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ORIGINAL PAPER

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

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Abstract The effect of Cd pollution (50 mg kg^{-1}), with and without sewage sludge (Sw) and PO_4^{3-} 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 Sw and P additions resulting in the following order of treatments: Cd>P+Cd>Sw+Cd. With the exception of urease and *N*-acetylglucosaminidase activities, all enzyme activities were negatively correlated with available Cd. The total culturable bacterial population was significantly higher with the addition of Sw alone than in the control during the incubation period ($P<0.05$). The number of fluorescent pseudomonads decreased with time, but was significantly increased by the addition of Sw. The total fungal populations decreased with time in all treatments, whilst the addition of Sw and PO_4^{3-} fertilisers relatively increased the fungal population. Addition of Sw 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 that the effects of Cd are reduced by P and Sw additions.

Keywords Cadmium · Pollution · Sewage sludge · Phosphate fertiliser · Enzyme activity

Introduction

In recent years, several reports have documented the harmful effects on soil microorganisms and microbial activity of the long-term heavy metal contamination of agricultural soils due to sewage sludge (Sw) and PO_4^{3-} fertiliser application. Of the heavy metals found in Sw and PO_4^{3-} fertilisers, Cd is one of the most toxic and has been recognised as an environmental contaminant with a significant role in various human and animal diseases (Bramley 1990; Loganathan et al. 1996). Soil microbial growth and enzyme activities are affected by high concentrations of Cd (Reber 1992; McGrath et al. 1995; Dar 1996; Moreno et al. 1998, 1999).

Large amounts of Cd are found in various PO_4^{3-} fertilisers (Williams 1974), e.g. the main rock phosphate sources in Australia are from oceanic sedimentary deposits, containing between 42 and 99 mg Cd kg^{-1} (McLaughlin 1991). Over 80% of the Cd added in PO_4^{3-} 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 effects 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 topsoil and were associated with the application of PO_4^{3-} fertilisers. However, Richards et al. (1998) found no evidence of Cd enrichment of either soil or crop after 25 years of PO_4^{3-} fertiliser applications.

Fliessach et al. (1994) reported that sludge containing low levels of metals had beneficial effects on the soil microbial activity. Furthermore, Dar (1996) found that Cd added at 10 μg g^{-1} soil (in Sw) caused no significant changes in soil enzyme activities. However, the addition of Cd at 50 μg g^{-1} soil decreased the soil enzyme activities, and this effect was greater in sandy loam than in loam or clay loam soil.

Moreno et al. (1999) reported that the effect of a high Cd content (815 μg g^{-1}soil) in Sw varied with the enzyme studied. Cd negatively affected dehydrogenase activity whereas β-glucosidase activity was unaffected and urease and phosphomonoesterase activities were stimulated. However, Brendecke et al. (1993) found no effects

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on several microbial parameters (populations or activities) after 4 years of Sw 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). Chaudri et al. (1992) found that of the metals in Sw, Zn and Cd were the most toxic to *Rhizobium leguminosarum* bv. *trifolii* in soil. Other workers have also found reduced populations of *R. leguminosarum* bv. *trifolii* in metal-contaminated soils (Martensson and Witter 1990; Obbard et al. 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).

McGrath et al. (1995) found that microbial activity and populations of cyanobacteria and *R. leguminosarum* 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 focused 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). However, it is important to obtain an assessment of soil enzyme activities that reflect the changes in soil metabolic processes using key enzymes involved in all four major nutrient cycles (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 Sw and PO_4^{3-} fertilisers in Cd-polluted soil. Since the application of organic wastes such as Sw 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 Cd contamination on the soil enzyme activities, microbial population structure and available Cd of sandy soil amended with Sw and PO_4^{3-} fertiliser.

Materials and methods

Soil and sludge description

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

The Sw used in this experiment came from an urban wastewater treatment plant in the city of Ankara. The properties of the Sw, analysed in the Faculty of Agriculture, Ankara University, are shown in Table 1.

Table 1 Physicochemical characteristics of sewage sludge (Sw). *CEC* Cation exchange capacity

EC (25°C)	2.10 dS m^{-1}
pH (1:2.5)	7.08
CEC	67 mEq 100 g^{-1}
Organic matter	25 (%)
Total N	1.54 (%)
Total P	4,079 (mg kg^{-1})
Total Cd	1.8 (mg kg^{-1})
Total Pb	144 (mg kg^{-1})
Total Zn	276.2 (mg kg^{-1})
Total Cu	123.9 (mg kg^{-1})

Experimental design

The experiment was conducted in pots, each containing 300 g coarsely sieved soil with various amendments. There were five replicates of each of the following treatments:

1. The control pots were unamended.
2. Soils were supplemented with analytical reagent grade $CdCl_2.H_2O$ to yield 50 mg Cd kg^{-1} soil (treatment Cd).
3. Sw was added to soil at 11.65 g^{-1} kg^{-1} soil, equivalent to 2 t ha^{-1} (treatment Sw).
4. Sw-amended soils were supplemented with analytical reagent grade $CdCl_2.H_2O$ to yield 50 mg Cd kg^{-1} soil (treatment Sw+Cd).
5. PO_4^{3-} fertiliser ($Na_4P_2O_7.10H_2O$) was added to soil at 2.914 g^{-1} kg^{-1} soil, equivalent to 250 kg P ha^{-1} (treatment P).
6. P-amended soils were supplemented with analytical reagent grade $CdCl_2.H_2O$ to yield 50 mg Cd kg^{-1} soil (treatment 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 the addition of distilled water.

Sampling and analysis

Samples (5 g) were taken at 7, 14, 28, 56 and 112 days using a cork borer and the samples were passed through a 2-mm sieve. Samples were analysed for their β-galactosidase activity (C cycle), urease activity (N cycle), *N*-acetylglucosaminidase (NAGase) activity (C and N cycle), acid and alkaline phosphomonoesterase activities (P cycle) and arylsulphatase activity (S cycle) by the methods of Naseby and Lynch (1997a).

Soil samples were analysed for water-soluble Cd contents by adding 9 ml water to 1 g soil in a 10-ml centrifuge tube. The soil suspensions were mixed for 1 h on a carousel rotor before being centrifuged at 4,000 *g* for 15 min. The supernatant was decanted off into clean test tubes and kept at 4°C until required on the same day. A Pye Unicam SP9 atomic absorption spectrophotometer was used to determine the concentrations of Cd in samples of the supernatant fluid.

A soil sample (1 g) from each replicate was added to 9 ml sterile quarter-strength Ringers solution; a tenfold dilution series of each soil sample was then used for the enumeration of the following microbial populations. Filamentous fungal populations were quantified on 10% malt extract agar containing 50 mg rose Bengal kg^{-1}. Plates were incubated at 20°C for 7 days before enumeration. The P1 medium of Katoh and Itoh (1983) was used for the enumeration of indigenous, fluorescent *Pseudomonas*. P1 plates were incubated at 25°C and enumerated after 5 days of growth. Tryptone soy agar (10%) was used for the enumeration of total culturable bacterial populations after incubation at 25°C for 7 days.

Table 2 Changes in water-available Cd in a sandy soil as affected by Sw and PO_4^{3-} fertiliser amendments. Significant differences between treatments at each time point ($P<0.05$ level) indicated by *different letters*. *Control* Non-Sw and non-PO_4^{3-} amended, *Cd* supplemented with 50 mg $CdCl_2.H_2O$ kg^{-1}, *Sw* Sw alone, *Sw+Cd* Sw supplemented with 50 mg $CdCl_2.H_2O$ kg^{-1}, *P* PO_4^{3-} fertiliser alone, *P+Cd* PO_4^{3-} fertiliser supplemented with 50 mg $CdCl_2.H_2O$ kg^{-1}

Incubation time (days)	Control	Cd	Sw	Sw+Cd	P	P+Cd
	Cd (mg kg^{-1})					
7	0.00f	39.00a	0.16e	23.97b	2.03d	12.20c
14	0.00f	34.11a	0.15e	13.49c	2.13d	25.08b
28	0.00f	30.98a	0.91e	10.76c	1.47d	22.36b
56	0.00f	28.38a	1.25d	8.94c	0.31e	20.16b
112	0.04f	26.49a	0.84d	7.08c	0.12e	14.09b
$LSD_{0.05}$	1,633					

Statistical analysis

Treatments (raw data) were compared by ANOVA and least square difference ($P<0.05$). The relationships between variables were investigated using the Spearman correlation coefficient followed by a test of significance. All statistical analyses were conducted with SPSS for windows (SPSS).

Results and discussion

Available Cd

Available Cd decreased with incubation time in all treatments (Table 2). Available Cd was reduced by the sludge and PO_4^{3-} additions resulting in the following significant differences in Cd availability: treatments Cd>P+Cd >Sw+Cd. The reduced availability of Cd with the addition of Sw is not surprising, as Dar (1996) also found that Sw reduced Cd availability and the primary cause of this effect was the formation of organic complexes of the Cd with the organic matter.

The cause of the reduction in Cd availability by the addition of PO_4^{3-} fertiliser is more deceptive. The mechanisms of this effect are described by Bolan et al. (1999), who also found a decrease in Cd availability with PO_4^{3-} fertiliser application. They concluded that specific sorption of PO_4^{3-} to soil particles leads to an increase in negative charge which results in an increase in Cd sorption to soil particles.

The initial large reduction in available Cd at 7 days in the P+Cd treatment, followed by an increase in Cd availability at 14 days, is the result of successive mechanisms. Firstly the initial addition of P provides a large sink of negative charge on the soil particles, thus making a large amount of the added Cd unavailable. Secondly the Cd sink is reduced over time by microbial P desorption, causing an increase in available Cd, which subsequently falls over time (from 28 to 112 days) as it is slowly rendered unavailable by adsorption to the soil and incorporation into the biomass/organic matter. Landi et al. (2000) also found a reduction in Cd availability over time through adsorption to the soil and accumulation into the biomass; however, this was over a much smaller time period without the addition of P.

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).

Phosphomonoesterases are important agronomically because they catalyse the hydrolysis of organic P to inorganic P, which can be assimilated by plants.

Acid phosphomonoesterase activity increased with time until the last sampling and was significantly reduced by the addition of Cd at the beginning of the incubation. Addition of Cd in the presence of Sw and P treatments reduced the acid phosphomonoesterase activity with respect to the Sw and P treatments (Table 3). By the end of the incubation period, Cd caused significant reductions in acid phosphomonoesterase activities

Table 3 Acid and alkaline phosphomonoesterase activities in a sandy soil as affected by Sw and PO_4^{3-} fertiliser amendments. Significant differences between treatments at each time point ($P<0.05$ level) indicated by *different letters*. *pNP* *p*-Nitrophenol, *Acid phos.* acid phosphomonoesterase, *Alk phos.* alkaline phosphomonoesterase; for other abbreviations, see Table 2

Enzyme	Class/EC no.	Incubation time (days)	Control	Cd	Sw	Sw+Cd	P	P+Cd
			(mg pNP h^{-1} g^{-1} dry soil)					
Acid phos.	3.1.3.2.	7	2.24d	1.42f	5.74a	2.91c	4.34b	1.88e
		14	2.91d	1.95f	6.67a	3.21c	5.71b	2.35e
		28	2.86d	1.24f	7.95a	3.02c	5.94b	2.16e
		56	2.82d	0.93f	9.51a	2.90c	6.13b	1.92e
		112	2.04d	0.53f	13.07a	2.51c	5.96b	1.46e
$LSD_{0.05}$			0.0516					
Alk phos.	3.1.3.1.	7	0.76d	0.51e	1.80a	1.02b	0.96c	0.76d
		14	0.76e	0.48f	6.83a	2.75c	3.90b	1.41d
		28	0.93e	0.34f	5.41a	1.97b	1.83c	1.24d
		56	0.81e	0.40f	6.07a	2.15c	2.75b	1.36d
		112	0.72e	0.12f	4.12a	1.77b	1.16c	0.76d
$LSD_{0.05}$			0.0365					

Table 4 Arylsulphatase and β-galactosidase activities in a sandy soil as affected by Sw and PO_4^{3-} fertiliser amendments. Significant differences between treatments at each time point ($P<0.05$ level) indicated by *different letters.* For abbreviations, see Table 2

Enyzme	Class/EC no.	Incubation time (days)	Control	Cd	Sw	Sw+Cd	P	P+Cd
			(mg pNP h^{-1} g^{-1} dry soil)					
Arylsulphatase	3.1.6.1.	7	0.16e	0.27d	0.49b	0.35c	0.65a	0.29d
		14	0.18f	0.23e	0.56b	0.38c	1.04a	0.30d
		28	0.36c	0.20e	0.43b	0.28d	0.95a	0.21e
		56	0.33c	0.14f	0.40b	0.22d	0.77a	0.17e
		112	0.31c	0.09e	0.39b	0.20d	0.69a	0.11e
$LSD_{0.05}$			0.0292					
β-Galactosidase	3.2.1.23.	7	0.26c	0.30b	0.38a	0.22d	0.38a	0.30b
		14	0.33c	0.19f	0.48b	0.29d	0.53a	0.26e
		28	0.31b	0.11e	0.31b	0.24c	0.40a	0.19d
		56	0.25c	0.09f	0.29b	0.19d	0.36a	0.12e
		112	0.18c	0.07e	0.23b	0.15d	0.30a	0.09e
$LSD_{0.05}$			0.0253					

($P<0.05$), whilst, Sw and P amendments in the absence of Cd resulted in significantly greater acid phosphomonoesterase activity than all other treatments. This contradicts the results of Moreno et al. (1999), who reported an increase in acid phosphomonoesterase activity caused by amendment with Sw containing high levels of Cd. The correlation coefficient between the activity of acid phosphomonoesterase and available Cd in this study was –0.531 ($P<0.01$).

Alkaline phosphomonoesterase activities were significantly reduced by the addition 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 phosphomonoesterase activity than in all other treatments ($P<0.05$). The activity of this enzyme was negatively correlated with available Cd ($r=–0.438$, $P<0.01$). However, it must be noted that the contrasting effects of inhibition caused by heavy metals and the stimulation caused by organic matter addition in the same systems are difficult to interpret (Nannipieri 1994).

Arylsulphatase is the enzyme involved in the hydrolysis of arylsulphate esters by fission of the O-S bond. This enzyme is believed to be involved in the mineralisation of ester sulphate in soils (Tabatabai 1994). All treatments with Cd had significantly greater arylsulphatase activities than the control at the beginning of the incubation ($P<0.05$), whereas the same treatments at the end of the incubation period showed significantly lower arylsulphatase activities than the control (Table 4), due to a decrease in the activity in the respective treatments and an increase in the activity in the control. The addition of P resulted in significantly greater arylsulphatase activity than in the Sw treatment. A significant negative correlation was found between arylsulphatase activity and available Cd ($r=–0.430$, $P<0.01$).

β-galactosidase catalyses the hydrolysis of β-galactosidase bonds and thus the breakdown of complex carbohydrates and important constituents of organic residues such as Sw, crop remains, animal manure, and 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 with the addition of Sw+Cd. The addition of P resulted in significantly greater β-galactosidase activity than all other amendments 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 the enzyme responsible for the hydrolysis of urea to NH_3, which can be assimilated by microbes and plants. The Sw 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, in the Sw+Cd treatment urease activity was significantly greater than in the Sw treatment at the end of the incubation. The Cd and P+Cd treatments also resulted in significantly greater urease activities than the controls at the end of the incubation (Table 5). Urease 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 the addition of Sw with a low heavy metal content. After incubation, urease activity increased in soil amended with the high dose of sludge.

NAGase releases *N*-acetylglucosamine subunits from chitin polymers, components of fungal cell walls. NAGase activity has been correlated to fungal biomass (Miller et al. 1998); however, 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 amendment significantly reduced the NAGase activity (Table 5). At the end of the incubation period, the Cd and P+Cd treatments had significantly

Table 5 Urease and *N*-acetylglucosaminidase (*NAGase*) activities in a sandy soil as affected by Sw and PO_4^{3-} fertiliser amendments. Significant differences between treatments at each time point ($P<0.05$ level) indicated by *different letters*. For other abbreviations, see Tables 2 and 3

Class/EC no.	Incubation time (days)	Control	Cd	Sw	Sw+Cd	P	P+Cd
		Urease (mg NH_3 released h^{-1} g^{-1} dry soil)					
3.5.1.5.	7	73.00f	99.30e	856.00b	873.00a	169.00c	129.00d
	14	62.80f	67.50e	1,132.30b	1,226.00a	136.50c	90.30d
	28	42.20e	71.00d	1,345.70b	1,373.70a	119.00c	75.00d
	56	38.50f	65.00e	1,116.00b	1,210.00a	100.95c	70.17d
	112	26.30f	60.30e	1,013.00b	1,134.30a	103.70c	67.10d
$LSD_{0.05}$		4.003					
		NAGase (mg pNP released $h^{-1}g^{-1}$ dry soil)					
3.2.1.50	7	0.49d	0.67c	0.71b	0.95a	0.47d	0.35e
	14	0.37d	0.51c	0.64a	0.56b	0.32e	0.27f
	28	0.15d	0.10e	0.41a	0.20c	0.23b	0.11e
	56	0.13d	0.07e	0.35a	0.17c	0.22b	0.09e
	112	0.11d	0.01e	0.29a	0.14c	0.21b	0.01e
$LSD_{0.05}$		0.0268					

Table 6 Log total soil bacterial, pseudomonads and fungal populations as affected by Sw and PO_4^{3-} fertiliser amendments. Significant differences between treatments at each time point ($P<0.05$ level) indicated by *different letters*. *CFU* Colony-forming units; for other abbreviations, see Table 2

Populations	Incubation time (days)	Control	Sw	Sw+Cd	P	P+Cd	Cd
		(CFU g^{-1} dry soil)					
Total bacteria	7	7.48b	7.01e	7.94a	7.32c	7.30c	7.17d
	14	7.38b	7.04d	7.67a	7.27c	7.26c	6.88d
	28	7.32b	6.90d	7.63a	7.27c	7.27c	7.00d
	56	7.25b	6.85e	7.59a	7.19c	7.22c	7.00d
	112	7.17b	6.79f	7.59a	7.12c	7.06d	6.93e
$LSD_{0.05}$		0.0357					
Total pseudomonads	7	5.88c	5.66d	6.97a	5.93c	6.04b	5.52e
	14	5.47e	5.66d	6.86a	6.32b	5.83c	5.12f
	28	5.12d	4.52e	6.52a	6.12b	5.66c	5.10d
	56	5.07d	4.36e	6.25a	6.00b	5.27c	5.10d
	112	5.00d	4.12e	5.98a	5.14b	5.10b	5.06c
$LSD_{0.05}$		0.0588					
		Control	Cd	Sw	Sw+Cd	P	P+Cd
Total fungi	7	5.03d	5.12c	5.23b	5.21b	5.33a	5.19b
	14	4.95d	5.00c	5.25a	5.07b	5.02c	4.96d
	28	4.70e	4.67e	5.23a	5.06b	4.97c	4.78d
	56	4.52d	4.42e	5.12a	4.75b	4.60c	4.53d
	112	4.40d	4.21e	4.61b	5.00c	4.52a	4.41d
$LSD_{0.05}$		0.0476					

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 Sw alone 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 Sw therefore had a direct effect on the bacterial numbers, 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 Cd had a detrimental effect on the bacterial population.

The supposition that Cd addition had a detrimental effect on the bacterial community is supported by the fact that a significant negative correlation was found between the total culturable bacterial population and available Cd ($r=-0.604$, $P<0.001$). 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 available Cd found in PO_4^{3-}-treated soil, where an initially large bacterial population on 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 mg kg^{-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-contaminated Sw.

The total number of bacteria was significantly and positively correlated with the soil enzyme activities. The correlation coefficient was greatest for acid phosphomonoesterase (r=0.692, P<0.001), followed by alkaline phosphomonoesterase (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 Sw alone in comparison to the control (Table 6) (P<0.05). However, the soil pseudomonad populations were not significantly affected by P treatments with and without Cd. Therefore, Sw alone increased the soil pseudomonad populations, whilst the addition of Cd alone decreased the pseudomonad population throughout the incubation period.

There were no significant correlations between the total soil pseudomonad populations and available Cd. However, the total soil pseudomonad 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 phosphomonoesterase (r=0.652, P<0.001), β-galactosidase (r=0.644, P<0.001) and acid phosphomonoesterase (r=0.568, P<0.01). However, it must be noted that culturable populations are not a true measure of microbial biomass due to the problems that culturability presents [for instance, the high proportion of non-culturable cells in soil (Colwell et al. 1985)].

The total fungal populations significantly decreased with time in all treatments. The addition of Sw and PO_4^{3-} fertilisers with and without Cd significantly increased the numbers of fungi at the start of the incubation (Table 6) (P<0.05). However, after 7 days of incubation, the addition of P with Cd resulted in a similar value to that of the control. Culturable fungal populations were not correlated to the levels of available Cd. However, significant positive correlations were found between the total fungal populations and the soil enzyme activities. The correlation coefficient was 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 phosphomonoesterase (r=0.446, P<0.01) and acid phosphomonoesterase (r=0.409, P<0.01). The correlation coefficients indicate that the enzyme activities correspond closely with the microbial structure in soil.

Cd addition had dramatic effects on most of the properties studied. The negative correlations between the available Cd and β-galactosidase, arylsulphatase, acid and alkaline phosphates and total bacterial populations indicate that Cd contamination has a large detrimental effect on nutrient cycling and microbial activity. In general, the organic matter added with the Sw had a positive effect on the enzymatic activities, which, in some cases, counteracted the negative effect that a high level of Cd contamination might have had on them.

Many of the effects of Cd were reduced by the Sw and PO_4^{3-} fertiliser amendments. Therefore, reducing the input of PO_4^{3-} fertilisers and Sw 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 Sw/organic matter amendments, which are low in pollutants, on a limited basis. For example, if 80% of Cd added to soils each year remains in the topsoil (Taylor 1997), the addition of P or OM resulting in less than a 20% increase in the soil Cd content will eventually result in a reduction of Cd in the soil. This will also reduce the availability of Cd resulting in the soil being less toxic and less Cd being sequestered by 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 of soil stress or health and quality.

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