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Microbial indicators of heavy metal contamination in urban and rural soils

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Abstract

Urban soils and especially their microbiology have been a neglected area of study. In this paper, we report on microbial properties of urban soils compared to rural soils of similar lithogenic origin in the vicinity of Aberdeen city. Significant differences in basal respiration rates, microbial biomass and ecophysiological parameters were found in urban soils compared to rural soils. Analysis of community level physiological profiles (CLPP) of micro-organisms showed they consumed C sources faster in urban soils to maintain the same level activity as those in rural soils. Cu, Pb, Zn and Ni were the principal elements that had accumulated in urban soils compared with their rural counterparts with Pb being the most significant metal to distinguish urban soils from rural soils. Sequential extraction showed the final residue after extraction was normally the highest proportion except for Pb, for which the hydroxylamine-hydrochloride extractable Pb was the largest part. Acetic acid extractable fraction of Cd, Cu, Ni, Pb and Zn were higher in urban soils and aqua regia extractable fraction were lower suggesting an elevated availability of heavy metals in urban soils. Correlation analyses between different microbial indicators (basal respiration, biomass-C, and sole C source tests) and heavy metal fractions indicated that basal respiration was negatively correlated with soil Cd, Cu, Ni and Zn inputs while soil microbial biomass was only significantly correlated with Pb. However, both exchangeable and iron- and manganese-bound Ni fractions were mostly responsible for shift of the soil microbial community level physiological profiles (sole C source tests). These data suggest soil microbial indicators can be useful indicators of pollutant heavy metal stress on the health of urban soils.

Keywords: Physiological profiles; Sequential extraction; Bioavailability, catabolic, diversity metals

1. Introduction

Research on urban soils is receiving increasing attention (Bullock and Gregory, 1991; Madrid et al., 2002; Markiewicz Patkowska et al., 2005). Most of this research has concentrated on the distribution of potentially toxic elements in urban soils (Chen et al., 1997), plants (Kimberly et al., 1999) and dusts (Li et al.,

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2001), and their chemical forms (Lottermoser, 1997; Ge et al., 2000; Li et al., 2001), distribution and movement (Charlesworth and Lees, 1999). Cu, Pb, Zn and Cd are common anthropogenic elements in the urban environment (Pouyat and Mcdonnell, 1991; Carlosena et al., 1995; Alexandrovskaya and Alexandrovskiy, 2000). The distribution, source and fate of organic pollutants have also been studied (Kawano et al., 1992; Schuhmacher et al., 1997). Soil fauna in urban soils have also been studied due to their important role in the transformation of nutrient cycles (Steinberg et al., 1997) but there has been little work on the microbiology of urban soils with the exception of studies on the changes in organic constituents as influenced by the soil microbial biomass (Beyer et al., 1995a). This is despite the fundamental importance of micro-organisms to soil function (Insam, 1991). The size of the microbial biomass, its turnover and the microbial utilisation efficiency of organic carbon can be important indicators of soil health and environmental quality (Bardgett and Saggar, 1994) and metals are known to reduce the size and activity of the microbial biomass. Changes in microbial community structure and a reduction in catabolic versatility have also been observed (Wenderoth and Reber, 1999a,b) in agricultural soils and linked to metal stress. Consequently, in this paper, we studied the relationship between microbial activity and heavy metal levels in an urban environment to assess the usefulness of microbial indicators for evaluating the health and functioning of urban soils.

One problem with assessing soil biological properties is that they can be significantly affected by the vegetation, land use and management, as well as the inherent soil physical and chemical properties, irrespective of pollutant levels. Thus, importantly, we selected soils from the same soil association, i.e. the same parent material, and ensured that all soils were under grassland to minimize the differences due to mineralogical composition and vegetation in order to assess more clearly the effect of anthropogenic inputs and urban use. We hypothesized that if urban soils were more polluted than rural soils this should be manifested in the microbial parameters and thus microbial parameters may reflect altered functionality. Because the potential toxicity of the metals is determined by their availability not by the total amount present we fractionated the metal content using sequential extraction procedures to determine whether specific fractions explained any biological response.

2. Materials and method

2.1. Soil preparation

Fourteen samples were taken using an auger from each of three location types: roadsides, city parks (parkland) in Aberdeen city, Scotland, UK and rural agricultural fields in the vicinity of Aberdeen city. All soils came from the same soil association (Countesswells) developed on glacial till derived from granite rocks (Wilson et al., 1984). Fresh soils were sieved (<2 mm) and all visible plant remains, animals and stones removed. Subsamples (~100 g) were air-dried at 30 °C and sub-sampled again into smaller quantities using a Rotary Sample Splitter. Portions were milled using a Fritsch Pulverisette No. 06.102 type milling machine and agate bowls and balls to avoid metal contamination in preparation for chemical analysis. Fresh sub-samples were stored at 4 °C for microbiological analysis.

2.2. Soil properties

Soil pH in water was determined using a pH meter after mixing 15 g soil in 45 ml deionised water for 2 h on a roller and subsequently allowing the mixture to equilibrate for 20 h. pH in calcium chloride was also determined by adding 5 ml of 0.1 M $CaCl_2$ solution to the suspension to give a 0.01 M $CaCl_2$ solution, and the suspension was mixed for 30 min and read after 2 h equilibration. Soil moisture was determined on samples (10 g) by oven drying at 105 °C, to constant weight. Loss on ignition (LOI) was determined on oven dried soils by heating in a muffle oven for 12 h at 900 °C. Total soil organic carbon (TOC) was calculated from the loss on ignition using a conversion equation of $TOC = 0.476LOI - 1.87$.

2.3. Microbial parameters

Soil microbial biomass-C (C_{mic}) was determined by the fumigation-extraction method (Wu et al., 1990) except that the chloroform used was stabilized with amylene. The carbon contents, extracted with K_2SO_4 from the $CHCl_3$-treated and untreated soils, were determined by an automated TOC Analyser (Shimazu, TOC-500) and a K_{ec} value of 2.22 was used to convert the measured flush of C to biomass-C. All biomass measurements were carried out in triplicate. Basal respiration (CO_2 evolution) was measured in duplicate on 20 g samples of soil in 100 cm^3 soil jars after 7 days incubation by using gas chromatography to measure the headspace CO_2 that accumulated over 48 h at 25 °C. The ratio of soil microbial respiration (R_{mic}) to soil microbial biomass (C_{mic}) was used to express the metabolic quotient (qCO_2), which is the amount of CO_2–C produced per unit microbial biomass carbon (Anderson and Domsch, 1986).

2.4. Community level physiological profiles (CLPP) by sole carbon source utilisation tests

CLPPs were determined by direct incubation of fresh soil extracts in 96-well plates containing different C

sources in individual wells (BIOLOG) to determine changes in relative and absolute rates of utilisation of individual substrates. Fresh soil (10 g) was added to 100 ml of distilled water in a 250 ml flask and shaken on a wrist action shaker at full speed for 10 min. Tenfold serial dilutions were made and the 1 in 10^3 dilution was used to inoculate the Biolog plates. The dilution was centrifuged at 500*g* for 10 min to separate the soil and 150 μl of supernatant was inoculated into each well of a 'MT' type plate (Biolog Inc., Hayward, California, USA) prepared with additional carbon sources herein referred to as MT8 (Campbell et al., 1997). The MT8 plate consisted of mainly aliphatic acid and aromatic compounds (Table 1) that are known to be breakdown products of organic contaminants found in soils and in the case of the aromatic compounds their degradation is known to be sensitive to heavy metals (Burkhardt et al., 1993). The 15 carbon sources and one blank were distributed in two columns in the MT8 plate so that six samples could be tested in one 96 well plate.

Plates were incubated at 25 °C for 7 days and well colour development was measured as absorbance (A) at 590 nm using an automated plate reader (VMAX, Molecular Devices, Crawley, UK) and the data were collected using Microlog Rel 3.5 software (Biolog Inc., Hayward, California, USA). Plates were read twice daily and ANOVA of the average well colour development (AWCD) over time was used to select comparable time points to avoid confounding effects of inoculum density differences between treatments in the multivariate analysis (Garland, 1996). The AWCD for all carbon sources was calculated as a measure of activity.

Table 1
List of carbon sources and volumes of standard solutions added to wells of 'MT8' plates

Carbon sources		Volume of stock solution added (μl)
Sugar	Glucose	60
Amino acid	Asparagine	60
Phenolic acids	Salicylic acid	150
	Toluic acid	150
	Naphthoic acid	125
	Protocatechuic acid	130
	p-OH Benzoic acid	150
	Benzoic acid	165
Aliphatic acids-saturated	Myristic acid	90
	Palmitic acid	80
	Stearic acid	75
Aliphatic acids-unsaturated	Palmitoleic acid	80
	Oleic acid	75
	Linoleic acid	75
	Linolenic acid	75

2.5. Sequential extraction of heavy metals

Sequential extraction of heavy metals in soils was carried out according to the modified BCR sequential extraction procedure (Rauret et al., 1999) such that each sample was extracted by 0.11 mol/l acetic acid solution followed by 0.5 mol/l hydroxylamine chloride solution extraction, then extracted by 1.0 mol/l ammonium acetate after digestion in hydrogen peroxide. In the final step, the residues were digested in aqua regia. Standard reference soil samples were used as quality control throughout the whole extraction process. The concentrations of Cu, Ni and Zn in the extracts were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) and those of Cd and Pb by graphite furnace atomic absorption spectrometry (GFAAS) after McGrath and Cunliffe (1985).

2.6. Statistical analyses

All statistical analyses were performed using Genstat Rel 5.3 software (NAG Ltd, Oxford, UK). Means and the least significant differences at the 5% level were calculated by a one way ANOVA for each soil type (Parkland, Roadside and Rural). For multivariate analysis of the CLPP data, the absorbance values at equivalent AWCD from different times of incubation (Garland, 1996) were compared and were also transformed by dividing by the AWCD to avoid bias between samples with different inoculum density (Garland and Mills, 1991). The absorbance data were analysed by canonical variate analysis (CVA), after first reducing the dimensionality by principal component analysis (PCA) and by comparison of mean inter group Mahalanobis distances with simulated confidence limits (SCL). AWCD, individual C source utilisation, and catabolic versatility (Wenderoth and Reber, 1999a,b) were calculated and analysed by a one-way ANOVA.

3. Results

3.1. Soil physico-chemical properties and microbial parameters

Total soil organic C was highest in the rural soils compared to the urban parkland and roadside soils (Table 2) but the parkland and roadside soils were not significantly different from one another (Table 2). The soils from three zones were not significantly different in moisture content. The parkland soil had the lowest pH_{H_2O} and pH_{CaCl_2} while roadside and rural soils were higher but not significantly different from one another.

The respiration rate of roadside soils was significantly higher than rural and parkland soils while the C_{mic} was significantly higher in the rural soils than the parkland

Table 2
Average soil physico-chemical and biological properties for soils sampled from parkland, roadsides and rural soils in and around Aberdeen city

Soil	% Total organic Carbon	pH ($CaCl_2$)	pH (H_2O)	% Moisture Content	Respiration rate (μg CO_2 g^{-1} soil h^{-1})	C_{mic} (μg C g^{-1} soil)	C_{mic}/C_{org} (mg C_{mic} g^{-1} C)	Mq (qCO_2) × 10^3 (μg CO_2 μg C^{-1} h^{-1})	Catabolic versatility
Parkland	2.58	4.46	5.28	23.9	0.607	321	13.6	1.89	1.39
Roadside	2.49	4.76	5.73	21.6	0.787	296	20.6	2.79	1.30
Rural	3.41	5.10	5.80	22.2	0.620	411	13.1	1.50	1.27
$LSD_{0.05}$	0.80	0.46	0.42	2.19	0.136	54.6	14.1	0.36	0.09

soils which, in turn, was higher than the roadside soils. There were no significant correlations between soil pH and the microbial parameters measured (basal respiration rate and C_{mic}, Table 3) suggesting the differences in soil pH were not responsible for the observed variation.

The ratio of microbial biomass carbon to soil total organic carbon (C_{mic}/C_{org}) and the metabolic quotient, qCO_2, are microbial parameters that are often used to assess changes in ecological succession and/or environmental stress. The C_{mic}/C_{org} values of the parkland and rural soils were significantly different, but these were different from the roadside soils (Table 2).

Variation in qCO_2 showed the sequence of roadside > parkland > rural soils, and significant differences were observed between them (Table 2).

Correlation analyses between microbial indicators and basic soil physico-chemical properties, based on 42 samples from the three zones, showed that basal respiration had poor relationships with soil pH and moisture but a significant positive relationship with soil total organic carbon (Table 3). This might reflect the observation that the soil microbial basal respiration was mainly associated with soil organic matter and moisture under normal circumstances, but might be impaired under urban environment.

Soil microbial biomass is usually controlled by soil moisture, organic matter and pH value (Arnold et al., 1999). In our study soil microbial biomass was closely related to soil organic matter, and also to moisture but did not show any significant relationship with soil pH (Table 3).

The C_{mic}/C_{org} ratio was mainly correlated to soil total organic carbon (TOC) and decreased greatly with an increase in TOC (Table 3). Negative relationships with moisture, but positive with soil pH, were also observed.

Correlations of qCO_2 with soil pH, TOC and moisture were not significant, in contrast to the data of Anderson and Domsch (1993) who found that qCO_2 usually had a negative correlation with soil pH but this probably reflects the narrow range in pH and uniformity of sol properties in our samples.

3.2. Biolog-CLPP

All Carbon sources except linolenic acid and toluic acid were utilised to lesser or greater extents. These two C sources were therefore omitted from any further analysis. Of the three soil groups, roadside samples had the highest average well colour development (AWCD) for all C sources (Fig. 1) and rural soils the lowest but these differences were not significant. Given a certain AWCD value of 0.8, it will take 97, 112 and 131 h for roadside soil, parkland soil and rural soil respectively, which might suggest micro-organisms would consume carbon sources much more quickly in urban soils than in rural soils. The utilisation of different types of carbon sources however, in the three soil types did differ significantly ($p < 0.05$, Fig. 1). Rural soils had the highest utilisation of sugar, glucose while roadside soils had the lowest. In contrast, roadside soils had the highest utilisation for the amino acid, asparagine, while parkland had the lowest. Greater differences between the soils were seen in the utilisation of phenolic acids which were used fastest in roadside soils and parkland soils. Similarly, there were some differences between the three

Table 3
Correlations between microbial indicators and soil properties

	pH_{H_2O}	pH_{CaCl_2}	Moisture	TOC
Basal respiration	−0.04	−0.10	0.28	0.35*
Microbial Biomass	−0.08	0.03	0.39**	0.63**
C_{mic}/C_{org}	0.31*	0.30*	−0.58**	−0.52**
qCO_2	0.13	−0.01	−0.21	−0.26

Superscripts *, ** represent $\alpha = 0.05$ and 0.01 significant levels of correlation, respectively based on 42 samples of the three soil types. TOC refers to total organic carbon.

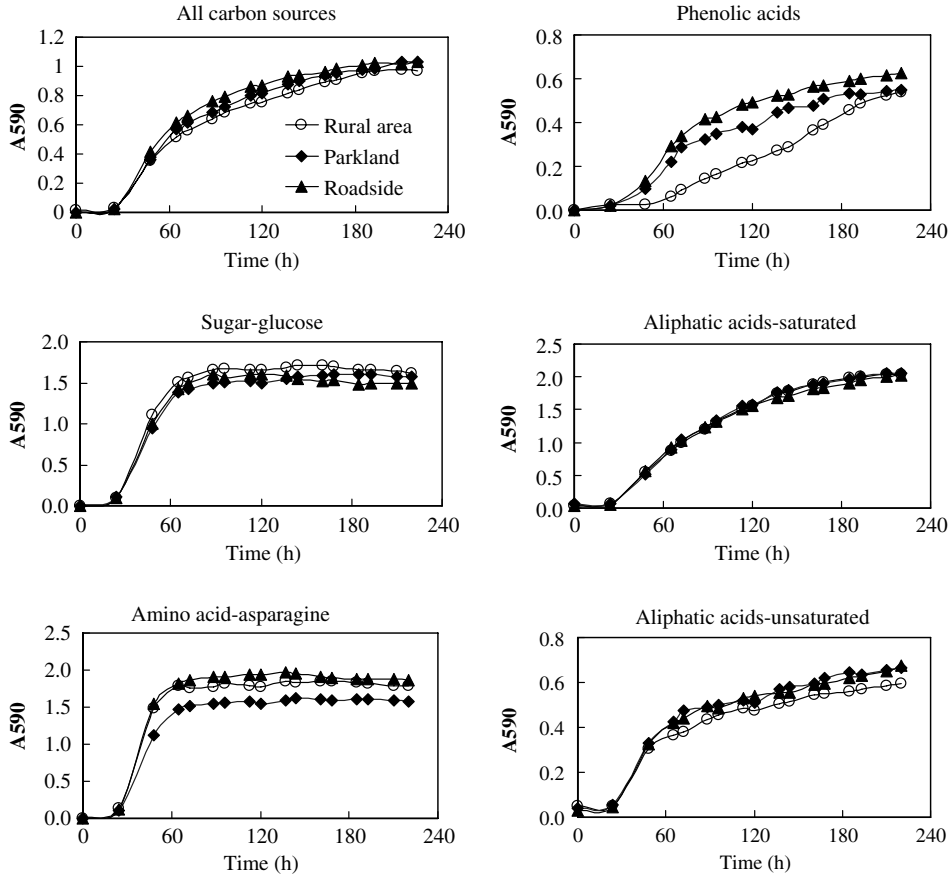

Fig. 1. Average well colour development (AWCD) over time of all and different groups of carbon sources in different type soils.

soil types for utilisation of unsaturated aliphatic acids as well as some slight differences for saturated aliphatic acids.

Differences in C source utilisation patterns were found in the canonical variate analysis of the 13 C sources (Fig. 2). The three zones had an average separation distance (Mahalanobis distance) of 2.1 ($p = 0.028$) suggesting the microbial communities were significantly different.

There were also significant differences ($p < 0.05$) in the catabolic versatility of the C sources between parkland soils and either roadside or rural soils (Table 2). The latter two soils were not significantly different from one another.

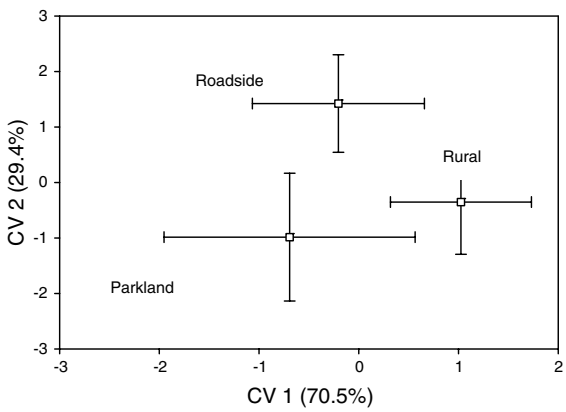

Fig. 2. Ordination score plot of Biolog-community level physiological profiles for 13 C sources analysed by canonical variate analysis in rural, parkland and roadside soils.

3.3. Potentially toxic element (PTE) accumulation and their availability in urban soils

The average Cd concentration of all samples (Table 4) was close to the world soil background value of 0.35 mg kg^{-1} (NEPB, 1990). The other metals show different accumulations in the urban soils compared with rural soil, especially the elements Pb and Zn, known to be anthropogenic pollutants in urban environment. Parkland soils had intermediate Pb and Zn concentra-

Table 4
Average soil total metal and acetic acid extractable metals in soils sampled from parkland, roadsides and rural soils in and around Aberdeen city

Soil	Total (mg kg^{-1})					Acetic acid extractable (step 1) (mg kg^{-1})				
	Cd	Cu	Ni	Pb	Zn	Cd	Cu	Ni	Pb	Zn
Parkland	0.342	32.2	21.2	138	101	0.118	1.75	1.75	9.45	27.4
Roadside	0.408	49.4	18.3	181	111	0.200	5.31	1.87	17.4	34.4
Rural	0.340	13.1	12.1	32.4	44.4	0.116	0.542	0.508	0.495	4.26
$LSD_{0.05}$	0.130	21.9	5.37	81.4	38.5	0.063	2.69	0.620	11.1	15.2

tions, but the largest standard deviations (data not shown), which might be attributable to the location of some parkland soils close to the arterial traffic routes.

There were no significant differences between urban soils and rural soils in Cd contents, but Cu, Ni, Pb and Zn contents were significantly higher in urban soils (roadside soils and parkland soils) than in rural soils ($p < 0.05$). Except for Ni, the highest metal concentrations were normally observed in roadside soils. Soil physico-chemical parameters were generally not responsible for heavy metal accumulation in soils except for significant positive relations between soil pH_{H_2O} and Cd and Pb contents (Table 5).

Principal component analyses (PCA) for soil PTE data (transformed by dividing by overall mean) showed the first two components explained 85.85% and 12.55% of the variation, respectively. Pb had the highest negative score for first principal component, while Zn and Cu could be attributed mainly to the second principal component with highest positive scores (Fig. 3). The first principal component clearly differentiated between the urban and rural soils, while for the second component the difference was not so obvious, which might suggest that the main factor controlling the difference between urban and rural soils was the Pb content.

The sequential extraction procedures showed that in contrast to total Cd the acetic acid extractable Cd, was significantly higher in roadside soils than both parkland and rural soils. Similar to total metal concentrations, significantly higher acetic acid extractable Cu, Ni, Pb and Zn concentrations were found in urban soils than in rural soils (Table 4) indicating a higher level of available metal in the urban environment. The hydroxylamine-hydrochloride extractable (step 2) had the largest

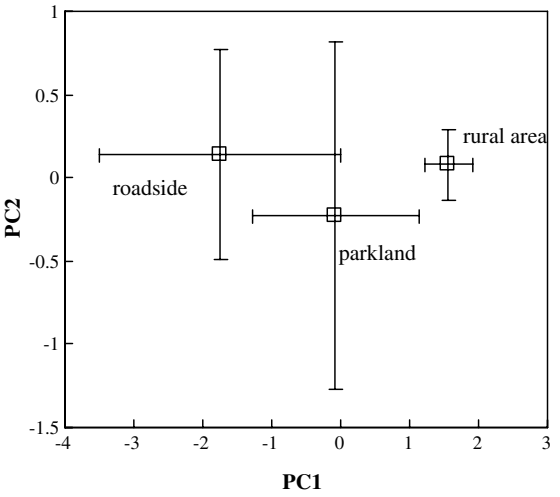

Fig. 3. Ordination score plot from the principal component analysis with heavy metal concentration data. Each plot is the Mean ± STDEV of 14 samples.

amount of Pb, accounted for more than 60%, suggesting a strong fixation of Pb by Fe and Mn oxides in these soils (Fig. 4). However, the main portions of Zn and Ni in soils were residual fractions which may have accounted for over 40% and 70%, respectively. Peroxidation/ammonium acetate extractable (step 3) Cu, which accounted for about 30%, was the main fractions of Cu, indicating strong binding of Cu to soil organic matter. A relative large amount of Cd (usually over 35%) in these soils were acetic acid extractable. Interestingly, the acetic acid extractable portions of Cd, Cu, Ni,

Table 5
Correlations of soil physico-chemical properties with soil cumulative heavy metal contents

	Cd	Cu	Ni	Pb	Zn
pH_{H_2O}	0.39**	0.10	0.01	0.32*	0.21
pH_{CaCl_2}	0.29	−0.02	−0.05	0.22	0.10
Moisture	0.18	0.00	0.23	0.11	0.21
Total organic carbon	0.02	0.14	0.15	−0.14	−0.07

Superscripts *, ** represent $\alpha = 0.05$ and 0.01 significant levels of correlation, respectively based on 42 samples.

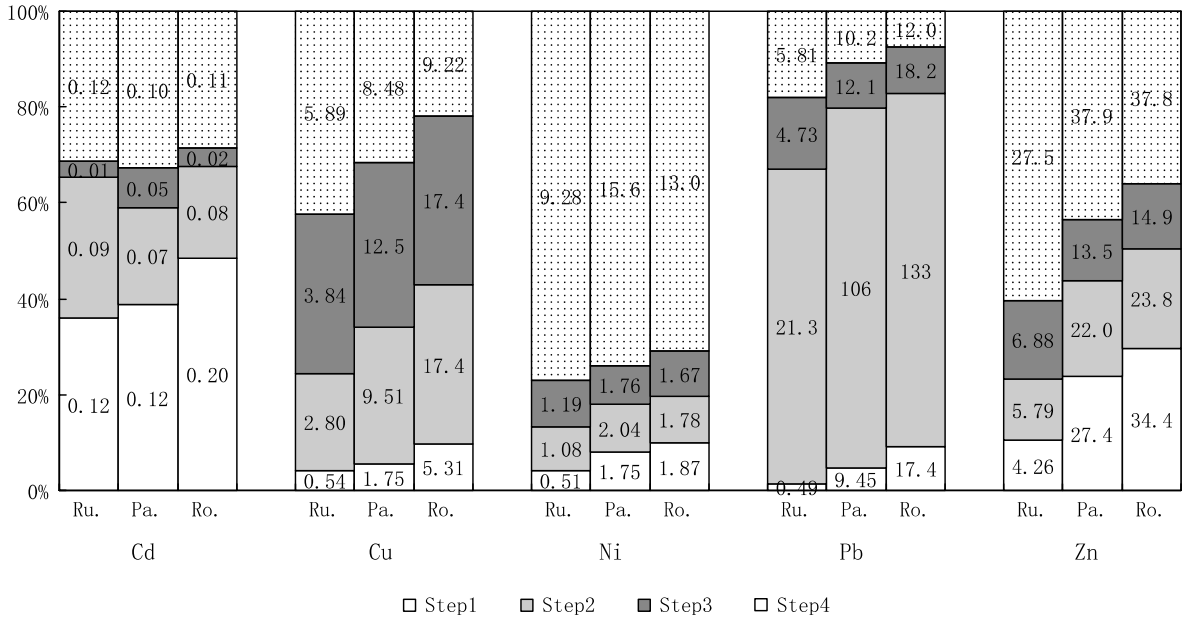

Fig. 4. Percentages of Cd, Cu, Ni, Pb and Zn extracted in the four step sequential extraction procedures. Ru—rural soils; Pa—parkland soils; Ro—roadside soils. Data in the bars are metal concentrations extracted in each step.

Pb and Zn increased from rural soils to parkland soils to roadside soils and the aqua regia extractable portions of these metals decreased (Fig. 4). This might suggest an elevated availability of heavy metals going from rural to urban soils.

PCA analyses for the heavy metal sequential extraction data were carried out after dividing by their overall average. The first two principal components explained 79.67% and 6.77% of the variation respectively (Fig. 5). The main contributor to these two principal components was extractable Pb, but with a negative weighting for the first principal component and a positive one for the second. Clearly, significant differences can be found between rural soils and urban soils, especially in the first principal component. This implied that extractable Pb could be more effective than other metal speciation to distinguish urban soils from rural soils in heavy metal accumulation.

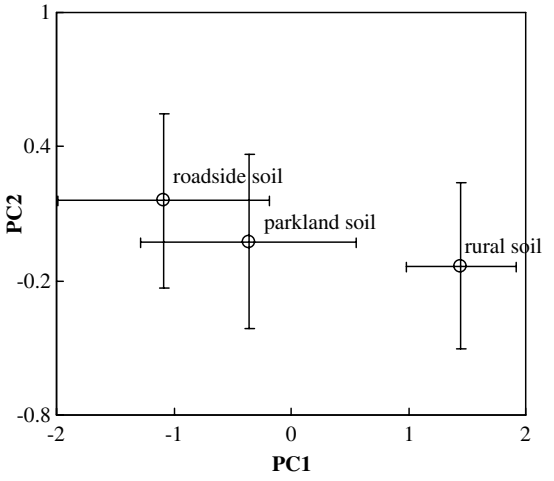

Fig. 5. Score plot from the principal component analysis with heavy metal sequential extraction data. Each value is the Mean ± STDEV of 14 samples.

3.4. Correlations of heavy metal speciation with microbial indicators

Soil basal respiration rates were significantly positively correlated with soil acetic acid extractable metals Cd, Cu, Ni and Zn, and with both hydroxylamine-hydrochloride extractable and peroxidation/ammonium acetate extractable Cu in total 42 soil samples (Table 6). Similarly, positive correlations with soil cumulative total Cu and Zn were also found. This implies basal respiration rates might be one of the most influential soil microbial indicators responsive to heavy metals Cd, Cu, Ni and Zn, especially Cu inputs. Noticeably, the soil microbial biomass only showed significant negative correlations with all Pb fractions in soils but no significant correlations with others metals could be found. This suggests microbial biomass might be the most sensitive microbial indicator to metal Pb inputs in soils. As inferred from PCA results (Figs. 3 and 5), Pb was the main variable responsible for the discrimination of the rural soils from urban soils, therefore, anthropogenic Pb could be the most responsible metal for lower micro-

Table 6
Correlations between heavy metal speciation concentrations and microbial parameters

	Cd	Cu	Ni	Pb	Zn
Acetic acid extractable (step 1)					
Basal respiration	0.34*	0.39**	0.34*	0.18	0.40**
Microbial biomass	−0.13	−0.27	−0.30	−0.38*	−0.23
C_{mic}/C_{org}	−0.12	−0.11	−0.17	−0.08	−0.12
qCO_2	0.38*	0.54**	0.47**	0.44**	0.47**
CV1-Biolog-CLPP	−0.06	0.09	0.43**	0.12	0.29
CV2-Biolog-CLPP	0.14	0.26	0.18	0.18	0.13
Hydroxylamine-hydrochloride extractable (step 2)					
Basal respiration	−0.08	0.41**	0.16	0.28	0.25
Microbial biomass	−0.16	−0.21	−0.07	−0.35*	−0.18
C_{mic}/C_{org}	−0.02	−0.20	−0.27	−0.18	−0.17
qCO_2	0.03	0.46**	0.11	0.49**	0.29
CV1-Biolog-CLPP	−0.21	0.20	0.46**	0.25	0.29
CV2-Biolog-CLPP	−0.21	0.23	0.08	0.17	0.09
Peroxidation/ammonium acetate extractable (step 3)					
Basal respiration	−0.08	0.41**	0.16	0.28	0.25
Microbial biomass	−0.16	−0.21	−0.07	−0.35*	−0.18
C_{mic}/C_{org}	−0.02	−0.20	−0.27	−0.18	−0.17
qCO_2	0.03	0.46**	0.11	0.49**	0.29
CV1-Biolog-CLPP	0.12	0.27	0.27	0.18	0.17
CV2-Biolog-CLPP	−0.06	0.17	−0.02	0.13	0.06
Soil cumulative heavy metal concentrations					
Basal respiration	0.32	0.40**	0.20	0.23	0.32*
Microbial biomass	−0.03	−0.22	−0.18	−0.39**	−0.21
C_{mic}/C_{org}	−0.23	−0.17	−0.23	−0.15	−0.16
qCO_2	0.26	0.48**	0.22	0.47**	0.36*
CV1-Biolog-CLPP	−0.06	0.23	0.45**	0.24	0.29
CV2-Biolog-CLPP	−0.04	0.20	0.09	0.17	0.09

Superscripts *, ** represent $\alpha = 0.05$ and 0.01 significant levels of correlation, respectively based on 42 samples.

bial biomass levels in urban soils than in rural soils. However, soil C_{mic}/C_{org} ratios showed no correlations with any of the fractions of heavy metals and they might be only related to the soil physico-chemical properties (Table 3). Similar to soil basal respiration rates, soil qCO_2 values were closely positively correlated with acetic acid extractable Cd, Cu, Ni, Pb and Zn, and with Cu and Pb in the hydroxylamine-hydrochloride extractable and peroxidation/ammonium acetate extractable fractions, and with soil cumulative Cu and Pb concentrations. In contrast to the above microbial parameters, soil Biolog-CLPP was only positively influenced by acetic acid extractable, hydroxylamine-hydrochloride extractable Ni and total Ni concentration. This suggests exchangeable- and iron- and manganese-bound Ni fractions might be the dominant influential metal affecting the microbial community level physiological profiles.

As shown in Table 7, the variation on CV1 of the Biolog-CLPP data was negatively correlated with soil pH value and microbial biomass. Consequently, as well as Ni, soil pH and microbial biomass might also affect the microbial community level physiological profiles.

Table 7
Correlations between CVA loading scores of Biolog-CLPP data (CV1 and CV2 in Fig. 2) and soil physico-chemical properties and microbial parameters

	Respiration ($\mu g\ CO_2\ g^{-1}\ h^{-1}$)	Microbial biomass ($\mu g\ C\ g^{-1}$)	Soil organic C ($g\ kg^{-1}$)	pH_{CaCl_2}
CV1	−0.03	−0.33*	−0.24	−0.40**
CV2	0.22	−0.30*	−0.19	0.10

Superscripts *, ** represent $\alpha = 0.05$ and 0.01 significant levels of correlation, respectively, based on 42 samples.

4. Discussion

The data from this study demonstrated elevated levels of Pb, Zn, Cu, Cd and Ni in urban soils in Aberdeen compared with the rural soils, especially in roadside soils. Considering the sampling locations, high levels of heavy metals were mainly found in roadside soils alongside arterial and city center routes, partly in parkland soils that are close to arterials, where these routes

are connected with dense traffic. Although, there is little heavy industry in Aberdeen city, disturbance of soil chemistry could also be attributed to anthropogenic influences (such as petroleum combustion, Paterson et al., 1996).

The toxicity of heavy metals is determined by their availability. Chemical speciation, extracted by sequential procedures or by single chemical extractants (such as EDTA) is widely applied to assess the availabilities of heavy metals. BCR (Rauret et al., 1999) and Tessier's sequential extraction (Tessier et al., 1979) methods are of two most popularly applied protocols and have been widely used to characterize soils contaminated with heavy metals (Verner et al., 1996). Bacon et al. (2005) evaluated the BCR method and found good reproducibility. In this study large proportions of acetic acid extractable Cd and Zn (generally larger than 10%, Fig. 4) might indicate a higher mobility and availability for these metals from urban soils, especially from the roadside soils. Small proportions of acetic acid extractable Cu, Ni and Pb (less than 10%) suggest a low risk of metal release and/or toxicity for these elements. However, large carbonate-bound fractions of Pb and Cu (>10%) increase the possibility of metal release and toxicity under decreasing pH conditions.

The urban soil microbial biomass was significantly reduced compared with that of rural soils. Even though soil microbial biomass was positively correlated with soil total organic content, negative correlations with Pb concentration for all fractions were still pronounced and this might be related to leaded petroleum combustion. Pb has been shown to accumulate to high levels in urban environments from a range of sources including that derived from leaded petrol (Markus and McBratney, 2001; Wong and Li, 2004; Moller et al., 2005) but only a relatively small proportion (<2.5%) of the Pb is in an exchangeable form (Wilcke et al., 1998; Lu et al., 2003; Smejkalova et al., 2003) similar to the levels found in this study. It is known that the accumulation of Pb can adversely affect soil microbial activity (Smejkalova et al., 2003) but few studies have assessed the relative impacts of specific chemical fractions of Pb on microbial activity and diversity although Leita et al. (1995) found that water soluble fraction of Pb was not significantly related with microbial biomass-C. This result is also consistent with the studies of Barajas Aceves et al. (1999), Kuperman and Carreiro (1997) and Kandeler et al. (1996) who all reported a smaller microbial biomass size in heavy metal-contaminated soils.

Heavy metal accumulation can also influence the soil microbial community structure (Baath et al., 1998) and many previous studies have shown that the Biolog method is a useful tool for assessing long-term effects of heavy metals on soil microbial community structure (Pennanen et al., 1996; Kelly and Tate, 1998; Kelly et al., 1999). However, it seems that there are no previous studies that report Ni acting as the most influential metal on soil microbial CLPP under complex metal pollution conditions.

We found no difference in the catabolic versatility of C sources used (Table 2). This is contrary to the findings of Wenderoth and Reber (1999a,b) who found catabolic versatility was reduced in metal-contaminated soils (heavy metal levels were higher in urban soils than in rural soils in this study, Table 4). There were, however, differences between the soil types, method and C sources used in their studies compared with our study. For example Wenderoth and Reber (1999a,b) tested agricultural soils which had much more elevated metal concentrations than our study and they also used a different set of phenolic acids. Nevertheless the fact no significant differences in catabolic versatility of C source utilisation was found is inconsistent with the effects observed on the Mq (qCO_2) and microbial biomass where roadside soil had the lowest microbial biomass and highest Mq (Table 2).

Normally, microbial basal respiration rate can be expected to have a significant positive correlation with soil microbial biomass. A significant positive correlation was found in the rural soils in this study, with a correlation coefficient of $r = 0.88$ ($p < 0.01$). However, this value reduced from 0.72 to 0.49 in soils from parkland to roadside, indicating a poor relationship between microbial basal respiration rate and microbial biomass in the urban soils. Although several factors might change this relationship elevated metal concentrations in urban soils are one likely influential factor, as exemplified by the significant positive relationships between basal respiration and heavy metal concentrations (especially acetic acid extractable metal concentrations, Table 6). Similarly, microbial ecophysiological parameters C_{mic}/C_{org} and qCO_2 were negatively correlated with soil microbial biomass in urban soils (parkland soils and roadside soils), with correlation coefficients of −0.48 and −0.52 respectively. No such significant correlations were found in the rural soils. Previous research has suggested that one of the main reasons for qCO_2 variance was a change in the ecophysiological state of the micro-organisms induced by environmental stress (Insam et al., 1996). Interestingly, the qCO_2 of the roadside soils was higher than that of the rural soils which had the lower content of heavy metals.

These findings might suggest that the size of soil microbial biomass was insufficient to attain the same community level microbial physiological activities in the urban soils. It is reasonable to deduce therefore that heavy metal accumulation might shift the soil microbial activity from a low and stable level in low metal soil to an unstable and more energy demanding (higher carbon source consumption) level in the higher metal-contaminated soil. The urban soils only showed this for phenolic substrates, however, and the effect on the overall

catabolic versatility was different from previous studies (Wenderoth and Reber, 1999a,b). It is interesting to speculate that one might also expect higher phenolic substrates in urban soils from organic contaminants, which commonly contain phenolic moieties, although this cannot be proved as we did not analyse any such compounds.

Changes in C_{mic}/C_{org} are usually related to the organic matter content and microbial activity. Higher C_{mic}/C_{org} is usually associated with increased decomposition of organic matter (Hoffmann et al., 1997) and lower C_{mic}/C_{org} with low levels of decomposition and stable low levels of microbial activity (Beyer et al., 1995b, 1999). Our results, of higher C_{mic}/C_{org} ratios in roadside soils than in parkland and rural soils, possibly reflected the poorer soil organic matter quantity in roadside soils due to comparatively poorer vegetation coverage.

In conclusion, the heavy metals Pb, Zn, Cu and Ni were significantly accumulated in Aberdeen city urban soils and were also associated with increased metal availability. Some microbial parameters (biomass-C and respiration) were significantly correlated with Pb and different Pb fractions but others such as community level physiological profiles gave different results from previous studies and were mostly associated with Ni. Consequently further work on the effects of metals in such soils on physiological profiling by sole C source tests is required. Accumulation of heavy metals Pb, Zn, Cu and Ni in urban soils did lead to a decrease in microbial biomass and elevate the microbial basal respiration suggesting these parameters are useful indicators of pollutant levels in such soils.

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