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Influence of cadmium on the metabolic quotient, L-:D-glutamic acid respiration ratio and enzyme activity:microbial biomass ratio under laboratory conditions

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Abstract This study was carried out to investigate the effect of very high cadmium concentrations (50 and 500 μg Cd g^{-1} soil) on some biochemical and microbiological measurements under laboratory conditions involving daily soil samplings. The data for both DTPA- and water-soluble Cd showed two distinctive patterns during soil incubation; from 0 to 4 days, values were about 50–500 and 1–100 μg g^{-1} dry weight soil, whereas they decreased markedly after 7 days. Both daily respiration and the ATP content but not the microbial biomass C determined by the fumigation–extraction method were lowered by high DTPA- and water-soluble Cd concentrations. Dehydrogenase and phosphatase activities as well as both enzyme activity:microbial biomass ratios were decreased by the high DTPA- and water-soluble Cd concentrations. In the first 2 days of incubation, the metabolic quotient (qCO_2) was also decreased by the highest values of available Cd. The early (after 6 h) mineralization of L- but not D-glutamic acid to CO_2 was inhibited during the 0–4 day incubation period by the highest Cd concentration. Possibly the L-enantiomer was used by a larger fraction of soil microorganisms than the D-enantiomer or, if they were used by the same fraction of soil microorganisms, the D-enantiomer was mineralized at a lower rate. The L-:D-glutamic acid respiration ratio was decreased by the high available Cd content because under polluted conditions soil microorganisms probably discriminated less between the two stereoisomers of glutamic acid.

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Introduction

Cadmium is characterized by a relatively high environmental mobility so that it can easily be transferred to plants and humans. It was the most toxic heavy metal when the effects of Cd, Zn, Cu and Pb on both nitrification and denitrification activities in soil were compared (Babich and Stotzky 1985). However, a better understanding of its effect on the microbiological activity of soil is required. There is a general consensus that soil microbiological activity may hold potential as an early and sensitive indicator of stress in soil quality (Brookes 1995). The main reasons are that soil microbes react quickly to stressful factors and that they are evenly distributed in the soil.

Investigations on the effect of heavy metals on microbial activity and the composition of the microbial community are generally based on measurements of microbial biomass, composition of phospholipids, whole soil fatty acid profiles, enzyme activities, respiration, nitrification and mineralization of added substrates caused by particular microbial species (Hattori 1989; Frostegård et al. 1993; Nannipieri 1994; Brookes 1995; Leita et al. 1995; Giller et al. 1998).

The soil microbial biomass can be accurately estimated by different methods such as the adenosine 5′-triphosphate (ATP) content, substrate-induced respiration and the fumigation–extraction method; it is affected by heavy metal exposure under field conditions (Brookes 1995). However, biomass and activity of the soil microbial communities are not the same in principle and early effects on microbial processes may be detected by measurements of microbiological activities rather than by the determination of the microbial biomass content. Indeed some of the microbial processes can be negatively affected by heavy metal exposure, al-

though these negative effects do not necessarily cause microbial death and consequently a decrease in microbial biomass. Microbiological activity is a complex picture of metabolic processes and thus it cannot be evaluated by measuring a single parameter but instead requires the simultaneous determination of more parameters (Nannipieri et al. 1990). However, it is not feasible to measure many parameters and a selection needs to be made by choosing the parameters that are the most sensitive. Another problem is to find indices combining various measured parameters to quantify changes in soil quality. The ratio between two measured parameters represents an easy combination of two different measurements in a single criterion which can give some indications on changes occurring in microbiological activity.

Anderson and Domsch (1990) proposed two ecophysiological parameters: qCO_2 (the metabolic quotient: which is the ratio between the respiration and the microbial biomass) and qD (the C-loss quotient based on microbial-C loss determined weekly), for studying the effect of pollution on microbial energy demand and microbial biomass-C loss. The qCO_2 has been considered a good index of heavy metal-induced stress in soil (Brookes 1995) because high qCO_2 values were found in long-term contaminated soils (Brookes et al. 1986). However, lower qCO_2 values have been also found in metal-contaminated soils (Bââth et al. 1991; Insam et al. 1996) probably as the result of the utilization of different substrates by microorganisms of polluted soils than those used by microorganisms of unpolluted soils (Insam et al. 1996). A decrease in qCO_2, especially in Cd-polluted soils, was observed under laboratory and field conditions (Palmborg and Nordgren 1996). Both qCO_2 and qD values were increased in heavy metal-polluted soils under laboratory conditions (Leita et al. 1995). Addition of heavy metals to soils can kill part of the soil microbial biomass and the following increased respiration may be due to the decomposition of killed microbial cells by the surviving microorganisms.

The substrate induced respiration (SIR) of different soils amended with some amino acid isomers was significantly higher for L-isomers than for D-isomers (Hopkins and Ferguson 1994). This depends on the fact that L-amino acids are more abundant than D-amino acids as constituents of microbial polymers. The most common D-isomers, D-alanine and D-glutamic acid, are present in bacterial cell walls. It was proposed that the L-:D-amino acid respiration ratio (L:D ratio) is a sensitive parameter of microbial stress in soil because it decreased when qCO_2 increased under acidic conditions (Hopkins et al. 1997). Under such conditions soil microorganisms probably discriminated between amino acid enantiomers to a lesser extent than non-stressed microorganisms as a response to a greater demand for energy supplier substrates.

Information on the relative toxicity of different metals on microbial activity and soil microflora composition can be obtained by short-term ecotoxicological laboratory studies. According to Giller et al. (1998) these studies provide insights on microbial processes and biodiversity of soil microflora population but they are not comparable to the field situation where the metal exposure persists with time and usually there is a gradual increase in the soil metal concentration. In addition, under field conditions the polluted soil is often contaminated with more than one metal. Studies involving the incorporation of heavy metaL-polluted sewage sludge into soil are problematic because it is difficult to separate the adverse effect of heavy metals from the stimulatory effect of organic compounds on microbial activity (Brendecke et al. 1993; Witter et al. 1993).

The aim of this study was to investigate the toxicity of Cd on several microbiological and biochemical parameters under laboratory conditions with unusually high Cd concentrations so as to artificially stress the soil microbial community. Very high Cd concentrations were used because a previous experiment under the same laboratory conditions showed that no changes were induced in the biomass and microbiological activity of different soil by the addition of lower Cd concentrations (Landi et al. 1997). A comparison of Cd effects on qCO_2, the L-:D-glutamic acid respiration ratio and the enzyme:microbial biomass ratio was carried out to assess the sensitivity of these ratios to the heavy metal. Among the amino acids we chose glutamic acid for its central role in microbial metabolism and because its mineralization was found to be retarded in heavy metal-polluted soils (Nordgren et al. 1988).

Materials and methods

Soil used and soil treatment

A forest soil was surface sampled (0–10 cm) from Fregene (20 km from Rome), sieved (2 mm) and stored moist in plastic bags at 4 °C. The soil had the following characteristics: 6.7% meq/100 g CEC, 2.27% organic C, 0.09% total N, 4.8 pH (H_2O), 87% sand, 8% silt and 5% clay. Before treatment, the soil sample was kept at 25 °C for 4 days to reduce the effects of sampling and storage disturbance. Moist soil samples (corresponding to 100 g d.w. soil) were treated with distilled water (control) or $CdSO_4$ solution to give 0, 50 or 500 μg Cd g^{-1} d.w. soil and 50% of the maximum water-holding capacity (WHC). The addition of the Cd solution did not change the soil pH. Soils were then incubated at 25 °C for 0, 1, 2, 3, 4, 7, 14, 21 and 28 days, in sealed 1-l flasks equipped with two vials containing 4 ml 1 M NaOH to trap the evolved CO_2 and one with 5 ml of water to prevent soil desiccation, respectively. Flasks incubated for a period longer than 7 days were aerated weekly to ensure a sufficient oxygen supply and the NaOH solution was removed, titrated and replaced by a fresh one. At each sampling time, the soil (100 g) was split into subsamples for the different analyses.

Glutamic acid mineralization

Soil subsamples (equivalent to 20 g d.w. soil) were placed in plastic beakers and amended with a fine powder of 2 mg of L- or D-glutamic acid and 0.5 mg of talc (inert carrier) g^{-1} d.w. soil; the amount of added substrate was that inducing the maximum rate of respiration. The soil was mixed thoroughly with a spatula so as to ensure an even distribution of the amendment. The plastic

beakers were placed in 1-l flasks containing two vials, one with 1 M NaOH to adsorb the evolved CO_2 and the other one with distilled water. The flasks were closed, incubated at 25 °C and the evolved CO_2 determined after 6 and 24 h. The amount of mineralized amino acid at 6 and 24 h was calculated by subtracting the value of basal respiration assuming that the addition of the amino acid did not influence the decomposition of native soil organic matter.

Measurements of microbial biomass, respiration, ATP content and enzyme activities

Microbial biomass-C was determined by the fumigation–extraction (FE) method (Vance et al. 1987). Respiration was determined following the procedure by Stotzky (1965) after trapping CO_2 in 1 M NaOH, precipitation of carbonates was with 0.375 M $BaCl_2$ and automated titration with 0.1 M HCl. All samples were corrected for the CO_2 content of blanks. The ATP content of soil was determined by the firefly luciferin–luciferase enzyme assay as described by Ciardi and Nannipieri (1990). Respiration of L- and D-glutamic acid was determined as reported by Anderson and Domsch (1978). Phosphatase and dehydrogenase activities were measured as reported by Tabatabai and Bremner (1969) and von Mersi and Schinner (1991), respectively.

Chemical analyses

Water- and DTPA-soluble Cd were determined in the following way: 20 g soil was shaken with 100 ml of water or DTPA-TEA solution (pH 7.3) according to Lindsay and Norwell (1978), for 2 h; the mixture was then centrifuged (5000 rev min^{-1}) and the supernatant filtered through Whatman 42 filter paper. Cd concentrations were measured by atomic absorption spectroscopy (Perkin-Elmer 5500). The soil pH was measured potentiometrically in water (1:2.5 w:v).

Statistical analysis

The data reported are mean values of three replicates with relative standard deviations. LSD analysis was also carried out, followed by the Tukey test (0.5 level) in order to assess the significance of the differences.

Results and discussion

The available Cd, K_2SO_4 extractable organic C, L- and D-glutamic acid respiration showed a distinctive behaviour when data for days 0–4 and days 7–28 were compared. Differences between values within each period were generally not significant whereas they were significant between the two periods. For this reason the percentage of available Cd with respect to the amount added to soil (Table 1), K_2SO_4 extractable organic C (Table 2), and L- and D-glutamic acid respiration (Table 4) were averaged in the two periods considered.

Cd availability

The amount of the total Cd added to soil extracted by DTPA was higher than the respective amount extracted by water (Table 1). However, both the fractions showed similar patterns as the water-soluble and DTPA-extractable Cd markedly decreased after 4 days. This was probably due to adsorbtion of Cd by mineral and organic soil colloids, accumulation by microorganisms or precipitation as insoluble compounds.

Table 1 Average percentage of the available Cd respect to the total metal added to the soil and extracted by either DTPA or water in the 0–4 or 7–28 day incubation periods

Incubation time (days)	DTPA-extractable Cd (%) Treatments		Water-soluble Cd (%) Treatments	
	50 μg Cd g^{-1}	500 μg Cd g^{-1}	50 μg Cd g^{-1}	500 μg Cd g^{-1}
0–4	100 ± 1.2[a]	100 ± 2.5	2 ± 0.2	20 ± 5.6
7–28	5 ± 2.3	5 ± 1.3	ND[b]	2 ± 0.4

[a] Standard deviation
[b] ND = below the detection limit

Extraction of Cd from soil either by water or by DTPA is thought to give an estimate of the plant-available Cd (Haq et al. 1980) and also of the heavy metal fraction potentially affecting the soil microflora. In particular, the use of DTPA mimics that of either plants or microorganisms, because it forms chelates with the free metal in solution, lowering its ionic activity so that an additional metal quantity can be released from the soil until equilibrium is obtained (Adriano 1986). The use of DTPA is not recommended in very acidic soils (Norwell 1984), unless it is buffered as it was here at pH 7.3 (Lindsay and Norwell 1978). Inaccurate extraction may occur in Cd-enriched soil when the metal concentrations exceed the capacity of DTPA to chelate Cd. This was not the case because DTPA was capable of extracting 100% of the 500 μg g^{-1} Cd added to soil on day 0.

Basal respiration, microbial biomass-C and qCO_2

The addition of 500 but not of 50 μg Cd g^{-1} soil markedly decreased the soil respiration rate after the first day of incubation; the inhibition effect disappeared after 2 days (Fig. 1) before the marked decrease in the amount of water- or DTPA-soluble Cd (Table 1). The presence of Cd might have inhibited the microbial activity and/or caused changes in the composition of soil microflora. Heavy metals added to soils can produce a shift from a prokaryote- toward an eukaryote-dominated population and it is well known that growth and reproductive rate of eukaryotes are lower than those of prokaryotes (Doelman 1985). Thus the decline in the CO_2–C evolution of the Cd-contaminated soil (Fig. 1) could be due to a decrease in the prokaryote population. Hattori (1989) also reported a negative correlation between CO_2 evolution and the content of water-soluble Cd in soil. However, usually no clear trend has been observed between metal contamination and soil respiration in agricultural soils (Giller et al. 1998), whereas negative effects have been reported in forest

Table 2 Average organic C extracted by 0.5 M K_2SO_4 from the unfumigated and fumigated soils in the 0–4 or 7–28 day incubation periods

Incubation time (days)	Unfumigated soils (μg organic C g^{-1} soil) Treatments			Fumigated soils Treatments		
	Control	50 μg Cd g^{-1}	500 μg Cd g^{-1}	Control	50 μg Cd g^{-1}	500 μg Cd g^{-1}
0–4	94.7 (LSD = 13.2)	99.5	112	302.2 (LSD = 29.6)	314.2	303.3
7–28	102.7 (LSD = 19.7)	116.2	101.8	264.7 (LSD = 28.9)	276.5	277.0

soils (Bââth 1989). The contrasting results may be due to the fact that various processes, directly or indirectly involved in soil respiration, can be affected by heavy metals. The toxicants may reduce the substrate availability for respiration by forming complexes with the substrate or by killing microorganisms. In the latter case, higher respiration rates may be due to the respiration of killed microbial cells by surviving microorganisms.

Microbial biomass showed no significant differences between the control and the Cd-contaminated soil throughout (Fig. 1). Generally microbial biomass-C, which usually ranges from 1% to 4% of total soil organic C, is a sensitive indicator of changes in soil conditions (Brookes 1995). Chander and Brookes (1991a) reported that the percentage of organic C present as microbial biomass C decreased from 1.5–1.6% of the unpolluted soil to 0.4–0.7% in Zn- and Cu-polluted soil whose contamination was due to repeated sludge additions. However, more organic C was extracted by K_2SO_4 from unfumigated soils after the Cd addition compared with the control in the 0–4 day incubation period (Table 2). This "extra carbon" may originate from cell death and lysis of the active and/or Cd-sensitive portion of the microbial community occurring in the Cd-amended soils. No differences were found between the fumigated soils (Table 2).

The qCO_2 has been considered to be an index of microbial stress in soil (Anderson and Domsch 1993; Nannipieri et al. 1997), and it was reported to be higher in metal-contaminated than in uncontaminated soils (Chander and Brookes 1991b; Leita et al. 1995). The increase was attributed to an increased amount of organic C metabolized for the maintenance of soil microorganisms followed by a lower amount of organic C incorporation into the microbial biomass (Chander and Brookes 1991b). According to this hypothesis the qCO_2 is a simplistic measurement of the maintenance energy. However, the increase in qCO_2 values of heavy metal-contaminated soils incubated under controlled conditions has been hypothesized to be due to the disturbance effect. Indeed, heavy metals partly kill microbial biomass, resulting in a flush of decomposition of dead microbial cells by the surviving microorganisms (Giller et al. 1998).

We observed a decrease in qCO_2 in the heavily Cd-contaminated soil with the respect to the control in the first 2-day incubation period, whereas no significant differences were found afterwards (Table 3). The observed decrease probably depended on the lower respiration rate during the first 2 days because no decrease in microbial biomass was observed in the same period. Both Bââth et al. (1991) and Hattori (1992) have found lower qCO_2 values in metal-polluted soils. According to Insam et al. (1996), the lower qCO_2 values of metal-polluted soils may depend on the fact that soil microorganisms may utilize different substrates than those used in the unpolluted soils.

ATP content

The ATP content decreased in the most Cd-contaminated soil compared with the control during the 0–4 day incubation period; then it fluctuated throughout regardless of treatment (Fig. 1). The ATP level of soil has been used to evaluate the effect of heavy metals on the soil microbial biomass (Zibilske and Wagner 1982); in this study, negative correlations were found between the heavy metal content and microbial biomass, ATP content or respiration. The different behaviour observed in our work between the ATP content and microbial biomass-C determined by the FE method may be due to the following factors: (1) the ATP technique is more sensitive than the FE method in detecting changes in microbial biomass under laboratory conditions involving daily measurements; and (2) the intracellular ATP concentration is sensitive to physiological changes deriving from reductions in the active metabolism even if the microbial biomass content is unaffected. It has been hypothesized that the ATP is an independent measurement of microbial biomass when soil samples are preincubated under controlled conditions before analysis (Nannipieri et al. 1990). Our data further confirm the need for a better understand of the meaning of ATP measurements when ATP level has been suggested to represent an index of microbial activity (Nannipieri et al. 1990): these are carried out immediately after soil sampling.

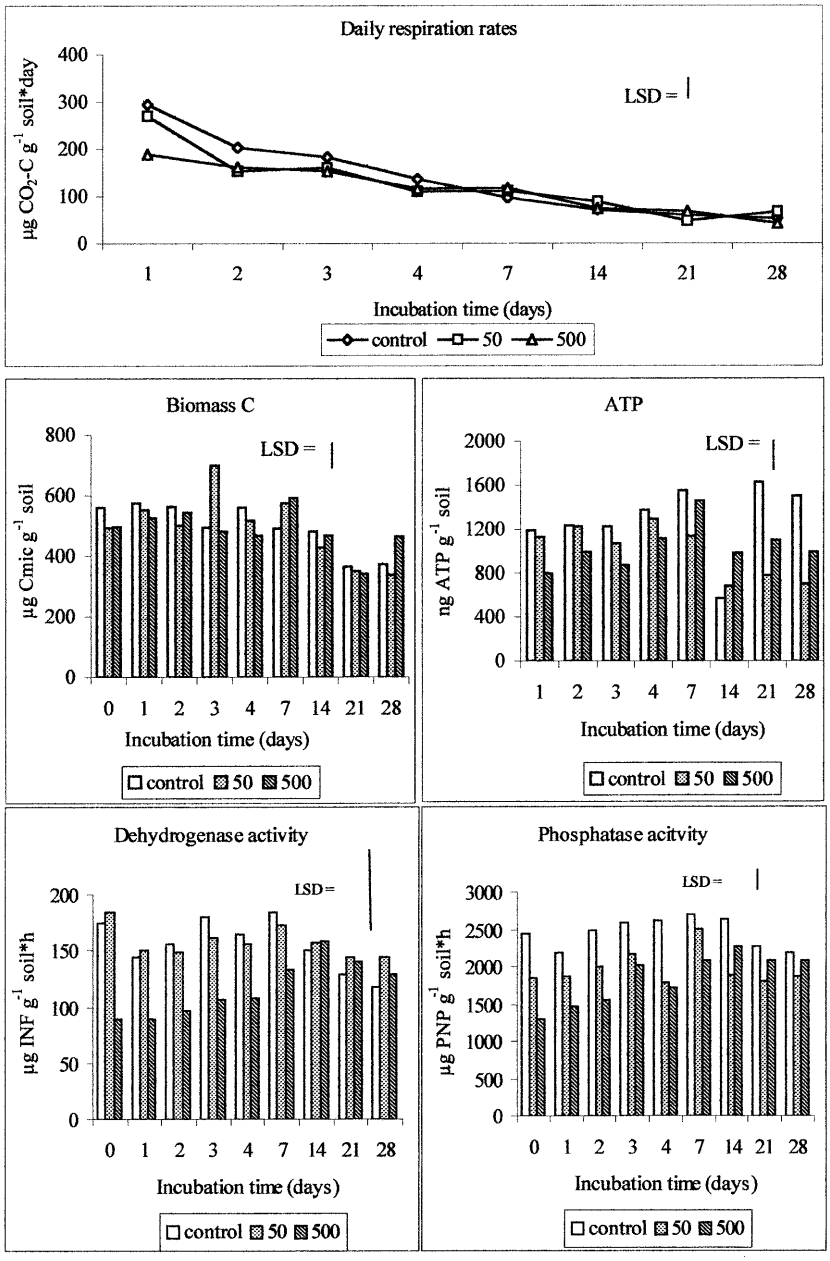

Fig. 1 Cd effects on soil basal respiration, microbial biomass, ATP content and enzymatic activities

The ATP:C_{mic} ratio generally fluctuated around a value of 2 in both Cd-contaminated and non-contaminated soils.

Dehydrogenase and phosphatase activity

A reduction in dehydrogenase activity was only observed at the highest Cd concentration (500 µg g^{-1} soil), during the 0–7 day incubation period (Fig. 1). Dehydrogenase activity is related to a group of intracellular enzymes present in active microorganisms in the soil (Nannipieri et al. 1990). Conversely, the acid phosphatase activity was generally reduced by both Cd concentrations during the whole incubation period (Fig. 1). We cannot explain with the present approach why phosphatase was more sensitive than dehydrogenase activity to the presence of Cd. Both the enzyme activities were inhibited by the highest Cd concentration as showed by the decrease in both activities at day 0. Heavy metals can reduce enzyme activity by interacting with the enzyme–substrate complex, denaturing the enzyme protein or interacting with the protein-active groups (Nannipieri 1994). They can also affect the synthesis of the enzyme by the microbial cells. An indirect effect is also possible because changes in the community structure can modify the enzyme activity (Nannipieri 1994).

Generally the values of dehydrogenase activity:microbial biomass C ratio (DH:B_{mic} ratios) were lower in

Table 3 qCO_2 and ATP:microbial biomass, dehydrogenase activity:microbial biomass and phosphatase activity:microbial biomass ratios under different Cd concentrations

Incubation time (days)	qCO_2 ($\mu g\ CO_2$–C $\mu g\ C_{mic}^{-1}*h^{-1}$) (LSD = 0.01) Treatments			ATP:C_{mic} (ng ATP $\mu g\ C_{mic}^{-1}$) (LSD = 0.79) Treatments			DH:C_{mic} (μg INTF $\mu g\ C_{mic}^{-1}*h^{-1}$) (LSD = 0.08) Treatments			PH:C_{mic} (μg PNP $\mu g\ C_{mic}^{-1}*h^{-1}$) (LSD = 2.2) Treatments		
	Control	50 μg Cd g^{-1}	500 μg Cd g^{-1}	Control	50 μg Cd g^{-1}	500 μg Cd g^{-1}	Control	50 μg Cd g^{-1}	500 μg Cd g^{-1}	Control	50 μg Cd g^{-1}	500 μg Cd g^{-1}
1	0.022	0.023	0.016	1.89	1.93	1.72	0.31	0.38	0.18	4.4	3.8	2.6
2	0.018	0.016	0.014	2.19	2.45	1.83	0.25	0.27	0.17	3.8	3.4	2.8
3	0.013	0.016	0.014	3.06	1.63	1.91	0.28	0.30	0.18	4.4	4.0	2.9
4	0.014	0.008	0.013	2.49	2.50	2.39	0.36	0.23	0.22	5.3	3.1	4.2
7	0.008	0.012	0.013	3.12	2.03	2.47	0.29	0.30	0.23	4.7	3.5	3.7
14	0.006	0.005	0.004	1.51	1.63	2.00	0.38	0.30	0.23	5.5	4.3	3.5
21	0.005	0.005	0.006	4.50	2.21	3.02	0.31	0.37	0.34	5.5	4.4	4.9
28	0.006	0.008	0.005	4.00	2.2	2.25	0.35	0.41	0.41	6.2	5.2	6.1

the most Cd-contaminated soils (Table 3). Also the lowest phosphatase activity per unit of biomass (PH/B_{mic}) was found in the presence of 500 μg Cd g^{-1} soil (Table 3). The differences were reduced at longer incubation times (21–28 days).

Mineralization of glutamic acid isomers and their effect on microbial biomass

The initial respiration response (within 6 h) is attributed to the native microbial biomass while that measured after 24 h could indicate the start of the microbial growth phase (Sparling 1995).

The averages of the early (6 h) L- or D-isomer mineralization to CO_2–C were lower in the polluted soils collected during the 0–4 day incubation period, but the only significant difference was observed for the highest pollution level in the case of L-glutamic acid mineralization (Table 4). The average values of the 7–28 day incubation period measured were not influenced by the Cd after 6 or 24 h (Table 4). We hypothesize that Cd did not affect the mineralization of either L- or D-glutamic acid to CO_2–C when active microbial growth started (after 24 h). The early (after 6 h) inhibitory effect on L- or D-glutamic acid mineralization to CO_2–C was only observed in soils sampled in the 0–4 day incubation period, that is when DTPA- and water-extractable Cd were 100% and 2–20% of the Cd amounts initially added to soil, respectively (Table 1). Probably, in the soils sampled from 7 to 28 days the amount of DTPA- and water-extractable Cd were too low to inhibit the early (after 6 h) mineralization of L-glutamic acid. Hattori (1992) showed that the early mineralization of substrates added to soil is more sensitive to the metal presence than later measurements. The addition of metals prolonged the lag phase before the onset of the apparent exponential growth when glutamic acid was added to soil (Doelman and Haanstra 1979; Nordgren et al. 1988).

The amount of CO_2–C evolved was significantly higher from L-glutamic acid than from the corresponding D-isomer. These results agree with those reported by Hopkins and Ferguson (1994) and may possibly de-

Table 4 Average mineralization of L- and D-glutamic acid in Cd-polluted and unpolluted soils measured after 6 and 24 h on samples collected in the 0–4 or 7–28 day periods

Incubation time (days)	L-Glutamic acid ($\mu g\ CO_2$–C g^{-1} soil) Treatments			D-Glutamic acid Treatments		
	Control	50 μg Cd g^{-1}	500 μg Cd g^{-1}	Control	50 μg Cd g^{-1}	500 μg Cd g^{-1}
After 6 h						
0–4 (LSD = 20.8)	98.6	79.5	41.6	35.5 (LSD = 13.9)	33.3	22.6
7–28 (LSD = 22.6)	78.2	69.4	77.0	36.7 (LSD = 15.7)	46.4	51.5
After 24 h						
0–4 (LSD = 18.4)	549.2	491.0	411.2	329.0 (LSD = 16.3)	331.5	302.5
7–28 (LSD = 86.5)	507.8	504.2	517.7	361.6 (LSD = 59.6)	382.5	431.7

Table 5 L-:D-glutamic acid respiration ratio of soils

Incubation time (days)	After 6 h Treatments			After 24 h Treatments		
	Control	50 μg Cd g^{-1}	500 μg Cd g^{-1}	Control	50 μg Cd g^{-1}	500 μg Cd g^{-1}
0	2.9	2.5	1.7	2.3	1.7	1.2
1	3.0	2.6	1.7	2.1	1.7	1.2
2	3.1	2.6	2.1	1.6	1.6	1.0
3	2.8	2.6	2.2	1.6	1.6	1.9
4	2.9	2.4	1.6	1.7	1.3	1.6
7	2.8	1.4	1.7	1.7	2.0	1.5
14	2.6	2.0	1.3	1.4	1.1	0.9
21	2.5	1.8	1.8	1.2	1.2	1.3
28	1.8	1.4	1.4	1.5	1.3	1.1
	LSD = (0.87)			LSD = (0.68)		

pend on the higher range of microorganisms utilizing L-amino acids in soil. It was also suggested, alternatively, that both enantiomers were utilized by a similar fraction of the microbial community but at different rates (Hopkins and Ferguson 1994).

Hopkins et al. (1997) hypothesized that under stressful conditions soil microorganisms are less discriminative between the two stereoisomeric forms of amino acids. They found that the L:D respiration ratio of a range of amino acids decreased when the microbial biomass was stressed by low soil pH. Our L:D respiration ratios of glutamic acid measured after 6 and 24 h after the amino acid addition to soils are presented in Table 5. No clear trend was observed in the ratio values measured after 24 h because it fluctuated for the same treatment throughout. The highest Cd contamination significantly decreased the values of the ratio with respect to the control, during the 0–14 day incubation period, when respiration was measured after 6 h.

Cd-contaminated soils also showed lower microbial biomass C content than the control, when microbial growth was induced by the L-glutamic acid addition to soils sampled during the 0–4 day period. No clear trend was observed at longer incubation times (Table 6). Possibly the presence of a high amount of available Cd decreased the microbial growth induced by the L- and D-glutamic acid. Later, when the available Cd was decreased (Table 1), the inhibitory effect disappeared. Generally the addition of the D-isomer induced a lower microbial growth than the L-isomer treatment (Table 6). As mentioned above, this may be explained by the two hypotheses suggested by Hopkins and Ferguson (1994). It is likely that the soil microflora must isomerize the D-isomer before its incorporation and presumably this process takes time, with a consequent delay in D-glutamic acid-induced growth.

Conclusions

Both the daily respiration rate and the ATP content, but not the microbial biomass C, determined by the FE method, were sensitive to high DTPA- and water-soluble Cd. A better understanding of these results requires further research on the meaning of ATP measurements as an indicator of microbial biomass or microbial activity when soils are analysed immediately after sampling. Dehydrogenase and phosphatase activities

Table 6 Microbial biomass C measured after 24 h in samples treated with L- or D-glutamic acid

Incubation time (days)	L-Glutamic acid (μg C_{mic} g^{-1} soil) Treatments			D-Glutamic acid Treatments		
	Control	50 μg Cd g^{-1}	500 μg Cd g^{-1}	Control	50 μg Cd g^{-1}	500 μg Cd g^{-1}
0	892	799	534	692	535	431
1	751	656	488	496	494	434
2	691	644	544	531	548	560
3	797	667	757	556	482	481
4	691	541	569	466	446	348
7	548	703	757	353	426	547
14	686	577	573	500	530	604
21	548	586	574	482	456	447
28	590	444	443	357	361	405
	(LSD = 161)			(LSD = 139)		

and their respective enzyme activity:microbial biomass ratios were also decreased by the high concentration of Cd available. The decrease in both enzyme activities could be due to enzyme inhibition, inhibition of their synthesis as well as changes in the composition of soil microflora. Decreases in qCO_2, observed under laboratory conditions, could indicate disturbance rather than microbial stress. The early (after 6 h) mineralization of L- but not of D-glutamic acid confirmed that the heavy metal pollution increased the lag time in amino acid mineralization (Nordgren et al. 1988). The L:D respiration ratio measured after 6 h was sensitive to high Cd concentrations. However, further research is needed to evaluate the early response of the L-:D-glutamic acid respiration ratio. It remains to be clarified whether the response of the ratio is due to the fact that microorganisms were less likely to discriminate between the two stereoisomer forms of amino acids under stress or to changes in the composition of soil microbial communities.

Caution is also required in extending data from laboratory experiments to the in situ situation because the latter should involve responses of adapted communities whereas generally the former concerns the community resistance and resilience after acute stress.

References

Adriano DC (1986) Trace elements in the terrestrial environment. Springer, Berlin Heidelberg New York

Anderson JPE, Domsch KH (1978) A physiological method for the quantitative measurement of microbial biomass in soil. Soil Biol Biochem 10:215–221

Anderson TH, Domsch KH (1990) Applicaton of eco-physiological quotients (qCO_2 and qD) on microbial biomass from soils of different cropping histories. Soil Biol Biochem 22:251–255

Anderson TH, Domsch KH (1993) The metabolic quotient for CO_2 (qCO_2) a specific activity parameter to assess the effects of environmental conditions, such as pH, on the microbial biomass of forest soils. Soil Biol Biochem 25:393–395

Bââth E (1989) Effects of heavy metals in soil on microbial processes and populations: a review. Water Air Soil Pollut 47:335–379

Bââth E, Arnebrandt K, Nordgren A (1991) Microbial biomass and ATP in smelter-polluted forest humus. Bull Environ Contam Toxicol 47:278–282

Babich H, Stotzky G (1985) Heavy metal toxicity to microbe-mediated ecologic processes. A review and potential application to environmental policies. Environ Res 36:591–594

Brendecke JW, Axelson RD, Pepper IL (1993) Soil microbial activity as an indicator of soil fertility: long-term effects of municipal sewage sludge on an arid soil. Soil Biol Biochem 25:751–758

Brookes PC (1995) The use of microbial parameters in monitoring soil pollution by heavy metals. Biol Fertil Soils 19:269–275

Brookes PC, Heijnen CE, McGrath SP, Vance ED (1986) Soil microbial biomass estimates in soils contaminated with metals. Soil Biol Biochem 18:383–388

Chander K, Brookes PC (1991a) Effects of heavy metals from past applications of sewage sludge on microbial biomass and organic matter accumulation in a sandy loam and silty loam U.K. soil. Soil Biol Biochem 23:927–932

Chander K, Brookes PC (1991b) Microbial biomass dynamics during the decomposition of glucose and maize in metaL-contaminated and non-contaminated soils. Soil Biol Biochem 23:917–925

Ciardi C, Nannipieri P (1990) A comparison of methods for measuring ATP in soils. Soil Biol Biochem 22:725–727

Doelman P (1985) Resistance of soil microbial communities to heavy metals. In: Jensen V, Kjoller A, Sorensen LH (eds) Microbial communities in soil. Elsevier, London, pp 369–383

Doelman P, Haanstra L (1979) Effects of lead on soil respiartion and dehydrogenase activity. Soil Biol Biochem 11:475–479

Frostegård A, Bââth E, Tunlid A (1993) Shift in the structure of the soil microbial communities in limed forest as revealed by phospholipid fatty acid analysis. Soil Biol Biochem 25:723–730

Giller KE, Witter E, McGrath SP (1998) Toxicity of heavy metals to micro-organisms and microbial processes in agricultural soils: a review. Soil Biol Biochem 30:1389–1414

Haq AU, Bates TE, Soon YK (1980) Comparison of extractants for plant-available zinc, cadmium, nickel and copper in contaminated soils. Soil Sci Soc Am J 44:772–777

Hattori H (1989) Influence of cadmium on decomposition of sewage sludge and microbial activities in soils. Soil Sci Plant Nutr 35:289–299

Hattori H (1992) Influence of heavy metals on soil microbial activities. Soil Sci Plant Nutr 38:93–100

Hopkins DW, Ferguson KE (1994) Substrate induced respiration in soil amended with different amino acid isomers. Appl Soil Ecol 1:75–81

Hopkins DW, O'Dowd RW, Shiel RS (1997) Comparison of D- and L-amino acid metabolism in soils with differing microbial biomass and activity. Soil Biol Biochem 29:23–29

Insam H, Hutchinson TC, Reber HH (1996) Effects of heavy metals on the metabolic quotient of the soil microflora. Soil Biol Biochem 28:691–694

Landi L, Renella G, Falchini L, Nannipieri P (1997) Effetto di elevate concentrazioni di Cadmio sul quoziente metabolico del suolo. Atti del Convegno Annuale SISS, Rome, pp 183–188

Leita L, De Nobili M, Muhlbachova G, Mondini C, Marchiol L, Zerbi G (1995) Bioavailability and effects of heavy metals on soil microbial biomass survival during laboratory incubation. Biol Fertil Soils 19:103–108

Lindsay WL, Norwell WA (1978) Development of a DTPA soil test for zinc, iron, manganese and copper. Soil Sci Soc Am J 42:421–428

Mersi W von, Schinner F (1991) An improved and accurate method for determining the dehydrogenase activity of soils with iodonitrotetrazolium chloride. Biol Fertil Soils 11:216–220

Nannipieri P (1994) The potential use of soil enzymes as indicators of productivity, sustainability and pollution. In: Pankhurst CE, Double BM, Gupta VVSR, Grace PR (eds) Soil biota: management in sustainable farming systems. CSIRO, Australia, pp 238–244

Nannipieri P, Grego S, Ceccanti B (1990) Ecological significance of the biological activity in soil. In: Bollag JM, Stotzky G (eds) Soil biochemistry, vol 6. Dekker, New York, pp 293–355

Nannipieri P, Badalucco L, Landi L, Pietramellara G (1997) Measurements in assessing the risk of chemicals to the soil ecosystem. In: Zelikoff JT (ed) Ecotoxitology: response, biomarkers and risk assessment. SOS, Fairhaven, N.J., pp 507–534

Nordgren A, Bââth E, Soderstron BE (1988) Evalutation of soil respiration characteristic to asses heavy metal effects on soil microorganisms using glutamic acid as a substrate. Soil Biol Biochem 20:949–954

Norwell WA (1984) Comparison of chelating agents as extractants for metals in diverse soil materials. Soil Sci Soc Am J 48:1285–1292

Palmborg C, Nordgren A (1996) Partitioning the variation of microbial measurements in forest soil into heavy metal and substrate quality dependent parts by use of near infrared spectroscopy and multivariate statistics. Soil Biol Biochem 28:711–720

Sparling GP (1995) The substrate-induced respiration method. In: Alef K, Nannipieri P (eds) Methods in applied soil microbiology and biochemistry. Academic Press, pp 397–404

Stotzky G (1965) Microbial respiration. In: Black CA (ed) Methods of soil analysis. American Society of Agronomy, Madison, Wis., pp 1550–1570

Tabatabai MA, Bremner JM (1969) Use of *p*-nitrophenil phosphate for assay of soil phosphatase activity. Soil Biol Biochem 1:301–307

Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring microbial biomass C. Soil Biol Biochem 19:703–707

Witter E, Màrtensson AM, Garcia FV (1993). Size of the soil microbial biomass in a long-term field experiment as affected by different N-fertilizers and organic manures. Soil Biol Biochem 25:659–669

Zibilske LM, Wagner H (1982) Bacterial growth and fungal genera distribution in soils amended with sewage sludge containing cadmium, chromium and copper. Soil Sci 134:364–370