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Microbial respiration as an indication of metal toxicity in contaminated organic materials and soil

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ABSTRACT

The effect of heavy metals on microbial respiration in organic materials used as soil amendments was evaluated to assess the stability of the materials. Solutions of Pb (II), Cu (II) and Zn (II) at rates of 5, 10 and 50 mg metal g^{-1} were added to green waste compost, peat, coir and wood bark. Metal toxicity led to a significant decrease in carbon dioxide evolved by the contaminated materials, up to 80% less at the highest rate of addition compared to the untreated material. There was a significant negative correlation between the organic carbon content of an amendment and the inhibition of CO_2 evolution by all three heavy metals. There was also a significant negative correlation between an amendment's cation exchange capacity and the inhibition of CO_2 evolution caused by Cu and Zn. The ability of the organic materials to enhance respiration in a soil from the vicinity of a Pb/Zn mine was also evaluated, by applying them to the soil at rates of 1, 10 and 20%. CO_2 evolution from the contaminated soil was enhanced significantly by the addition of all of the amendments, with coir causing up to 90% enhancement at high levels of addition.

1. Introduction

Organic materials such as compost, peat, coir and wood bark have been shown to adsorb heavy metal ions from solution, and have been suggested as possible amendments for contaminated soils [1,2]. However, the stability of such materials when added to soil is not known. In order to be effective as amendments, they must persist in soil in a form that can adsorb metal ions over a significant period of time. On the other hand, the addition of a degradable organic material will enhance the biological activity, which can be important in contaminated soils where toxicity may inhibit the natural microbial functions. This study aims to assess these two competing processes.

The microbial biomass is a significant component of soil and an important agent in the breakdown of organic matter, the degradation of pollutants, and the recycling of plant nutrients and heavy metals [3,4]. High metal concentrations can significantly reduce rates of decomposition because microbial activity is adversely affected by heavy metal toxicity [5,6]. Factors such as microbial biomass, specific respiration rate, and potential metabolic activity tend to be particularly affected [7,8]; while elevated levels of heavy metals in soils can decrease the population size of the microbial communities [9,10].

The impacts of pollutants can be extremely diverse, ranging from direct effects on micro-organisms, to indirect effects which determine biochemical processes in soils. Microbial biomass in highly metal contaminated soils has been found to be consistently lower than in uncontaminated or low metal conditions [11]. On the other hand, 'moderate' metal levels may have no significant effect, or a mild stimulatory effect, on microbial processes [12,13].

Microbial respiration is an effective measure of the rate of carbon mineralization, since about 70% of C added to the soil is lost as carbon dioxide, mainly as a product of microbial respiration [14,15]. The ability of bacteria to decompose complex substrates is significantly reduced by the presence of heavy metals [16], so the amount of CO_2 evolved is a very reliable indicator of the effect of metal contamination on microbial activity [17,18]. This is because respiration has been found to suffer the greatest reduction, while other microbial parameters like biomass may be reduced only slightly [17].

Incubation periods that have been used to assess the effect of metal toxicity on microorganisms cover a very wide range. Some studies have been as few as 20 days [4], while others as much as 300 days [19]. The response of microorganisms to metals in spiked soil has been found to differ greatly from their response in field contaminated soils [20], as effects tend to be higher through spiking than that in aged field contaminated soils. This often leads to a large discrepancy in responses obtained at similar metal

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Table 1
Properties of the organic materials used as soil amendments.

	pH water	Loss on ignition (%)	Carbon (%)	Nitrogen (%)	Cation exchange capacity ($cmol_c\ kg^{-1}$)	Pb ($mg\ kg^{-1}$)	Cu ($mg\ kg^{-1}$)	Zn ($mg\ kg^{-1}$)
GW compost	7.9	24	14	1.1	64	176	102	201
Peat	3.6	95	55	1.0	197	23	12	22
Coir	5.6	93	54	0.6	120	28	33	56
Woodbark	4.9	95	55	0.5	149	28	25	90

concentrations but where mode of contamination differs [21]. Microbial respiration under both types of contamination was examined in this study, in which the objectives were:

i. to determine the effect of metal toxicity on CO_2 evolved in organic materials that had been manually contaminated by Pb (II), Cu (II) and Zn (II)
ii. to determine the effect of added amendments on CO_2 evolved in a field contaminated soil.

2. Materials and methods

Amendment materials used were green waste compost, peat, coir and wood bark, and an initial characterisation of the materials before treatment is presented in Table 1 [2].

The contaminated field soil had a pH of 8.2, an organic matter content of 0.4% and cation exchange capacity of 2.4 $cmol_c\ kg^{-1}$, while total Pb, Cu and Zn were 2291, 127 and 1842 $mg\ kg^{-1}$, respectively [1].

2.1. Microbial respiration in metal spiked organic materials

Fresh organic material equivalent to 20 g oven dry weight was weighed individually into 500 ml jars, and brought to 50% moisture saturation by adding in an appropriate volume of a single metal solution. Solutions of $Pb(NO_3)_2$, $CuCl_2$ and $ZnSO_4{\cdot}7H_2O$ were added to achieve concentrations of zero (deionised water only), 5, 10 and 50 mg metal g^{-1} organic material. The experiment was set up as a 4 × 4 randomised design, with the variables being the four organic materials and the four metal concentrations. The effect of each metal was evaluated separately. Each sample was thoroughly mixed manually, after which the jars were sealed and left for two weeks as a conditioning incubation to allow microbial activity to stabilize before any readings were taken. Aerobic conditions were ensured by opening the jars for 2 h each week to allow replenishment of oxygen [11].

After two weeks, 10 ml of 1 M NaOH solution was placed in small jars on top of the moist organic materials and the bigger jar sealed. A jar with no organic material was used as the blank. After one week, the jar containing the NaOH was removed, 5 ml 1 M $BaCl_2$ added, and the NaOH solutions titrated with 1 M HCl to determine the amount of CO_2 released. 0.1% phenolphthalein was used as the indicator.

The CO_2 released per week was then measured as:

$$\text{total } CO_2 - C\text{ evolved (mg/kg)} = \frac{(\text{blank titre mls} - \text{amendment titre mls}) \times 6}{\text{amendment weight (g)}} \times 1000 \quad (1)$$

After the NaOH was taken out and titrated, the jars containing the organic materials were left open for an hour to replenish the air, and the moisture of the material brought back to 50% saturation with deionised water where necessary. A fresh NaOH solution was then placed into each jar, and sealed. The total incubation period was ten weeks, and CO_2 release was measured each week.

The inhibition effect of metals on CO_2 evolved was calculated as:

$$\%\ \text{inhibition} = \left(1 - \frac{B}{A}\right) \times 100 \quad (2)$$

where A is the cumulative CO_2 evolved in non contaminated amendment; B is the cumulative CO_2 evolved in contaminated amendment.

Thus, % inhibition is a comparison of respiration in individual amendments to their non contaminated counterparts.

2.2. Microbial respiration in field contaminated soil

The field contaminated soil was collected from the vicinity of a disused Pb mine in Tyndrum, Scotland. Fresh green waste compost, peat, coir and wood bark were added as amendments at the rates of 0, 1, 10 and 20% of soil weight (all weights on oven dry basis). 100 g of amended soil was weighed into 500 ml screw top jars, and allowed to pre-incubate for four weeks before CO_2 readings began, with weekly replenishment of oxygen as before. Amount of CO_2 evolved was measured through back titration as described above.

Amount of CO_2 evolved in contaminated soil was enhanced by the addition of amendments, and this enhancement was expressed as:

$$\%\ \text{enhancement} = \left(1 - \frac{C}{D}\right) \times 100 \quad (3)$$

where C is the cumulative CO_2 evolved in non amended soil; D is the cumulative CO_2 evolved in amended soil.

2.3. Statistical analyses

All statistical analyses were carried out with the programme MINITAB version for windows. The data were analysed using a general linear model of ANOVA, considering the treatments as the independent variable. Where F test was significant, Tukey's test at $p < (0.05)$ was used to determine LSD for the comparison of means. Correlation was by Pearson's coefficients at $p < 0.05$.

3. Results and discussion

3.1. Effect of metal toxicity on microbial respiration in contaminated organic materials

The quantity of CO_2 evolved by all the organic materials was adversely affected by the presence of Pb, Cu and Zn (Figs. 1–3). After 10 weeks of incubation, the rate of CO_2 evolved from amendments contaminated by 50 mg Pb g^{-1} was in the order wood bark > peat > coir > green waste compost: for contamination by 50 mg Cu g^{-1} it was coir > wood bark > peat > green waste compost: and for 50 mg Zn g^{-1}, it was in the order wood bark > coir = peat > green waste compost.

Weeks 6–10 were used to estimate the weekly CO_2 evolved to obtain an estimation of the microbial respiration rate, because at that stage, amendments had reached a relatively constant, linear rate of CO_2 production [22]. The mean weekly CO_2 production is presented in Table 2.

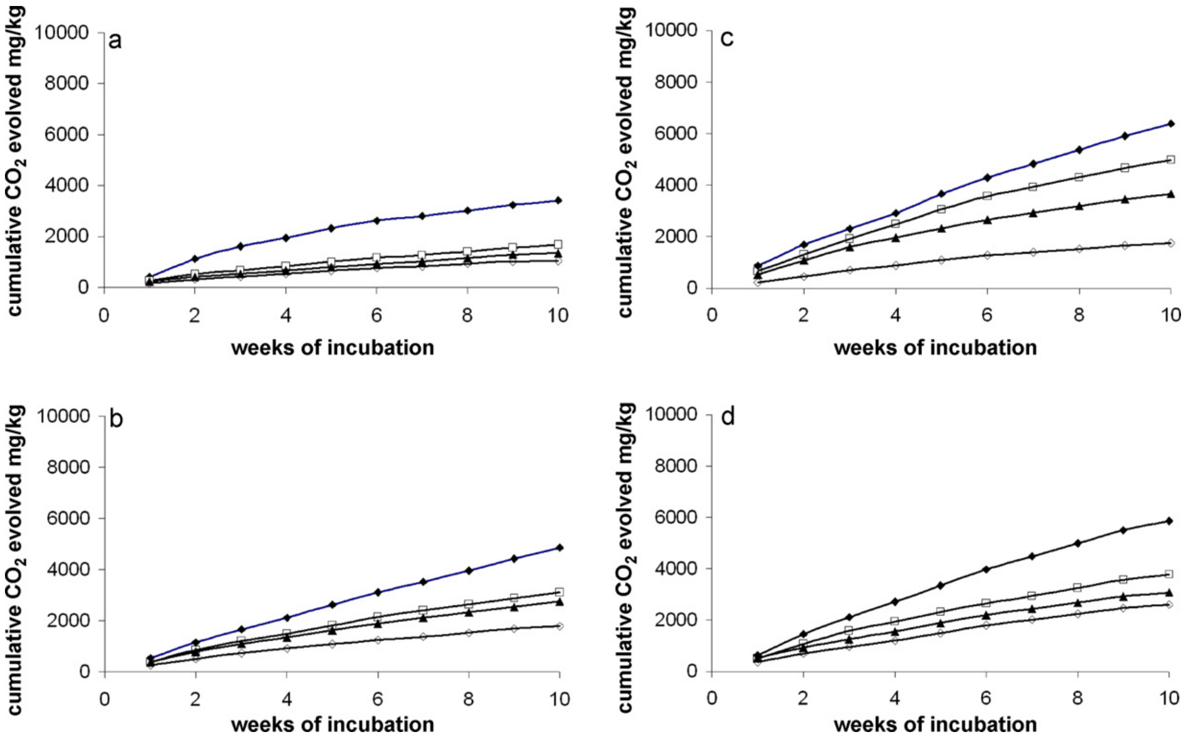

Fig. 1. Effect of Pb addition on CO_2 evolution in (a) green waste compost, (b) peat, (c) coir, and (d) wood bark. (♦) No metal addition: (□ 5) mg Pb g^{-1}: (▲) 10 mg Pb g^{-1}: (◊) 50 mg Pb g^{-1}.

3.1.1. Comparison of amendments (row comparisons)

In each of the separate metal incubations, CO_2 evolution in the non-contaminated materials was generally in the order coir > wood bark ≥ peat > green waste compost. Comparing amendments contaminated with the same metal concentration, the respiration in green waste compost was significantly lower for all metals, while CO_2 evolved in the coir, peat and wood bark were not significantly different from each other at Pb and Zn concentrations above 5 mg g^{-1} (Table 2), and not significantly different at 50 mg Cu g^{-1}. Thus, as metal concentrations increased, differences between amendment materials became less significant.

3.1.2. Comparison of metal concentrations (column comparisons)

For green waste compost, peat and wood bark, the amount of metal added had little effect, as in many cases the amount of CO_2 evolved by varying metal concentrations was not significantly

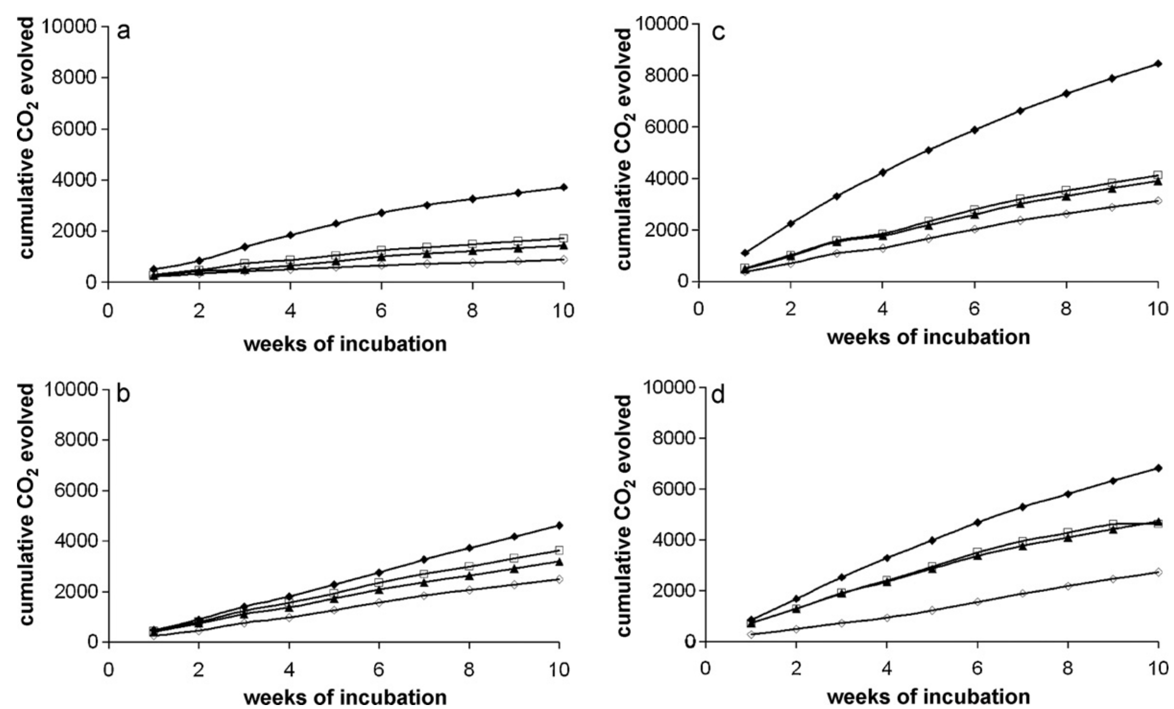

Fig. 2. Effect of Cu addition on CO_2 evolution in (a) green waste compost, (b) peat, (c) coir, and (d) wood bark. (♦) no metal addition: (□) 5 mg Cu g^{-1}: (▲) 10 mg Cu g^{-1}: (◊) 50 mg Cu g^{-1}.

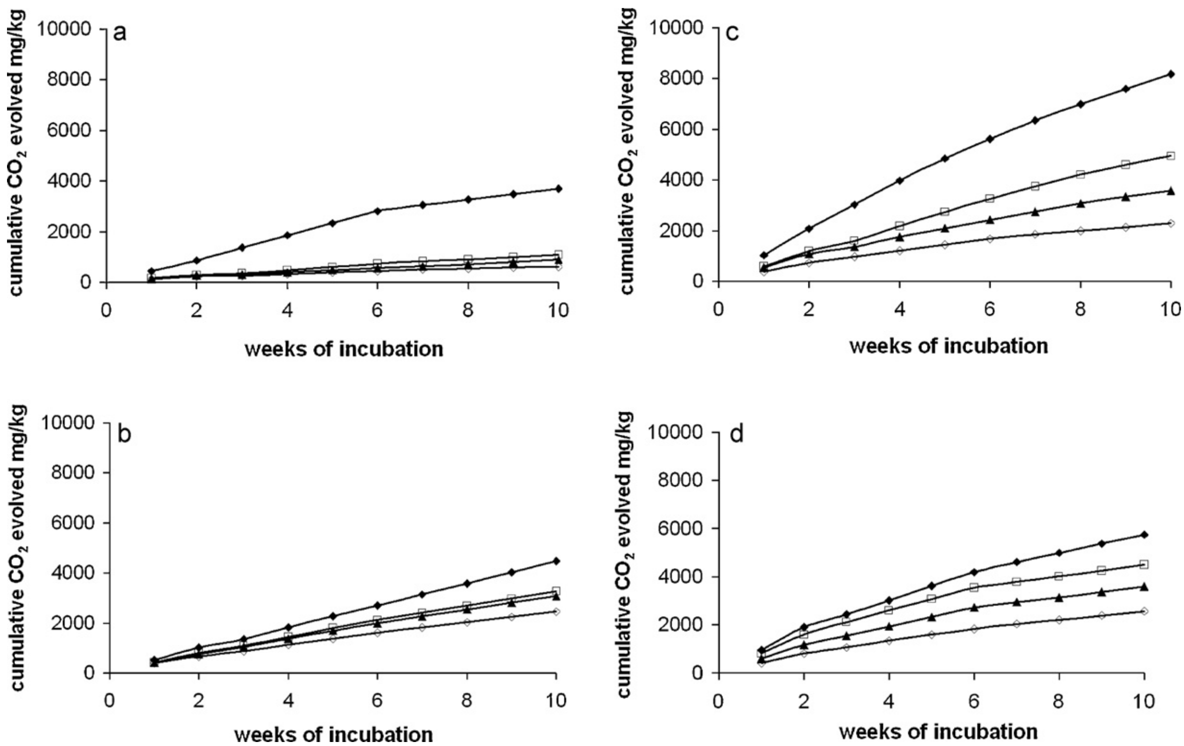

Fig. 3. Effect of Zn addition on CO_2 evolution in (a) green waste compost, (b) peat, (c) coir, and (d) wood bark. (♦) No metal addition: (□) 5 mg Zn g^{-1}: (▲) 10 mg Zn g^{-1}: (◊) 50 mg Zn g^{-1}.

different. Coir in all cases showed more stepwise effect of contamination, with CO_2 evolved becoming less as amount of metal increased. This suggests that coir is inactivating the metal.

3.2. Inhibition of microbial respiration by metals in 'spiked' amendments

The inhibition effect of metals on CO_2 evolved in metal spiked amendments is presented in Table 3. Inhibition of microbial respiration by metals did not follow a predictable pattern. At low concentrations of Pb the inhibitory effect was least in coir, but at 50 mg Pb g^{-1}, there was no significant difference in inhibition in peat, compost or coir. Inhibition in wood bark was significantly lower at this high concentration than in the other amendments. Inhibition in peat was significantly the least at all concentrations of Cu after 10 weeks of incubation. At 5 mg Cu g^{-1}, inhibition was highest in compost, but at 50 mg Cu g^{-1} inhibition was equally high in compost and coir after 10 weeks. In Zn contaminated amendments, inhibition was highest in compost, and least in peat and coir at 5 mg Zn g^{-1}. At 50 mg Zn g^{-1} however, % inhibition was in the order (highest to lowest) green waste compost > coir > wood bark > peat.

At 5 mg g^{-1} metal concentration microbial respiration was similar for all four amendments, but at 50 mg g^{-1} there was a clear separating out of the amendments (Figs. 1–3). Thus the ability of the amendments to sustain microbial respiration in the presence of toxicity was significantly different at high Cu and Zn concentrations, but not at low metal concentrations. Inhibition within a single treatment (i.e. same amendment/same metal/same concentration) was generally unchanged within the incubation period, being the

Table 2
Mean weekly rate of CO_2 evolved by metal-contaminated amendments.

	Amount of CO_2 evolved (mg C kg^{-1} $week^{-1}$)			
Lead mg/g	GW compost	Peat	Coir	Wood bark
0	203aB	441bC	528cD	480bC
5	131aA	240bB	356cC	288bB
10	111aA	213bAB	254bB	222bAB
50	77aA	140bA	123bA	207bA
Copper mg/g				
0	249aB	464bC	642dB	531cB
5	117aA	317bB	330bA	335bA
10	108aA	278bAB	321cA	288bcA
50	56aA	227bA	273bA	291bA
Zinc mg/g				
0	221aB	443bB	635cD	389bB
5	86aA	285bA	425cC	239bA
10	83aA	273bA	290bB	216bA
50	41aA	210bA	152bA	182bA

Data within a row compare the effect of the amendment used. Values in a row followed by the same lower case letter are not significantly different at $p < 0.05$. Data within a column compare the effect of metal concentration. Values in a column followed by the same upper case letter are not significantly different at $p < 0.05$. In both cases the least significant difference value was determined using the Tukey test.

Table 3
Inhibition by heavy metals of CO_2 evolution from organic amendments.

	Week	% Inhibition by Pb			% Inhibition by Cu			% Inhibition by Zn		
		5 mg Pb g^{-1}	10 mg Pb g^{-1}	50 mg Pb g^{-1}	5 mg Cu g^{-1}	10 mg Cu g^{-1}	50 mg Cu g^{-1}	5 mg Zn g^{-1}	10 mg Zn g^{-1}	50 mg Zn g^{-1}
GW compost	1	57.9d	66.4d	72.9c	46.7c	63.0b	68.5bc	73.9d	77.2d	80.4d
	5	55.4d	64.6d	70.9bc	54.1c	63.0b	75.7c	73.5d	79.9d	83.6d
	10	50.9d	60.5d	69.3bc	54.0c	61.3b	76.2c	70.4d	75.7d	83.0d
Peat	1	27.0ab	34.2ab	55.9a	11.7a	20.2a	45.7a	19.8ab	23.1a	35.2a
	5	30.9ab	39.1abc	59.9ab	14.7a	24.5a	42.9a	21.5ab	26.5a	40.3a
	10	36.1bc	43.5ab	63.3ab	21.4a	30.8a	46.1a	27.1b	31.1ab	45.2a
Coir	1	16.9a	30.5a	69.5bc	52.3c	53.6b	66.8bc	47.0c	55.0c	67.8c
	5	16.8a	38.1ab	70.3bc	52.6c	55.9b	65.6bc	42.1c	56.8c	70.1c
	10	22.1ab	42.7b	72.5c	51.2c	53.9b	62.9b	39.4c	56.1c	71.9c
Wood bark	1	24.8ab	40.4b	54.6a	24.4b	25.0a	71.4b	13.0ab	36.4b	56.2b
	5	33.2c	44.5b	54.7a	25.0b	27.9a	66.7b	15.4ab	35.1b	56.3b
	10	35.5c	47.6b	55.5a	32.3b	30.8a	60.0b	21.7ab	37.6b	55.4b

Values followed by the same letter within a single column are not significantly different at $p < 0.05$ using the Tukey test for least significant difference. GW: green waste.

same at the onset, midway, and at the end of the experiment after 10 weeks of the incubation.

In order to determine the relationship between organic carbon content and the cation exchange capacity (CEC) of an amendment, and the concentration of metal on the amount of inhibition in metal contaminated amendments, correlations between these parameters were determined using Pearson's correlation in the Minitab statistical package. Data for the entire incubation period of ten weeks were used, and the results are presented in Table 4.

There was significant negative correlation between the organic carbon content of each amendment and the amount of inhibition by all three metals. There was also significant negative correlation between an amendment's cation exchange capacity, and the inhibition of CO_2 evolved when contaminated by Cu and Zn. This indicates that the higher the organic carbon content or CEC, the lower the inhibitory effect of metals on respiration within the concentrations tested in the study. As expected, there was significant positive correlation between metal concentration and inhibition by Pb, Cu and Zn on all amendments, with the inhibition effect being in the order Pb > Cu > Zn. This confirms that respiration rate is negatively correlated with Zn, Pb and Cu content [23].

The effect of metals on CO_2 production may have been due to a direct inhibition of other related microbial processes, or decrease in substrate availability [24]. It has been established that at low metal concentrations, responses of microorganisms to contamination do not always lead to a reduction in amount of CO_2 evolved [5]. Such discrepancies may be explained by the fact that microorganisms differ in their sensitivity to metal toxicity [3].

It is possible that at lower metal concentrations or short-term exposure, the microorganisms may compensate by a higher C turnover and so lead to a gradual change in viability [25]. At much higher concentrations however, heavy metals cause immediate death [24]. Whenever there is any discrepancy at such high metal levels it is often due to a community shift, in which case the tolerance of the dominant microbial group will determine the respiration [25,26]. The combined effect of the acidity of the peat with an increase in metal concentrations led to a further constraint on respiration, confirming previous findings [27].

Table 4
Correlations between organic carbon, CEC, metal concentration, and inhibition of microbial respiration.

	% Organic carbon	CEC	Metal concentration
Inhibition by Pb	−0.480*	−0.374 ns	0.779***
Inhibition by Cu	−0.543**	−0.739***	0.556**
Inhibition by Zn	−0.749***	−0.825***	0.471*

* Represent significant Pearson's correlation $p < 0.05$.
** Represent significant Pearson's correlation $p < 0.01$.
*** Represent significant Pearson's correlation $p < 0.001$.

3.3. Effect of amendments on microbial respiration in field contaminated soil

Basal respiration in non-amended soil was very low, totalling only 93 mg C kg^{-1} over the entire incubation period of ten weeks (Fig. 4). Due to the very low organic matter content of the soil, it would offer very limited energy source to the microorganisms. There was however significant increase in CO_2 evolved once the soil was amended with organic materials (Fig. 4). As was observed in the metal spiked amendments in the first part of the study, the cumulative CO_2 evolved was highest in coir and wood bark amended soil, while that due to green waste compost was least. The green waste compost was a fine textured material which appeared to be already in an advanced stage of decomposition before it was added to the contaminated soil, and so microbial breakdown and consequently respiration, may have been greatly hampered in the soils amended with this material.

The chemical make up of the material will have a major influence on the rate of release of carbon dioxide. Komilis and Ham [28] have shown that the ratio of cellulose to lignin is important, and is used as a measure of maturity in composts. Cellulose is readily broken down by microbial action as it is a simple glucose polymer. Lignin on the other hand is a heterogeneous polymer of different phenolic monomers, so is much more recalcitrant and known to persist in soils [29]. In a study of the composting of a variety of municipal solid wastes [28], breakdown of cellulose and hemicellulose was shown to be responsible for >50% of the total dry mass loss, whereas lignin/humus breakdown accounted for only <22% of the weight loss. Thus, although not measured here, the cellulose:lignin ration of the materials used could have an influence on their breakdown, and hence the release of carbon dioxide.

Addition of amendments led to an enhancement of microbial respiration in the contaminated soil (Table 5). At 1% amendment, there was no significant difference between enhancement by compost, peat or wood bark after the first week of incubation. At this 1% rate of addition, the ability of compost to enhance respiration declined later in the incubation period, while coir and wood bark remained consistent throughout. At 10 and 20% additions, percent enhancement within individual materials did not differ significantly at any time during the incubation period.

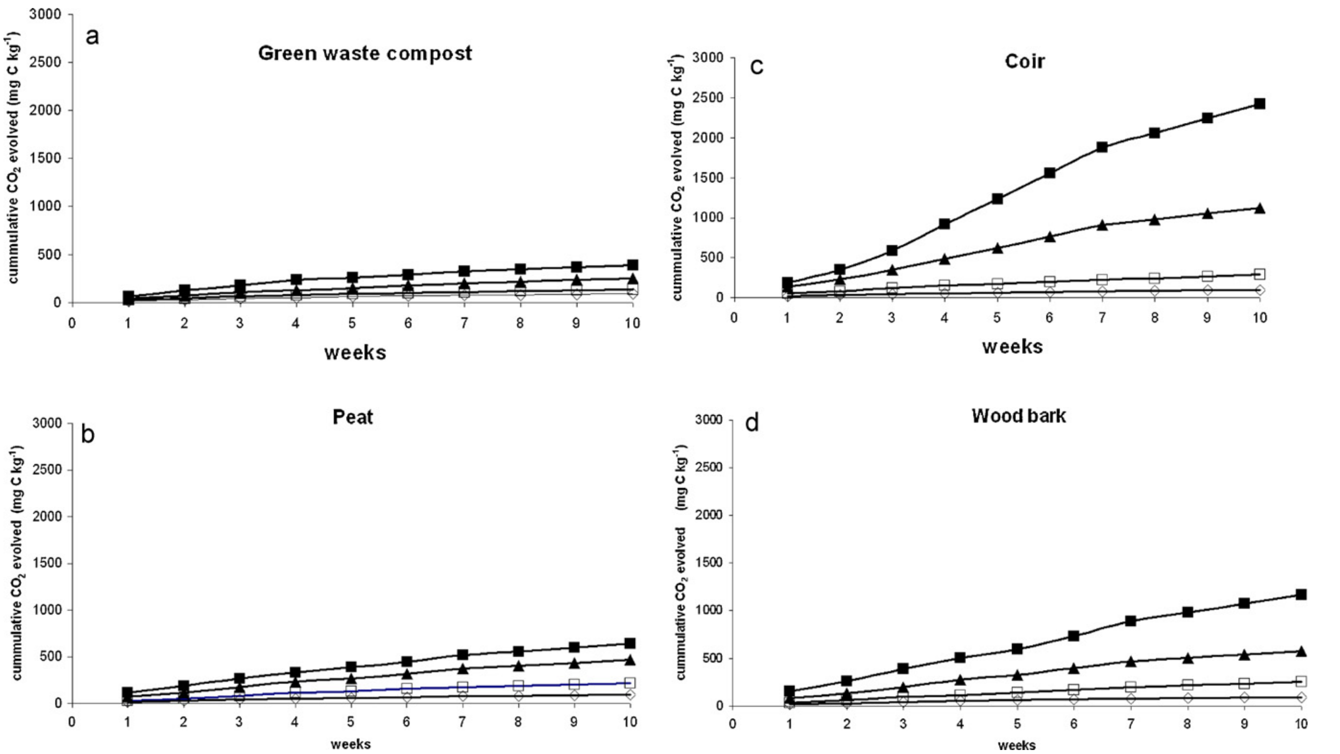

Fig. 4. Effect of amendment addition on CO_2 evolution in a Pb/Zn contaminated soil: (a) green waste compost, (b) peat, (c) coir, and (d) wood bark. (◊) No amendment addition: (□)1% amendment: (▲) 10% amendment: (■)20% amendment.

Comparing the different amendments however, enhancement was in the order coir > wood bark = peat > green waste compost (Table 5).

The addition of organic matter to Pb contaminated soil has been found to decrease the extent to which soil enzymatic activities are inhibited [30]. Other workers have also observed an increase in microbial activities after compost and wood chips were added to soils polluted with Cu and Ni [31]. That the addition of 20% amendment led to the greatest enhancement of microbial respiration may be due to the organic wastes causing an increase in the carbon and nutrient status of the soil [15]. Higher doses of waste material provide more organic matter to be degraded, thus delaying the appearance of metal toxicity, as the organic amendments bind the metal so decreasing its availability [32]. In the two separate incubation experiments, one with metal spiked amendments, and the other with contaminated soil, green waste compost performed poorest when compared with the other amendments. It is noted that it was lower in percent organic carbon than the other amendments [2].

After ten weeks of incubation with metal solution, water extraction of the contaminated amendments showed no significant difference in the amount of Pb extracted from all the amendments at any of the metal concentrations applied (data not shown). This may have been due to the insoluble nature of Pb (II) in water. Extractable Cu and Zn showed significant differences between amendments at applied Cu and Zn concentrations of 50 mg g^{-1} only, with water extraction in peat being significantly higher that the other amendments. This confirms the lower ability of peat to bind metals when compared to the other amendments as found previously [2].

Table 5
Amendment effect on enhancing microbial respiration in contaminated soil.

	Amendment rate	1%	10%	20%
	Week	% Enhancement		
GW compost	1	45.5b	60.0a	72.7a
	5	32.4a	61.7a	76.3a
	10	32.6a	63.5a	76.2a
Peat	1	40.0b	75.0b	84.2b
	5	56.6c	78.3b	84.6b
	10	56.9c	80.1bc	85.4b
Coir	1	64.7d	86.7cd	90.3bc
	5	65.2d	91.0d	95.6c
	10	67.7d	91.8d	96.2c
Wood bark	1	50.0bc	78.6b	88.2b
	5	59.6c	82.7bc	90.6b
	10	63.5cd	83.8c	92.1b

Values within a single column followed by the same letter are not significantly different at $p < 0.05$, using the Tukey test for least significant difference.

Table 6
Release of carbon from organic amendments and time required to release all carbon.

	Weight of carbon (mg)	Rate of C release (mg C wk^{-1})		% of total C released per week		Time required to release all carbon (years)	
		Unamended organic material	Field soil treated at 20% organic material	Unamended organic material	Field soil treated at 20% organic material	Unamended organic material	Field soil treated at 20% organic material
GW compost	2800	4.5	0.36	0.16	0.013	12	150
Peat	11,000	9.0	0.80	0.08	0.007	24	263
Coir	10,800	12.0	4.16	0.11	0.039	17	50
Wood bark	11,000	9.3	1.76	0.08	0.016	23	120

3.4. Comparison of respiration rates in uncontaminated organic materials and contaminated field soil

An average of the three controls for the mean weekly rates of CO_2 evolved (Table 2) gives values of 224, 449, 602 and 467 mg C kg^{-1} $week^{-1}$ for green waste compost, peat, coir and wood bark, respectively. These values can be directly compared with the release rates from the field contaminated soil that had been treated with 20% amendment, as in both cases 20 g of material were used. Table 6 shows the weight of carbon present in 20 g of each amendment, along with the release of CO_2, shown as mg C $week^{-1}$, and the % of the total C released. The very small amount of the total C released per week shows that the materials are sufficiently stable to persist in a soil. Indeed when added to a contaminated soil, such as the Pb/Zn mine soil used in this study, greater stability is conferred on the amendment due to the inhibition of microbial activity.

These figures allow an estimate to be made of the time required for all of the carbon in the amendment to be released. Such a calculation is very approximate, as it assumes that the rate of C release remains constant, and that all of the carbon can be released by microbial respiration. However, such an estimate does give an assessment of the relative persistence of each amendment material in a soil. The values suggest that they can remain in soil for considerable periods, although in practice it is likely that further additions would be made. Coir has proven to be the least stable of the materials used, and peat the most stable. However all of the materials could be effective for remediation of heavy metal contaminated soils.

4. Conclusions

The organic materials used as amendments for remediation of heavy metal contaminated soil can persist and act as sorbants for metals. Their rate of breakdown, as measured by CO_2 release is inhibited by heavy metals, although this can be modified as a result of their ability to immobilize the metals. When added to contaminated soil, they improve the microbial activity, which may influence other soil functions. However, concerns that the organic material would be too unstable to persist in soil have been shown to be unfounded.

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