^{e}	0.27^{d}	0.49^{b}	0.35^{c}	0.65^{a}	0.29^{d}
		14.	0.18^{f}	0.23^{e}	0.56^{b}	0.38^{c}	1.04^{a}	0.30^{d}
		28.	0.36^{c}	0.20^{e}	0.43^{b}	0.28^{d}	0.95^{a}	0.21^{e}
		56.	0.33^{c}	0.14^{f}	0.40^{b}	0.22^{d}	0.77^{a}	0.17^{e}
		112.	0.31^{c}	0.09^{e}	0.39^{b}	0.20^{d}	0.69^{a}	0.11^{e}
$LSD_{0.05}$		0.0292						
β-gal	3.2.1.23.	7.	0.26^{c}	0.30^{b}	0.38^{a}	0.22^{d}	0.38^{a}	0.30^{b}
		14.	0.33^{c}	0.19^{f}	0.48^{b}	0.29^{d}	0.53^{a}	0.26^{e}
		28.	0.31^{b}	0.11^{e}	0.31^{b}	0.24^{c}	0.40^{a}	0.19^{d}
		56.	0.25^{c}	0.09^{f}	0.29^{b}	0.19^{d}	0.36^{a}	0.12^{e}
		112.	0.18^{c}	0.07^{e}	0.23^{b}	0.15^{d}	0.30^{a}	0.09^{e}
$LSD_{0.05}$		0.0253						

† Activities expressed as mg pNP released $h^{-1}g^{-1}$ dry soil. Sulph, aryl sulphatase; β-gal, β -galactosidase; Cont, unammended; Cd, soil supplemented with 50 $mgkg^{-1}$ $CdCl_2.H_2O$; Sw, sludge alone; Sw+Cd, sludge soil supplemented with 50 $mgkg^{-1}$ $CdCl_2.H_2O$; P, phosphate fertiliser alone; P+Cd, phosphate fertiliser soil supplemented with 50 $mgkg^{-1}$ $CdCl_2.H_2O$. Significant differences between treatments (columns) at $P<0.05$ level indicated by different letters

Table 5. Urease and NAGase enzyme activities in a sandy soil as affected by sewage sludge and phosphate fertiliser amendments.

Enyzme †	Class/ EC number	Incubation Time (Days)	Cont	Cd	Sw	Sw+Cd	P	P+Cd
Urease	3.5.1.5.	7.	73.00^{f}	99.30^{e}	856.00^{b}	873.00^{a}	169.00^{c}	129.00^{d}
		14.	62.80^{f}	67.50^{e}	1132.30^{b}	1226.00^{a}	136.50^{c}	90.30^{d}
		28.	42.20^{e}	71.00^{d}	1345.70^{b}	1373.70^{a}	119.00^{c}	75.00^{d}
		56.	38.50^{f}	65.00^{e}	1116.00^{b}	1210.00^{a}	100.95^{c}	70.17^{d}
		112.	26.30^{f}	60.30^{e}	1013.00^{b}	1134.30^{a}	103.70^{c}	67.10^{d}
$LSD_{0.05}$		4.003						
NAGase	3.2.1.50	7.	0.49^{d}	0.67^{c}	0.71^{b}	0.95^{a}	0.47^{d}	0.35^{e}
		14.	0.37^{d}	0.51^{c}	0.64^{a}	0.56^{b}	0.32^{e}	0.27^{f}
		28.	0.15^{d}	0.10^{e}	0.41^{a}	0.20^{c}	0.23^{b}	0.11^{e}
		56.	0.13^{d}	0.07^{e}	0.35^{a}	0.17^{c}	0.22^{b}	0.09^{e}
		112.	0.11^{d}	0.01^{e}	0.29^{a}	0.14^{c}	0.21^{b}	0.01^{e}
$LSD_{0.05}$		0.0268						

† Urease activity expressed as mg ammonia released $h^{-1}g^{-1}$ dry soil and NAGase expressed as mg pNP released $h^{-1}g^{-1}$ dry soil. NAGase, N acetylglucosaminidase; Cont, unammended; Cd, soil supplemented with 50 $mgkg^{-1}$ $CdCl_2.H_2O$; Sw, sludge alone; Sw+Cd, sludge soil supplemented with 50 $mgkg^{-1}$ $CdCl_2.H_2O$; P, phosphate fertiliser alone; P+Cd, phosphate fertiliser soil supplemented with 50 $mgkg^{-1}$ $CdCl_2.H_2O$. Significant differences between treatments (columns) at $P<0.05$ level indicated by different letters

Table 6. Log total soil bacterial, pseudomonas and fungal populations as effected by sewage sludge and phosphate fertiliser amendments

Tot bact †	Incubation Time (Days)	Cont	Cd	Sw	Sw+Cd	P	P+Cd
	7.	7.48^{b}	7.01^{e}	7.94^{a}	7.32^{c}	7.30^{c}	7.17^{d}
	14.	7.38^{b}	7.04^{d}	7.67^{a}	7.27^{c}	7.26^{c}	6.88^{d}
	28.	7.32^{b}	6.90^{d}	7.63^{a}	7.27^{c}	7.27^{c}	7.00^{d}
	56.	7.25^{b}	6.85^{e}	7.59^{a}	7.19^{c}	7.22^{c}	7.00^{d}
	112.	7.17^{b}	6.79^{f}	7.59^{a}	7.12^{c}	7.06^{d}	6.93^{e}
$LSD_{0.05}$	0.0357						
Tot pseu †		Cont	Cd	Sw	Sw+Cd	P	P+Cd
	7.	5.88^{c}	5.66^{d}	6.97^{a}	5.93^{c}	6.04^{b}	5.52^{e}
	14.	5.47^{e}	5.66^{d}	6.86^{a}	6.32^{b}	5.83^{c}	5.12^{f}
	28.	5.12^{d}	4.52^{e}	6.52^{a}	6.12^{b}	5.66^{c}	5.10^{d}
	56.	5.07^{d}	4.36^{e}	6.25^{a}	6.00^{b}	5.27^{c}	5.10^{d}
	112.	5.00^{d}	4.12^{e}	5.98^{a}	5.14^{b}	5.10^{b}	5.06^{c}
$LSD_{0.05}$	0.0588						
Tot fungi †		Cont	Cd	Sw	Sw+Cd	P	P+Cd
	7.	5.03^{d}	5.12^{c}	5.23^{b}	5.21^{b}	5.33^{a}	5.19^{b}
	14.	4.95^{d}	5.00^{c}	5.25^{a}	5.07^{b}	5.02^{c}	4.96^{d}
	28.	4.70^{e}	4.67^{e}	5.23^{a}	5.06^{b}	4.97^{c}	4.78^{d}
	56.	4.52^{d}	4.42^{e}	5.12^{a}	4.75^{b}	4.60^{c}	4.53^{d}
	112.	4.40^{d}	4.21^{e}	4.61^{b}	5.00^{c}	4.52^{a}	4.41^{d}
$LSD_{0.05}$	0.0476						

† Microbial populations expressed as c.f.u.g^{-1} soil. Tot bact, total bacteria; Tot pseu, total pseudomonad; Tot fungi, total fungi; cont, non sludge and phosphate; Cd, soil supplemented with 50 mgkg^{-1} $CdCl_2.H_2O$; Sw, sludge alone; Sw+Cd, sludge soil supplemented with 50 mgkg^{-1} $CdCl_2.H_2O$; P, phosphate fertiliser alone; P+Cd, phosphate fertiliser soil supplemented with 50 mgkg^{-1} $CdCl_2.H_2O$. Significant differences between treatments (columns) at $P<0.05$ level indicated by different letters