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The Use of Microbial Respiration, Biomass Carbon and Metabolic Quotient for Assessing Soil Metal Pollution A-Review

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Abstract: With the intense industrial development and with the indiscriminate use of fertiliser and pesticides, soils in most part of the world have shown elevated levels of metals concentration. There is concern that metals present above certain levels in the soil may affect agricultural production. Furthermore, metals deposition in the soil might be a potential source of their introduction into food chain. Microbial parameters appear to be useful in monitoring soil metal pollution and could provide an early warning of deteriorating soil quality. Microbial activities and processes like soil microbial respiration, microbial biomass C, metabolic quotient (qCO_2), biomass N, community structure, N mineralisation, organic matter decomposition and enzymatic activities have been successfully used for studying metals effect on soil quality. This review, however, discussed the effects of soil metal pollution on microbial respiration, biomass carbon and metabolic quotient only. Short- and long-term studies and studies carried out under different environmental conditions were considered. Metals availability and toxicity differs in soil with different physicochemical properties. Effects of metals on microbial activity in soils with contrasting properties were also reviewed.

Key words: Metal pollution, microbial respiration, biomass carbon, metabolic quotient

Introduction

A large number of laboratory and field studies have been conducted to investigate the extent of pollution in soil caused by metals in sewage sludge applications, effluent and wastewater irrigation, smelter and vehicle emissions and mining activities etc. Microorganisms, having both mass and activity and being in intimate contact with the soil micro-environment can be affected by the disturbance caused by these metals (Brookes, 1995). Domsch *et al.* (1983) studied the effects of naturally occurring stresses such as fluctuations in temperature, extremes of water potential, extremes of pH, decreased supply of nutrients etc on soil microbial population and activities. Their conclusion was that these stresses singly or jointly, can markedly affect both the size and activity of soil microorganisms. On the basis of these results and many more, microbial indices have been frequently used to assess the extent of soil metal pollution.

The review of literature will concentrate on effects of metals on soil microorganisms and on metals behaviour in soils. Microbial activities and processes have been successfully used for studying metals effect on soil quality. The emphasis in this review will be on soil microbial respiration, microbial biomass C and metabolic quotient (qCO_2) in metal contaminated soils. The relative toxicity of different metals will also be considered. Available metals occur in the ionic form in the soil and can be bound strongly by organic materials such as humic acid and fulvic acid (Stevenson, 1976). Mathur (1983) reported that soils with high cation exchange capacity and more organic matter are known to bind metals and make them less available to microorganisms. Therefore effects of soil type on microbial responses to metal contamination will also be highlighted.

Soil respiration: Soil microbial respiration has been utilized for estimating microbial activity since the late 19th century (Domsch, 1961) and is the most studied variable in connection with metal pollution (Baath, 1989). Soils amended with contaminated sewage sludge, around smelters and roads, where metals have been added in field experiments and under laboratory conditions have been investigated for metal contamination by assessing effects on CO_2 evolution.

CO_2 evolution in the field is subject to enormous natural fluctuations (Domsch *et al.,* 1983) which may mask the effect of metals on soil microorganisms. However, it appears to be a sensitive measurement especially under standardised conditions with which to detect any disturbance in the soil (Baath, 1989). By standardizing the soil water content and minimized the variability among samples, Tyler (1974) was able to detect changes in respiration rate at lower contamination levels than Nordgren *et al.* (1983), who used soil samples at field moisture contents.

Higher microbial activity facilitates the measurement of the effects of soil pollutants. Therefore the addition of different substrates to the soil and the subsequent degradation of these substances measured as increased respiration rate has also been widely used to study metal effects on soil microorganisms. Glucose, acetate and many other compounds have been exploited to stimulate soil respiration as will be discussed latter.

In laboratory experiments where soils are contaminated with metal salts, with or without added substrate, results in most cases decreased CO_2 evolution with increased metal inputs. For example, Dar and Mishra (1994) found lower rates of CO_2 evolution in soil amended with sewage sludge (dried form) where this sludge treated with the equivalent of 25 and 50 µg Cd g^{-1} soil in a laboratory study. In another study Aoyama *et al.* (1993) applied Cu in the range of 100 to 1000 µg g^{-1} to soil, with or without plant residues (orchard grass) and found a significant decrease in CO_2 evolution with increased Cu level over the 12 weeks period of incubation. However, the reduction at the highest rate of Cu was more in the unamended soil (30%) than in the amended soil (2%), a result which could be explained the by effect of residues on metal avaiiabilities in soils. An earlier study by Bhuiya and Cornfield (1972) also reported somewhat lower values of CO_2 evolution in soil incubated without oat straw and pre-treated with 1000 µg g^{-1} of Cu. In contrast, Chang and Broadbent (1981) found a reduction in CO_2 evolution at lower concentrations of Cu (50 µg g^{-1}) in the soil where this was amended with alfalfa and sewage sludge. On the other hand Dar (1996) reported that sewage sludge added to soils did not mitigate the toxic effect of added Cd.

Responses of microbial respiration to metals in soils with different physicochemical properties vary. Babich and Stotzky (1977) reported that the toxicity of metal might be reduced by the specific abiotic properties of one soil, whereas in another soil with different physicochemical characteristics, the toxicity of an equivalent dose of the same metal may be greater. Doelman and Haanstra (1979) reported that a concentration of 375 µg Pb g^{-1} caused a 15% reduction in respiration of the sandy soil. In contrast the respiration of peat soil was not affected even up to 7500 µg Pb g^{-1}.

In laboratory experiments, metals are usually added as a single dose. However, in reality, soil pollution may be more or less continuous process. Therefore, Wilke (1991) investigated the effects of single and successive additions of Cd, Ni and Zn on soil respiration. Single doses were 100 peg Cd g^{-1}, 200 µg Ni g^{-1} and 200 pg Zn g^{-1} soil. Successive additions were 50 µg Cd g^{-1}, 100 kig Ni and Zn g- and then 10, 20 and 20 pg of Cd, Ni and Zn respectively were added at weekly intervals. Successive additions of metals inhibited soil respiration more than single doses, even before the same amount had been added. The greater effect on CO_2 evolution of successive additions was attributed to short-term increases of metal concentrations in the soil solution after each application. This suggests that metal of the same concentration accumulated over long period of time might be less toxic to microorganisms than in short-term laboratory experiments.

Soils contaminated due to smelter emissions also showed lower values of CO_2 evolutions compared with non-contaminated soils in most studies. Nordgren *et al.* (1986) studied soils which were smelters contaminated, with Cu and Zn the main polluting metals. Their results showed a significant negative relationship between increased metal contamination and respiration. Soil respiration rate decreased from 100 to 150 µg CO_2 g^{-1} dry soil h^{-1} to about 30 µg close to the smelter. Similar findings have been obtained elsewhere when smelters contaminated soils have been studied (Strojan, 1978; Laskowski *et al.,* 1994).

As discussed above, metal contamination tends to decrease soil microbial respiration. However, a substantial amount of evidence has indicated that increased metal inputs sometime increase CO_2 evolution. A series of experiments were carried out on a sandy loam soil at Woburn Experimental Station, UK, which received contaminated sewage sludge 30 years ago and where the soil total metal concentrations were at, or a little above, UK permitted limits (DoE, 1989). This study by Chander and Brookes (1991c) showed greater values of CD_C evolution from contaminated soil amended with glucose and maize straw compared with the non-contaminated soil during the first 5 days of incubation. Earlier study by Ausmus *et al.* (1978) has also shown that metal contamination significantly increased the rate of liner-soil CO_2 evolution compared with the control. However, this increase in CO_2 evolution was attributed to the disruption of fungal-root associations in the treated microcosms, which may have increased available substrate from fungal cells for a period of time. Chander and Brookes (1991c) suggested that where increases in respiration occurred, this might be the result of a shift of substrate utilization from biomass synthesis to maintenance.

Khan and Scullion (1999) added Cu (32, 112, or 182 mg kg^{-1}), Ni 138, 58 or 98 mg kg^{-1}) and Zn (177, 220 or 375 mg kg^{-1}) to grassland soil through sludges in a factorial design and monitored respiration for 7 weeks. They reported that respiration was reduced at 1 week by Ni input and by Cu in combination with Ni. Zinc inputs at 1 week and all metal inputs after 3 weeks increased respiration. Their conclusion was that respiration varied with time. Increased respiration mostly at tater stages of the experiments was attributed to stress of moderate metal contaminations on soil microorganisms. Furthermore, they reported that metals (Cu, Ni and Zn) effects were mostly non additive and that the effect of one metal was more pronounced (positive interaction) with the high level of the other (Khan and Scullion, 1999).

Similar results were also reported by Leita *et al*. (1995) who treated soil with Zn and Pb salts to yield a concentration of 600 µg Zn and 200 µg Pb g^{-1} of soil. The CO_2 evolution rate significantly increased in Zn contaminated soil in the first 3 weeks and thereafter the Zn effect tended to decrease. Pb, which did not affect microbial biomass C, also tended to increase CO_2 evolution. One reason given (Chander and Brookes, 1993; Fliessbach *et al.,* 1994; Leita *et al.,* 1995) by those who found increased CO_2 evolution rate in contaminated soil was the need of living organisms to consume more energy to survive.

Several studies have indicated that CO_2 evolution responses to metal inputs may change with time since application. For example Andreyuk *et al.* (1997) studied the effects of added Cu, Cd and Pb on CO_2 production and reported that in the first 2-3 days slight decreases in the Cu (2.6%) and Cd (19.4%) treated soils were observed, whereas Pb treated soil showed 50% increase at that time compared with the control. After 10 days the intensity of CO_2 production was higher (greater than 150%) in all metal treated soils than the control. After 30 days, increases in CO_2 production were less pronounced in Cu and Pb soils, but CO_2 evolution was still higher than in the control. However, Cd treatment did not show any effect at this time.

Doelman and Haanstra (1984) obtained similar results when studying CO_2 evolution responses to metal inputs, but these were inconsistent across five different soils. In the short-term (2 to 8 weeks), the effect of Cu in all soil was marked, except in the clay soil. In the longer-term (18 weeks), effects were less pronounced except for the sandy loam where they tended to increase. Among metals, Ni added at the rate of 3000 µg g^{-1} increased respiration by a factor of 4.5 in the sand and silty loam soil. In other soils (sandy loam, clay and peat) the effects were negative with increased Ni content. Zn on the other hand had a strong inhibitory short-term effect on CO_2 evolution in all soils. The long-term effect of Zn was less pronounced than the short-term but in most cases a significant inhibition was found at concentrations higher than 150 µg Zn g^{-1}. The increase found in CO_2 evolution due to some metals was either explained by the high pH or the high lime content in these soils.

A similar study by Khan and Scullion (2000) investigated the effect of soil types on microbial responses to same levels of metal contamination. Soils used were with varying clay and organic matter content and metals were added through sludges. Metal levels were control (sludge with no artificial metal contamination), medium (sludge contaminated with Cu = 112, Ni = 58 and Zn = 220 mg kg^{-1}) and high (sludge contaminated Cu = 182, Ni = 98 and Zn = 325 mg kg^{-1}). They reported that respiration rate In the early stage (1-2 weeks) of the experiment increased in all soils, however, at later stages high metal inputs caused significant reductions mainly in sandy loams compared with clay soils. The reduction in respiration in the sandy soils was attributed to the greater availability of metal in sandy soils with low cation exchange capacity and organic matter content compared with clay soils. They concluded that although soil pH influence metals availability and their toxicity, the role of organic matter and clay content should also be considered in assessing metals effect on microbial activities across different soils.

The inconsistencies reported above can sometimes be very difficult to interpret because it is not possible to distinguish a metal toxicity effect from an effect of metal addition on substrate availability. Some metals such as Pb may decrease the amount of substrate available to microorganisms through the formation of complexes and thus decrease the respiration rate (Tyler*,* 1974). Similarly death of microbial cells as a result of metal addition may explain the early increase in respiration in response to metal inputs as was reported by

Wilke (1991). He observed that Cd, Ni and Zn treated soils (individually) increased CO_2 evolution for the first 2 days and then a reduction in all metal contaminated soils was observed. It may be concluded from the literature reviewed that soil respiration was either unaffected or increased or decreased in the metal contaminated soils. These different CO_2 evolution responses in the metal contaminated soils could be due to the differences in the degree of contamination, to differences in soil properties and to variations in the source of C mineralized. Type of contamination (e.g. sewage sludge, smelters or road vehicle) could also be a factor responsible for the inconsistencies in the CO_2 evolution from metal contaminated soils.

Soil microbial biomass and related indices: Soil microbial biomass, the living part of soil organic matter, comprises the total mass of soil microorganisms and is defined as those that have volumes of less than 5000 μm^3 (Brookes, 1995). When soils are at near steady-state conditions with respect to total organic matter content, the soil microbial C comprises about 23% of the total organic C in a wide range of arable, grassland and woodland soils (Jenkinson and Ladd, 1981; Anderson and Domsch, 1989). Changes in soil management (e.g. due to inputs of organic matter manures such as sewage sludge) brings changes in the microbial biomass much faster than for the total soil organic matter content (Powlson *et al.,* 1987; Saffigna *et al.,* 1989). This and much other similar work supports the idea of Powlson and Jenkinson (1976) that measuring biomass could detect early changes in the soil before they are detectable in other ways. Similarly other authors have found that soil microbial biomass is the most labile fraction of soil organic matter and, therefore, can be a useful indicator of changes in soil conditions due to changes in soil management practices (McGrath *et al.,* 1994). Brookes (1995) reported that the microorganisms at the whole population level, at the functional group level, or at the single organism level could be a good potential indicator of metal contamination in the soil.

When metals are added to a soil, various microorganisms may vary in their response. Some may become intoxicated and lyse (a decrease in biomass), whereas resistant microorganisms may increase in number because of decreased competition (change in community structure) (Van Beelen and Doelman, 1997). Soil polluted with metals under laboratory studies have generally shown lower values of microbial biomass and a change in the community structure (Frostegard *et al.,* 1993; Leita *et al.,* 1995; Khan and Scullion, 1999, 2000).

Different metals may have different effects on microbial biomass. Leita *et al.* (1995) studied the influence of Zn (600 and Pb (20014 g^{-1}) on biomass survival and activity during a laboratory incubation of soil. Biomass C after 8 weeks was markedly decreased in the Zn contaminated soil. However, Pb did not have any inhibitory effect on the level of microbial biomass which may be because of its interaction with soil, which prevents toxic effects on the microbial biomass through a buffering effect (Mathur, 1983; Mathur and Farnham, 1985). In another short term laboratory study carried out by Khan and Scullion (1999) on grassland soils it was reported that close to UK permissible limits (DoE, 1989) Cu was more toxic to soil microorgaisms than Ni and Zn.

Like CO_2 evolution, microbial biomass seems to be less affected by metals in clay than in sandy soils. Dar and Mishra (1994) studied the effect of added Cd on microbial biomass in soil incubated with sewage sludge for a period of 2 months and found reductions of 25, 22 and 17% in sandy loam, loam and clay loam soil, respectively. Similarly, Haanstra and Doelman (1991) investigated metal effects on microbial activities in a range of five different soils and found that toxicity (defined as ED_{50}, ecological dose) was highest in sand and sandy loam soil and lowest in sandy peat soil.

In another experiment Dar (1996) treated sewage sludge amended and unamended clay-loam, loam and sandy-loam soils with cadmium chloride to yield 0, 10, 25, 50 µg Cd g^{-1} soil. After 2 months of incubation at 30 ± 1°C, Cd at 25 and 50 µg Cd g^{-1} soil caused a significant decline in microbial biomass in all soils. However, a comparatively smaller decrease in biomass was observed in the clay-loam and loam soils than in the sandy-loam soils. The author attributed this to the presence of more cation-exchange sites (clay minerals and organic complexes) in the clay-loam and loam soils. Earlier Babich and Stotzky (1977) also observed reductions in the toxicity of Cd to microorganisms attributable to clay minerals. Another study involving two different soils (Lee valley and Ludington) treated with single amendment (125 t dry solids ha^{-1}) of sludge contaminated predominantly with Zn (16000 mg kg^{-1}), Cu (8000 mg kg^{-1}), Ni (4000 mg kg^{-1}) (individually) 22 years ago was reported by Chander and Brookes (1991a). At Ludington site, the plots contained 1.5 times more Cu than is currently permitted for arable land receiving sludge in the UK (DoE, 1989) and had about 18% less biomass C than the uncontaminated plots. There was no effect of Ni at 138 mg kg^{-1} on the soil microbial biomass in either soil. The plots at Lee valley contained Cu 2.1 times the limit did not affect biomass. The differences between the two sites were ascribed by these authors to the effect of soil texture. The Lee valley soil was a heavier textured soil and had more organic matter. These findings (Chander and Brookes, 1991b) were confirmed in a recent study by Khan and Scullion (2000) where five different soils were contaminated with same levels of metals and incubated for 7 weeks. Biomass C measured both at 3 and 7 weeks decreased in all soils, however, treatment effects were greatest in sandy soils compared with clay soils.

A series of long-term experiments on soil subjected to repeated application of sewage sludge have shown that soil microorganisms exposed to long-term metal stress, even at modest metal concentrations, were not able to maintain the same overall biomass as in unpolluted soil (McGrath, 1994, 1984; McGrath *et al.,* 1995). For example Brookes and McGrath (1984) reported that microbial biomass in soil, which had received anaerobically digested sludge (between 1942-1967), was half that in the soil that had received farmyard manure during that time (control). Sludge application increased soil metal concentrations up to current limits (DoE, 1989) with the exception of Cd, which was three to five times the maximum limit. In contrast, the concentrations of metals in soil supplied with farmyard manure were small whereas the organic matter content and pH of both soils were the same. Reduced biomass in the sludged soil was therefore attributed to the toxic effects of metals in sludge on the soil microbial population. These differences were detectable 20 years after the last application of sewage sludge.

Similarly Witter *et al.* (1993) studied soil from a long-term field experiment at Upsalla, Sweden, which received sewage sludge and farmyard manure every 2 years from 1959 to 1988. Soil microbial biomass was found to be reduced by about 60%, in the soil treated with metal added sewage sludge compared with the soil treated with farmyard mannure. The total metal concentrations at which this reduction occurred were 125 µg Cu g^{-1}, 0.7 Cd g^{-1}, 35 µg g^{-1}, 40 µ Pb g^{-1} and 230 µg Zn g^{-1} dry soil. The procedure used for extracting these metals was not clear.

Two other field experiments at Braunschweig, Germany which received inorganic fertilisers, moderately contaminated and metal-spiked sewage sludge for 10 years from 1980-1990 showed that microbial biomass increased with increasing additions of moderately contaminated sewage sludge. In contrast, increases in biomass were less pronounced or even absent in the inorganic fertiliser treatments and for metal-contaminated sludge treatments. These effects on the biomass were apparent after 7 years of sludge additions (Fliessbach *et al.,* 1994). They also determined the efficiency of biomass synthesis (biomass C per unit soil C) and reported that the highest efficiency was found in the soil amended with low metal sludge (Fliessbach *et al.,* 1994).

On the other hand, Filcheva *et al.* (1996) did not observe reductions in biomass after 3 years in a field experiment on a granitic sandy loam soil with metal inputs. The highest rate of metals applied through sludge were 2 x the EU maximum permitted levels and soil sampling (0-7.5 cm) was done after growing grass and pasture for 3 years. However, unlike other experiments (Brookes and McGrath, 1984; Witter *et al*., 1993) sludge was left on the surface and not incorporated into the soil and the metals were added to the sludge.

Microbial biomass is sensitive to other abiotic and biotic stresses besides metals as discussed above. Knight *et al.* (1997) studied effects of Cu, Cd or Zn on microorganisms in soils with a range of pHs (4.5, 5.1, 6.3, 7.0) taken from a long-term liming experiment at the Woburn experimental farm in south east of England. Metal concentrations used were at around the maximum permissible values for these metals in agricultural land receiving sewage sludge. After a period of 3 years since metal inputs, the microbial biomass measured, did not show any reduction due to pH or metals except for the Cu treatments applied to soils with a pH 4.5, which caused a reduction in biomass C. At higher pH values the absence of toxic effects was correlated with absence of free Cu^{+2} from the soil solution. They also noticed a stimulation of biomass due to Cd treatment. Their conclusion was that this effect may be due to indirect effects of Cd on the availability of other essential micronutrients or that it was due to plant responses to Cd inputs.

Studies have shown that microbial responses to metals are easier to detect in a metabolically activated soil (Van Beelen and Doelman, 1997). This activation can be performed by the addition of substrates (like glucose) which can be used by a wide variety of microorganisms. Bardgett and Saggar (1994) studied the influence of metal contamination on the efficiency of conversion of fresh substrates (^{14}C glucose) into new soil microbial biomass. Through the incubation (4 weeks) the amount of added ^{14}C incorporated into the microbial biomass was consistently lower in the metal-contaminated than in the uncontaminated control soils. Their findings were similar to the earlier work reported by Chander and Brookes (1991c) and suggested that the microorganisms in contaminated soils were less efficient at using added glucose for synthesis of new microbial biomass.

Like CO_2 evolution, metals applied alone (without a carbon source) may cause greater reductions in soil biomass. For example Aoyama *et al.* (1993) artificially enriched soil (Brown Lowland) with different amount of Cu (ranges from 100 to 1000 $\mu g\ g^{-1}$) and incubated with or without pulverized orchard grass for 12 weeks at 25°C. A reduction in the microbial biomass C (measured by the fumigation-extraction method) was found at the maximum level of Cu of 58% (unamended with orchard grass) and 31% (amended with orchard grass) compared with the control. They also reported that the reduction in biomass C was much greater than the reduction in the CO_2 evolution and suggested that only a small part of the microbial biomass was involved in the decomposition of plant residues. Highly significant and negative correlations were also observed between $CaCl_2$ extractable Cu and biomass C. They concluded that water-soluble and exchangeable fractions of Cu in soil might control the size of the microbial biomass.

Analysis of soils contaminated with other sources such as run-off from timber treatment plants (Bardgett *et al.,* 1994), past applications of Cu-containing fungicide (Zelles *et al.,* 1994), Cu and Zn in animal manures (Christie and Beattie, 1989) and surrounding of metal-contaminated army disposal sites (Kuperman and Carreiro, 1997) have also revealed reductions in soil microbial biomass.

The ratio of microbial biomass carbon to soil carbon was reported by several authors to be a reliable index for evaluating metal contamination and was considered to be more sensitive than either microbial biomass C or total organic C (McGrath, 1994; Brookes, 1995; Leita *et al.,* 1995). There is a reasonably close linear positive relationship in uncontaminated soils between the soil organic C contents and their biomass C (Jenkinson and Ladd, 1981). However, Insam *et al.* (1996) did not find any significant effect on biomass C to organic C ratio for different soils whereas a significant decrease in CO_2 evolution per unit biomass (qCO_2) was detected in metal polluted compared with unpolluted soil. In contrast much greater reductions (up to 82%) in biomass C as a percentage of total organic C in soil contaminated with 900 mg Zn, 300 mg Cu, 150 mg Ni and 9 mg Cd kg^{-1} soil compared with controls soils and measured after 3 years of exposure were observed by Kandeler *et al*. (1996).

Bardgett *et al.* (1994) examined the effects of increasing metal contamination on soil microbial activity and found lower values of biomass C to total organic C. Valsecchi *et al.* (1995) studied three soils contaminated with irrigation water (urban wastewater) under different management practices and did not find any correlation between metals (NH_4-EDTA extractable) and biomass C. However, they found a close negative relationship between metals and microbial biomass C to soil C ratio. They concluded that, in accord with Anderson and Domsch (1993), a correct comparison between soils under different environmental conditions or subject to different agricultural practices cannot be made on the basis of absolute microbial biomass values but on the ratio of biomass C to soil organic C. In their earlier experiment Anderson and Domsch (1989) reported that microbial biomass C to organic C ratio may be high if a soil is in a state of C equilibrium. In contrast if a source of C (e.g. sludge) is applied to soil recently (e.g. short-term experiment), a greater proportion of it will not be utilized by microorganisms and therefore a lower biomass C to soil C ratio would be expected which would probably give a less clear picture of the metal toxic effect on microorganisms.

Specific microbial biomass respiration rate (metabolic quotient, qCO_2): In assessing the effects of metal on soil quality, there has been considerable interest in the development of easily measured bioindicators, which are sensitive to metal pollution. Microbial indices such as biomass C, soil respiration, community structure change, biomass C to organic C ratios etc all respond readily to soil disturbance and can provide an effective early warning on the deterioration of soil quality (Powlson *et al.,* 1987; Fliessbach *et al.,* 1994).

Anderson and Domsch (1990) proposed the ratio of soil basal respiration to microbial biomass (specific respiration of the biomass, metabolic quotient, qCO_2), based on the Odum's theory of ecosystem succession (Odum, 1985), as an alternative measure of changes in microbial biomass in response to disturbance. Some authors (Bardgett *et al.,* 1994; Fliessbach *et al.,* 1994; Leita *et al.,* 1995) have also suggested that microbial biomass and soil respiration are not sensitive parameters but the ratio between the respiration and biomass can be used as a moderately sensitive indicator of the effects of pollutants. Similarly Fliessbach *et al.* (1994) reported that differences in metabolic quotient between low and high metal soils were higher than those of biomass or respiration alone and suggested that this parameter was more sensitive to metal responses than biomass C and CO_2 evolution.

Brookes *et al.* (1984) found no effects of metal contamination on respiration rate in a long-term experiment at Woburn Farm experiment. But the metabolic quotient was considerably greater in the metal-contaminated soil. This was also confirmed by other authors (Brookes and McGrath, 1987; Chander and Brookes, 1991b) who studied soil from the same experimental site. This effect could be explained by the fact that metal-sensitive species have been replaced by other more tolerant groups which respire at a higher rate (Smith, 1991). Alternatively, the effect could be interpreted as a metal-induced stress response (Khan and Scullion, 1999). Another study reported an increase of 25% in metabolic quotient in soil, which was treated with sewage sludge between 1966 and 1989 and where the total metal concentrations (Cd, Cr, Cu, Pb and Zn) were below the current EC limits for soils (Dahlin *et al.,* 1997). In contrast, some authors (Baath *et al*., 1991) reported reductions in metabolic quotient in metal contaminated compared with uncontaminated soils and assumed it to be because of enhanced dormancy.

Other authors reported that the assumption that metal stress on the soil microorganisms may easily be detected by an increased metabolic quotient might not be correct all the time (Insam *et al*., 1996). Other soil properties such as texture, pH, organic matter or nutrient contents of the soils may mask the metal effect on metabolic quotient (Khan and Scullion, 2000). Anderson and Domsch (1993) were able to demonstrate that a decrease in pH caused an increase in the metabolic quotient of soil microorganisms.

Likewise, Wardle and Ghani (1995) reported that metabolic quotient has certain limitations, For example, metabolic quotient can be insensitive to disturbance and ecosystem development and may fail to distinguish between effects of disturbance (like ploughing) and stress.

Khan and Scullion (1999) reported that the net response of metabolic quotient depends on the degree of metals contamination and toxicity in the soil. At higher input rates metals may suppress microbial population markedly resulting lower respiration rate and metabolic quotient. Whereas in soils with moderate metals contamination population density may not be suppressed markedly and energy may be diverted from cells synthesis to maintenance resulting higher respiration per unit biomass (metabolic quotient).

These contradictory results regarding metabolic quotient could be because of the conflicting responses in CO_2 evolution in metal contaminated soils discussed earlier. An increase (Fliessbach *et al.,* 1994; Khan and Scullion, 1999) or decrease (Dar and Mishra, 1994; Khan and Scullion, 2000) in CO_2 evolution associated with lower biomass in response to metal contamination could lead to increased, decreased or unchanged metabolic quotient. It can be concluded from the above discussion that metals present above certain levels may cause reduction in soil microbial biomass. In contrast, soil respiration and metabolic quotient appear to be giving conflicting response to metal inputs. Within individual soils only, microbial respiration and metabolic quotient may be a valid parameter for monitoring soil metal pollution.

Due to the complex nature of the soil and different availability of metals, no single microbial parameter can be used universally. Similar metal inputs may be more toxic in sandy than in the clay soils. It can also be concluded from studies discussed above that the form in which metals are added to soil or the source of metal contamination affects the severity of toxic effects on microorganisms. It can be seen that limited research has been done on the interactive effects (synergistic and antagonistic) of metals on soil microorganisms. Also most studies used limited parameters for measuring metal effects on microorgansims and explained these results in terms of total soil metal contents rather than available fractions. In the light of the above discussion, studies assessing soil metal pollution must always take in to consideration the available metal rather total metal of the soil. Similarly, interactive effect of one metal on the availability and toxicity of the other and physicochemical of the soil which has contrasting effect on the responses of microorganisms to metal pollution (Khan and Scullion, 2000) should also be taken in to account.

References

Anderson, T.H. and K.H. Domsch, 1989. Ratios of the microbial biomass carbon to total organic carbon in arable soils. Soil Biol. Biochem., 21: 471-479.

Anderson, T.H. and K.H. Domsch, 1990. Application of eco¬physiological quotient (qCO_2 and qD) on microbial biomasses from soils of different cropping histories. Soil Biol. Biochem., 22: 251-255.

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

Andreyuk, E.I., G.A. Iutinskaya, E.V. Valagurova, V.E. Kozyritskaya, N.I. Ivanova and A.D. Ostapenko, 1997. The hierarchical system of the biological indication of soils contaminated by heavy metals. Eurasian Soil Sci., 30: 1338-1343.

Aoyama, M., S. Itaya and M. Otowa, 1993. Effects of copper on the decomposition of plant residues, microbial biomass and β-glucosidase activity in soils. Soil Sci. Plant Nutr., 39: 557-566.

Ausmus, B.S., G.J. Dodson and D.R. Jackson, 1978. Behaviour of heavy metals in forest microcosms: II: Effect on litter-soil-carbon metabolisms. Wet. Air Soil Poll., 10: 19-26.

Baath, E., 1989. Effects of heavy metals in soil on microbial processes and populations (A review). Water Air Soil Pollut., 47: 335-379.

Baath, E., K. Arnebrant and A. Nordgren, 1991. Microbial biomass and ATP in smelter-polluted forest humus. Bull. Environ. Contam. Toxicol., 47: 278-282.

Babich, H. and G. Stotzky, 1977. Effect of cadmium on fungi and on interactions between fungi and bacteria in soil: Influence of clay minerals and pH. Applied Environ. Microbiol., 33: 1059-1066.

Bardgett, R.D. and S. Saggar, 1994. Effects of heavy metal contamination on the short-term decomposition of labelled (^{14}C) glucose in a pasture soil. Soil Biol. Biochem., 26: 727-733.

Bardgett, R.D., T.W. Speir, D.J. Ross, G.W. Yeates and H.A. Kettles, 1994. Impact of pasture contamination by copper, chromium and arsenic timber preservative on soil microbial properties and nematodes. J. Biol. Fertil. Soils, 18: 71-79.

Bhuiya, M.R.H. and A.H. Cornfield, 1972. Effects of addition of 1000 ppm Cu, Ni, Pb and Zn on carbon dioxide release during incubation of soil alone and after treatment with straw. Environ. Pollut., 3: 173-177.

Brookes, P.C. and S.P. McGrath, 1984. Effect of metal toxicity on the size of the soil microbial biomass. Eur. J. Soil Sci., 35: 341-346.

Brookes, P.C. and S.P. McGrath, 1987. Adenylate energy charge in metal-contaminated soil. Soil Biol. Biochem., 19: 219-220.

Brookes, P.C., 1995. The use of microbial parameters in monitoring soil pollution by heavy metals. Biol. Fertil. Soils, 19: 269-279.

Brookes, P.C., S.P. McGrath, D.A. Klein and E.T. Elliott, 1984. Effects of Heavy Metals on Microbial Activity and Biomass in Field Soils Treated with Sewage Sludge. In: Environmental Contamination, Perry, R. (Ed.). CEP, Edinburgh, pp: 574-583.

Chander, K. and P.C. Brooks, 1991a. Effects of heavy metals from past applications of sewage sludge on microbial biomass and organic matter accumulation in a sandy loam and a silt loam UK soil. Soil Biol. Biochem., 23: 927-932.

Chander, K. and P.C. Brookes, 1991b. Plant inputs of carbon to metal-contaminated soil and effects on the soil microbial biomass. Soil Biol. Biochem., 23: 1169-1177.

Chander, K. and P.C. Brookes, 1991c. Microbial biomass dynamics during the decomposition of glucose and maize in metal contaminated soils. Soil Biol. Biochem., 23: 917-925.

Chander, K. and P.C. Brookes, 1993. Residual effects of zinc, copper and nickel in sewage sludge on microbial biomass in a sandy loam. Soil Biol. Biochem., 25: 1231-1239.

Chang, F.H. and F.E. Broadbent, 1981. Influence of trace metals on carbon dioxide evolution from a yolo soil. Soil Sci., 132: 416-421.

Christie, P. and J.A.M. Beattie, 1989. Grassland soil microbial biomass and accumulation of potentially toxic metals from long-term slurry application. J. Applied Ecol., 26: 597-612.

Dahlin, S., E. Witter, A. Martensson, A. Turner and E. Baath, 1997. Where's the limit? Changes in the microbiological properties of agricultural soils at low levels of metal contamination. Soil Biol. Biochem., 29: 1405-1415.

Dar, G.H. and M.M. Mishra, 1994. Influence of cadmium on carbon and nitrogen mineralization in sewage sludge amended soils. Environ. Pollution, 84: 285-290.

Dar, G.H., 1996. Effects of cadmium and sewage-sludge on soil microbial biomass and enzyme activities. Bioresour. Technol., 56: 141-145.

DoE., 1989. Code of practice for the agricultural use of sewage sludge. Department of Environment (DoE), HMSO, London.

Doelman, P. and L. Haanstra, 1979. Effect of lead on soil respiration and dehydrogenase activity. Soil Biochem., 11: 475-479.

Doelman, P. and L. Haanstra, 1984. Short-term and long-term effects of cadmium, chromium, copper, nickel, lead and zinc on soil microbial respiration in relation to abiotic soil factors. Plant Soil, 79: 317-327.

Domsch, K.H., 1961. Bodenatmung-sammelbericht Abe methoden und ergebnisse. Zentralblatt fur Bakteriologie, Mikrobiologie und Parasitenkunde, 116: 33-78.

Domsch, K.H., G. Jagnow and T.H. Anderson, 1983. An ecological concept for the assessment of side-effects of agrochemicals on soil microorganisms. Res. Rev., 86: 65-106.

Filcheva, E., M.V. Cheshire, C.D. Campbell and D.B. McPhail, 1996. Effect of heavy metal contamination on the rate of decomposition of sewage sludge and microbial activity. Applied Geochem., 11: 331-333.

Fliessbach, A., R. Martens and H.H. Reber, 1994. Soil microbial biomass and microbial activity in soils treated with heavy metal contaminated sewage sludge. Soil Biol. Biochem., 26: 1201-1205.

Frostegard, A., A. Tunlid and E. Baath, 1993. Phospholipid fatty acid composition, biomass and activity of microbial communities from two soil types experimentally exposed to different heavy metals. Applied Environ. Microbiol., 59: 3605-3617.

Haanstra, L. and P. Doelman, 1991. An ecological dose-response model approach to short-and long term effects of heavy metals on aryl sulfatase activity in soil. Biol. Fertility Soils, 11: 18-23.

Insam, H., T.C. Hutchinson and H.H. Reber, 1996. Effects of heavy metal stress on the metabolic quotient of the soil microflora. Soil Biol. Biochem., 28: 691-694.

Jenkinson, D.S. and J.N. Ladd, 1981. Microbial Biomass in Soil: Measurement and Turnover. In: Soil Biochemistry, Paul, E.A. and J.N. Ladd (Eds.). Marcel Dekker, New York, USA., pp: 415-471.

Kandeler, E., C. Kampichler and O. Horak, 1996. Influence of heavy metals on the functional diversity of soil microbial communities. Biol. Fertility Soils, 23: 299-336.

Khan, M. and J. Scullion, 1999. Microbial activity in grassland soil amended with sewage sludge containing varying rates and combinations of Cu, Ni and Zn. Biol. Fertility Soils, 30: 202-209.

Khan, M. and J. Scullion, 2000. Effect of soil on microbial responses to metal contamination. Environ. Poll., 110: 115-125.

Knight, B.P., S.P. Mcgrath and A.M. Chaudri, 1997. Biomass carbon measurements and substrate utilization patterns of microbial populations from soils amended with cadmium, copper, or zinc. Applied Environ. Microbiol., 63: 39-43.

Kuperman, R.G. and M.M. Carreiro, 1997. Soil heavy metal concentrations, microbial biomass and enzyme activities in a contaminated grassland ecosystem. Soil Biol. Biochem., 29: 179-190.

Laskowski, R., M. Maryanski and M. Niklinska, 1994. Effect of heavy metals and mineral nutrients on forest litter respiration rate. Environ. Pollut., 84: 97-102.

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

Mathur, S.P. and R.S. Farnham, 1985. Geochemistry of Humic Substances in Natural and Cultivated Peetlands. In: Humic Substances in Soil, Sediment and Water: Geochemistry, Isolation and Characterization, Aiken, G.R., D.M. McKnight, R.L. Warshaw and P. MacArthy (Eds.). John Wiley and Sons, New York, ISBN: 9780471882749, pp: 52-86.

Mathur, S.P., 1983. A lack of bactericidal effect of subsidence-mitigating copper in organic soils. Can. J. Soil Sci., 63: 645-649.

McGrath, S.P., 1984. Metal concentrations in sludges and soil from a long-term field trial. J. Agric. Sci., 103: 25-35.

McGrath, S.P., 1994. Effects of Heavy Metals from Sewage Sludge on Soil Microbes in Agricultural Ecosystems. In: Toxic Metals in Soil-Plant Systems, Ross, S.M. (Ed.)., Wiley, New York, USA., ISBN: 0-471-94278-0, pp: 247-273.

McGrath, S.P., A.M. Chaudri and K.E. Giller, 1994. Long-term effects of land application of sewage sludge. Proceedings of the 15th World Congress of Soil Science, July 10-16, 1994, Acapulco, Mexico, pp: 517-533.

McGrath, S.P., A.M. Chaudri and K.E. Giller, 1995. Long-term effects of metals in sewage sludge on soils, microorganisms and plants. J. Indian Microbiol., 14: 94-104.

Nordgren, A., E. Baath and B. Soderstrom, 1983. Microfungi and microbial activity along a heavy metal gradient. Applied Environ. Microbiol., 45: 1829-1837.

Nordgren, A., T. Kauri, E. Baath and B. Soderstrom, 1986. Soil microbial activity, mycelial lengths and physiological groups of bacteria in a heavy metal polluted area. Environ. Pollut. Ser. A: Ecol. Biol., 41: 89-100.

Odum, E.P., 1985. Trends expressed in stressed ecosystems. Bioscience, 35: 419-422.

Powlson, D.S. and D.S. Jenkinson, 1976. The effects of biocidal treatments on metabolism in soil-II. Gamma irradiation, autoclaving, air-drying and fumigation. Soil Biol. Biochem., 8: 179-188.

Powlson, D.S., P.C. Prookes and B.T. Christensen, 1987. Measurement of soil microbial biomass provides an early indication of changes in total soil organic matter due to straw incorporation. Soil Biol. Biochem., 19: 159-164.

Saffigna, P.G., D.S. Powlson, P.C. Brookes and G.A. Thomas, 1989. Influence of sorghum residues and tillage on soil organic matter and soil microbial biomass in an Australian vertisol. Soil Biol. Biochem., 21: 759-765.

Smith, S.R., 1991. Effects of Sewage Sludge Application on Soil Microbial Processes and Soil Fertility. In: Advances in Soil Science, Stewart, B.A. (Ed.). Springer-Verlag, New York, ISBN: 978-1-4612-7812-2, pp: 191-212.

Stevenson, F.J., 1976. Binding of Metal Ions by Humic Acids. In: Environmental Biogeochemistry: Metals Transfer and Ecological Mass Balances, Nriagu, J.D. (Ed.). Ann Arbor Science, USA., ISBN: 9780250401260, pp: 519-540.

Strojan, C. L., 1978. Forest leaf litter decomposition in the vicinity of a zinc smelter. Oecologia, 32: 203-212.

Tyler, G., 1974. Heavy metal pollution and soil enzymatic activity. Plant Soil, 41: 303-311.

Valsecchi, G., C. Gigliotti and A. Farini, 1995. Microbial biomass, activity and organic matter accumulation in soils contaminated with heavy metals. Biol. Fertil. Soils, 20: 253-259.

Van Beelen, P. and P. Doelman, 1997. Significance and application of microbial toxicity tests in assessing ecotoxicological risks of contaminants in soil and sediment. Chemosphere, 34: 455-499.

Wardle, D. A. and A. Ghani, 1995. A critique of the microbial metabolic quotient ($q$$CO_2$) as a bioindicator of disturbance nd ecosystem development. Soil Biol. Biochem., 27: 1601-1610.

Wilke, B.M., 1991. Effects of single and successive additions of cadmium, nickel and zinc on carbon dioxide evolution and dehydrogenase activity in a sandy luvisol. Biol. Fertil. Soils, 11: 34-37.

Witter, E., A.M. Martensson and F.V. Garcia, 1993. Size of the soil microbial biomass in a long-term field experiment as affected by different nitrogen fertilizers and organic. Soil Bol. Biochem., 25: 659-669.

Zelles, L., Q.Y. Bai, R.X. Ma, R. Rackwitz, K. Winter and F. Beese, 1994. Microbial biomass, metabolic activity and nutritional status determined from fatty acid patterns and poly-hydroxybutyrate in agriculturally-managed soils. Soil Biol. Biochem., 26: 439-446.