

# CHROMIUM ENVIRONMENTAL ISSUES

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## 1. INTRODUCTION

Soil micro-organisms (collectively the soil microbial biomass) and soil fauna mineralize both fresh organic inputs and the much larger pool of soil organic matter. The fertility of natural soil ecosystems therefore significantly depends on the rate of turnover of the soil organic matter, mediated by the soil microbial biomass. Agents that may suppress or poison soil organisms, or change the quality or quantity of organic matter (either fresh inputs or the soil organic matter itself), can damage the functioning of the soil-plant ecosystem either in the short-term or over much longer periods (Brookes and Verstraete, 1989).

In agricultural ecosystems soil fertility can be increased by applications of inorganic or organic fertilizer. The fertility of natural ecosystems however depends almost entirely on natural microbial processes, including  $N_2$ -fixation, the mineralization of organic forms of N, C, P and S, and organic matter transformations, all mediated by the soil microbial biomass. Any decline in natural fertility resulting from pollutants entering soils will therefore have proportionately greater effects on natural ecosystems.

Chromium can enter soils from a number of sources, common to all heavy metals, including wastes from mines and smelters, atmospheric deposition (following release of metals into the atmosphere from metal smelting or other industries) and sewage sludge. In most areas chromium is not a major problem in terms of soil pollution. In Italy, however, because of the very large leather tannery industry, producing chrome-tanned leather residues, there is a special situation. The residues are converted to leather-meal which is officially accepted as an organic nitrogenous fertilizer under Italian regulations. Indeed, leather meal is the most important organic N fertilizer in Italy. As well as containing high concentrations of mineralizable nitrogen (10 to 13%) and organic matter, it also contains from about 1 to 3% Cr (Ciavatta and Sequi 1989). While Cr is generally considered as one of the least toxic heavy metals in soil, being exceedingly immobile, in view of the high concentrations of Cr in the leather fertilizer, and the practically indefinite residence time of Cr in soil, it is important that all aspects of the possible environmental risks of Cr addition to soil by this route are carefully explored. For example, while the Cr(III) forms present in the residues are considered immobile, Cr(IV) and Cr(VI) forms are soluble, can be leached and are very toxic to plants (Turner and Rust 1971) and soil micro-organisms (Ueda *et al.* 1988). Currently, there is no mandatory European Union (EU) limit on permitted soil Cr concentrations in soil. Guidelines vary between Member States. For example, the maximum recommended limit for Cr in sludge-amended Danish soils is 30  $\mu\text{g g}^{-1}$  soil while it is 400  $\mu\text{g g}^{-1}$  in UK (MAFF and DoE, 1993).

For heavy metals such as Cu, Ni, Cd and Zn, mandatory EU limits restrict the amounts of metals permitted to accumulate in agricultural soils. The limits are based upon known effects of metals on

plant uptake and animal health. They take no account of effects on soil micro-organisms or important microbial processes, e.g. organic matter or soil N dynamics. This is because, at the time the limits were originally set, the methods necessary to investigate the effects of metals on these properties were not available. However, recently developed methods have indicated significant effects of heavy metals such as Cu, Ni, Cd and Zn at concentrations around, or below, current EU limits on both the standing crop of microbial biomass and its activity. Partly as a result of this work it has now been proposed that the maximum permitted concentration for Zn in soil in the UK treated with sewage sludge be lowered from 300 to 200  $\mu\text{g Zn g}^{-1}$  soil (MAFF and DoE, 1993; RCEP, 1996). Comparable systematic studies have not yet been done for Cr. However, we suggest that the same procedures could easily be tested with Cr and they are described with this in mind.

### 3. EVALUATION OF THE POTENTIAL OF MICROBIOLOGICAL PROPERTIES AS INDICATORS OF SOIL POLLUTION BY Cr

Detection of small, transient changes in microbial population size or microbial activity in the laboratory or field, as a response to a real or apparent pollutant should not, in the light of the large natural variations observed in the field, be accorded a great deal of weight (Domsch *et al.*, 1983). Based on these criteria, only those approaching or exceeding a recovery period of around 30-60 days should be considered 'critical', while over the first 30 d at least a depression in activity of 90% followed by recovery, still falls within the range considered 'normal' (Somerville *et al.*, 1987). It should be noted that this theoretical framework was originally developed for testing the side effects of pesticides. However, it would seem sensible to extend the same approach

to other soil pollutants, such as heavy metals. It also seems unlikely that field monitoring of the effects of heavy metals or other pollutants on microbial processes will permit detection of many effects, because of the likely spatial and temporal variation that will be encountered (See Cook and Greaves, 1987 for examples of this). However, once soil has been sampled, sieved and incubated moist at 20-25°C for several days, most microbiological activities approach some basal level and generally proceed at relatively constant rates for considerable periods, e.g. respiration and N mineralization (Jenkinson, 1988). Measurements of both microbial biomass and microbial activities may be conveniently measured under such conditions, but we must recognise the need for caution in extrapolation from the laboratory to the field.

#### 4. CRITERIA TO BE USED IN SELECTION OF POSSIBLE MICROBIOLOGICAL PARAMETERS AS INDICATORS IN SOIL POLLUTION MONITORING

Let us consider some basic criteria that a microbiological parameter might be expected to fulfil as an indicator in monitoring soil pollution by Cr or other heavy metals.

1. The parameter needs to be accurately and precisely measurable across a wide range of soil types and soil conditions.
2. Because a large number of samples usually have to be analyzed it is preferable that the parameter can be measured easily and economically.
3. The parameter needs to be of a nature that control or background measurements can also be made so that the effects of the pollutant can be precisely determined.

4. The parameter needs to be sufficiently sensitive to indicate pollution but also sufficiently robust not to give false alarms.
5. The parameter needs general scientific validity based upon reliable and contemporary scientific knowledge.
6. It may be that reliance upon a single parameter is unsafe. Two or more, preferably independent, might be chosen. If so, their interrelations in non-polluted environments should be known.

## 5. SELECTION OF POSSIBLE MICROBIOLOGICAL PARAMETERS AS INDICATORS IN MONITORING SOIL POLLUTION

Microbiological parameters as indicators of soil pollution by heavy metals fall into two main groups. The first contains those that measure the activity of the whole population or a very large proportion of it, e.g. respiration, N mineralization, or activity of individual members of its population or discrete groups or perhaps one of their enzymes e.g. nitrification,  $N_2$  fixation. The second type measures the size of the population; either the microbial biomass for the whole population or specific microbial groups. A third possibility also exists by combining both activity and biomass data, giving specific activities of the microbial population; see below.

The use of pure cultures of micro-organisms isolated from soil as indicators of soil pollution is rejected. Pure cultures of isolated organisms may be atypical of their form in soil or may undergo undetected change during storage. Their metabolic rate may be very different from that in soil as they are removed from their normal ecological interactions. In view of this, interpretation of results and extrapolation to field conditions is impossible (Greaves *et al.*, 1980).

## 6. MICROBIAL ACTIVITY MEASUREMENTS

Any measurement *in isolation* is of little use in deciding if an ecosystem is suffering from pollution. Microbial activity measurements are valuable in evaluating soil pollution, but the problem is always to decide what to compare the measurement with, i.e. what 'control' to use. The problem is removed if properly constructed field experiments with fully replicated treatments are available. A good example is the Woburn Market Garden Experiment at Rothamsted (see below), which has provided unique data on the effects of heavy metals on microbial biomass and microbial activity (Brookes and McGrath, 1984). Interpretation of data from the natural environment is much more difficult as the determination of basal levels of microbial activity is a real problem. In view of this most of the following data are from field or laboratory experiments. Much of the following data refers not to work with Cr, but to other heavy metals, especially Cu, Cd and Zn. The main aim is to illustrate the techniques that we consider could, and should, be used to evaluate the possible effects of Cr on microbial processes in future research.

## 7. MICROBIAL RESPIRATION

Under controlled laboratory conditions at suitable moisture, e.g. 40-50% water-holding capacity (WHC), and temperature, e.g. 15°-25°C, the respiration of sieved (2-6.25 mm) soil can be determined accurately and precisely (Jenkinson and Powlson 1976). This measurement is widely used by microbial ecologists as an index both of microbial activity and microbial biomass itself. However, microbial respiration appears unaffected at heavy metal concentrations at around current EU mandatory limits (e.g. Brookes and McGrath 1984; Brookes *et*

*al.* 1984). Only at very large heavy metal concentrations is CO<sub>2</sub> evolution decreased. Thus Tyler (1981) reported that microbial respiration was decreased at more than 1000 µg g<sup>-1</sup> of Cu or Zn. Similarly, Bardgett *et al.* (1994) reported that soil respiration declined linearly at up to about 15000 µg Cr g<sup>-1</sup> soil. However since the soils were also contaminated with closely correlated amounts of both Cu and As at up to similar soil concentrations it is impossible to attribute the effects to Cr alone.

## 8. NITROGEN AND CARBON MINERALIZATION

It seems that mineralization rates of soil organic N, as with microbial respiration, is unaffected at soil metal concentrations at around current EU limits (e.g. Brookes *et al.* 1984). This is supported by Doelman (1986) who reported that inhibition of both N mineralization and nitrification are inhibited at around 1000 µg g<sup>-1</sup> Zn, Cu and Ni, around 100-500 µg g<sup>-1</sup> Pb and Cr and around 10-100 µg g<sup>-1</sup> Cd. Benedetti *et al.* (1991) reported no adverse effects on N mineralization by Cr as Cr<sub>2</sub>O<sub>3</sub> or in leather meal, both at 100 µg Cr g<sup>-1</sup> soil.

There is a general consensus that interpretations of the effects of heavy metals on N mineralization and nitrification is difficult for several reasons (e.g. Båath 1989; Brookes and Verstraete 1989; Chander 1991). These include lack of standardization of experimental procedures and because of variation in soil properties which may alter the relative or absolute toxicity of the metal (e.g. Tyle: 1981). Similarly, Duxbury (1985) concluded that information on the effects of pollutants such as heavy metals on these processes is conflicting and recommended caution in generalizing about the effects of heavy metals on microbially mediated processes in natural environments.

There is evidence that heavy metals introduced in sewage

sludge, and giving soil metal concentrations several times the current EU mandatory limits, cause accumulation of soil organic matter compared with controls receiving uncontaminated sewage sludge. Thus Chander and Brookes (1991b) reported that soil at a site in the UK, Luddington (15% clay), which contained Cu at 3.7 times the current UK limit of  $140 \mu\text{g g}^{-1}$  contained 32% more organic matter than a soil receiving uncontaminated sludge. At Lee Valley (21% clay), soil contaminated with Zn at 3.4 times the permitted concentration contained 10% more organic matter than those treated with uncontaminated sludge. Similarly, plots containing Cu at 3.8 times the limit had about 14% more organic matter. These results strongly suggest that the heavy metals were decreasing the turnover rate of the organic matter, presumably because of inhibitory effects on the microbial biomass itself. Provided suitable sites could be found this work could easily be done with Cr also.

## 9. MICROBIAL POPULATION MEASUREMENTS

Measuring effects of heavy metals on the micro-organisms themselves, rather than their activity, is another possible means of monitoring soil pollution. There are two main ways in which pollutants can act upon the microbial population. One is by producing direct toxic effects, i.e. killing or biochemically disabling the organisms - antibiotics are a good example. The other way is by operating indirectly - for example by decreasing the availability of a substrate, e.g. plant root exudates. Thus the decreased energy available to the microbes could also result in a smaller population.

There are serious problems in trying to relate *in vitro* studies of heavy metals upon micro-organisms to the soil environment, and this will not be considered here, unless it provides useful background information.

For example, numerous papers refer to laboratory experiments where heavy metals were added to micro-organisms *in vitro* at concentrations far exceeding those present in soil solution. Studies of single species or groups of micro-organisms in soil are also difficult because most organisms cannot be easily isolated from soil.

With heavy metals, effects upon the microbial population are essentially permanent, both because of their toxicity and persistence. This is illustrated by reference to effects of heavy metals upon the soil microbial biomass.

#### 10. EFFECTS OF HEAVY METALS FROM PAST APPLICATIONS OF SEWAGE SLUDGE UPON THE SOIL MICROBIAL BIOMASS

The soil microbial biomass, the living part of soil organic matter, comprises the total mass of micro-organisms that live in soil, defined as those with volumes of less than  $5000 \mu\text{m}^3$ . Methods now exist for measuring the biomass as a single compartment, and these have been reviewed elsewhere (e.g. Jenkinson and Ladd 1981; Jenkinson 1988). The fumigation-extraction procedure for measuring the biomass, coupled with automated analysis (Vance *et al.* 1987; Wu *et al.* 1990) permits rapid and routine measurements of large numbers of samples.

Biomass measurements certainly have their limitations in soil pollution studies of Cr or any other pollutants. Being essentially 'black box' measurements they do not permit evaluation of changes in the community structure of the microbial population, e.g. shifts in the fungal:bacterial ratio in soil. Nevertheless, they have revealed damaging effects to the microbial population at or around current maximum EU permitted limits of heavy metal concentrations in soil (Brookes and McGrath, 1984).

## 11. RELATIONSHIP BETWEEN BIOMASS CARBON AND TOTAL SOIL ORGANIC CARBON

Generally, biomass C comprises about 1-4% of total soil organic C (e.g. Jenkinson and Ladd 1981). Thus there is an approximate linear relationship between these two variables, although it may vary somewhat between soils of different physical characteristics or between soils under different managements. Most clay soils contain several times more biomass and organic C than sandy soil under similar management and climate (Gregorich *et al.* 1991). Also the microbial biomass and organic C in forest and grassland soils are both generally higher than in arable soils (e.g. Adams and Laughlin 1981). This accords with the statement of Jenkinson and Ladd (1981) that situations favouring the accumulation of organic matter in soil increase both the amount of biomass and its proportion of the total soil organic matter.

Changes in soil management cause the microbial biomass to increase or decrease much faster than total soil organic matter content. Adams and Laughlin (1981) reported that changing from forest or grassland to arable management caused much greater decreases in biomass C than total soil organic C. Similarly, Powlson *et al.* (1987) reported that 18 y of straw incorporation in Danish soils caused about a 40-50% increase in biomass C whereas total soil organic C only increased by 5%. Similar results were also reported by Saffigna *et al.* (1989) for Australian soils. This, and much other similar research, supports the original idea of Powlson and Jenkinson (1976) that the biomass is a much more sensitive indicator of changing soil conditions than is total soil organic matter content, so that the biomass can serve as an 'early warning' of such changes long before they are detectable in other ways.

There is accumulating evidence that heavy metals at around, or a little in excess of, current permitted EU limits also decrease the

proportion of biomass C in total soil organic matter. Thus, Brookes and McGrath (1984) reported that soil from the Woburn Market Garden Experiment which was last treated with metal-contaminated sewage sludge more than 30 years ago (contaminated soils) contained about half as much microbial biomass as similar soil supplied with inorganic fertilizer or farmyard manure (uncontaminated soils) over this period. The contaminated soil now contains Cu, Ni and Zn at around current EU limits and Cd at up to three times the limit. The biomass content of contaminated soils showed no correlation with total soil organic C, unlike the uncontaminated soils from the same experiment. Similarly, Chander and Brookes (1991b) reported that biomass C as a percentage of total soil organic C was twice as large (1.5-2.0%) in non-sludged soils as in soils treated with sludges which were principally contaminated with Cu or Zn (0.7-1.0%) in field experiments on Luddington and Lee Valley experimental farms.

There is very little comparable data on the effects of Cr on the soil microbial biomass. Bardgett et al. (1994) reported a decrease in the ratio (biomass C)/(soil organic C) with increasing soil Cr concentration up to 1300  $\mu\text{g Cr g}^{-1}$  soil. However the soils were also contaminated with similar concentrations of Cu and As, so effects specific to Cr alone could not be determined. Since biomass measurements have proved so useful in detecting adverse effects of other heavy metals on the soil ecosystem they should also now be used to investigate Cr similarly, particularly in Italian soils where Cr may accumulate due to repeated application of Cr-loaded leather residues.

Long-term trials will be necessary. Following incorporation of Cr-loaded leather meal fertilizer to soil there will initially be a rapid increase in microbial activity as the organic matter in the fertilizer is mineralized by the microbial biomass. Thus the first stage of microbial dynamics is driven by carbon. As discussed by Benedetti et al. (1994) any

adverse effects of Cr on the soil microorganisms will thus be masked during this period. They considered that long-term experiments were required to determine if Cr released into the environment following mineralization of the leather could have adverse effects.

## 12. EFFECTS OF HEAVY METALS ON THE SPECIFIC RESPIRATION OF THE BIOMASS

While measurements of soil respiration and microbial biomass made alone can, in certain circumstances, be useful indicators of environmental stress, combining the two measurements to give amounts of  $\text{CO}_2$  evolved per unit of biomass (biomass specific respiration) has been shown to be a more subtle environmental indicator of stress. Because standard conditions of temperature, soil moisture and substrate availability are required, these measurements are probably only valid under laboratory conditions.

There is now evidence that biomass specific respiration is a good indicator of adverse effects of heavy metals on the soil microbial community. Thus, Brookes and McGrath (1984) reported that rates of  $\text{CO}_2$  evolution ( $\mu\text{g CO}_2\text{-C g}^{-1}$  soil) from both uncontaminated and metal-contaminated soil from the Woburn Market Garden Experiment were indistinguishable when the unamended soils were incubated at 50% WHC and 25°C. However, biomass specific respiration (measured as  $\text{mg C respired g}^{-1}$  biomass  $\text{C day}^{-1}$ ) was twice as fast in the metal-contaminated soils than in the uncontaminated ones.

In a later experiment about 10% more total and 20% more  $^{14}\text{C}$ -labelled  $\text{CO}_2$  were evolved from the contaminated than uncontaminated soil (Chander and Brookes 1991a) during the first 5 days after addition of  $^{14}\text{C}$ -labelled glucose and maize (about 10% and 20% respectively). In

contrast, about 30% less  $^{14}\text{C}$ -labelled biomass was synthesised per unit of added substrate. Similarly, Chander and Brookes (1991c) showed that plant-derived inputs of organic  $^{14}\text{C}$  were about 20% less in the contaminated soil than the uncontaminated soil. Also, the biomass in the contaminated soil contained about 35% less of this  $^{14}\text{C}$ -labelled organic C than in the uncontaminated soil. These results indicate that two mechanisms operate in decreasing the biomasses in metal-contaminated soils. These are decreased C inputs from growing plants and decreased efficiency of conversion of this C into new biomass C. The latter mechanism appears to be the more important.

It is therefore clear from this review of current literature that the methodology already exists to determine if Cr has similar effects on the soil ecosystem and this work should now be given priority. The leather industry is an important part of the Italian economy and any possible problems caused by applying Cr to soil therefore need to be determined or discounted so that the industry is perceived to act in accord with good environmental practices.

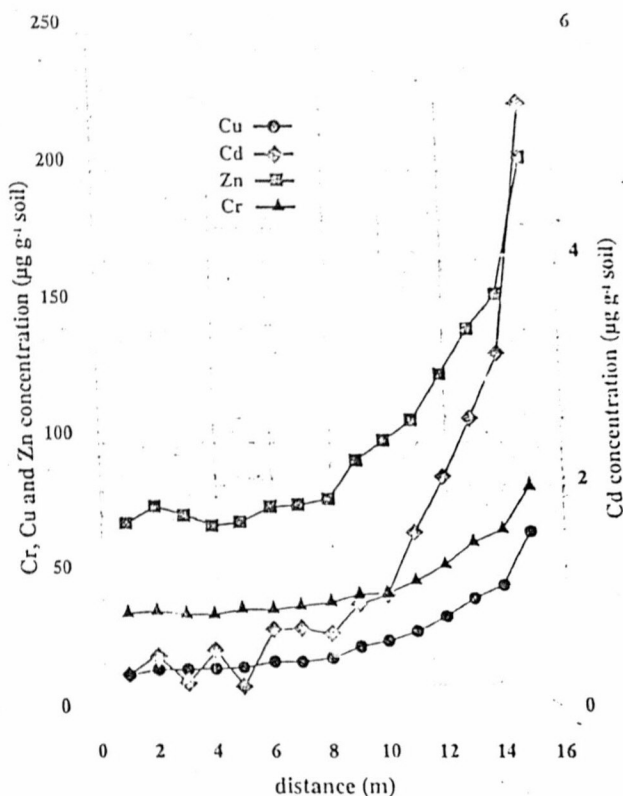
### 13. MICROBIAL BIOMASS AND ACTIVITY MEASUREMENTS IN SOILS FROM THE WOBURN MARKET GARDEN EXPERIMENT

In 1942 an experiment was started on a sandy loam soil (9% clay, pH 6.5) at Woburn Experimental Farm UK. Treatments, each of four replicate plots, included: (1) inorganic fertilizers (NPK) between 1942-67 (2) Farmyard Manure (FYM) applied at  $10.4 \text{ t ha}^{-1} \text{ y}^{-1}$  and anaerobically digested, metal-contaminated sewage sludge applied at  $16.4 \text{ t ha}^{-1} \text{ y}^{-1}$  (Johnston and Wedderburn, 1974). After 1967, all plots received annual uniform rates of inorganic fertilizer only. Vegetable and arable crops were grown during 1942-72 and grass from 1973-82, which was then ploughed up for arable crops in 1983.

In September 1995 and March 1996 we sampled across the middle of three adjacent plots receiving NPK, FYM and sewage-sludge, respectively at the above rates. Eight soil cores (2cm diameter, 0-23 cm depth) were taken at one metre intervals along the middle of the plots and bulked separately at each sampling point. The three plots were chosen to provide a gradient of heavy metal concentrations ranging from the lowest in the NPK plot to the highest in the sludged plot. The soils were then analyzed for heavy metals and microbial biomass C by fumigation-extraction and soil  $\text{CO}_2$  evolution was measured. A complete set of analyses is not yet available. However, current results clearly show that a gradient of increasing metal concentration exists from the NPK plot, through the FYM plot and to the sludged plot (Fig.1).

FIGURE 1

Cd, Cr, Cu AND Zn CONCENTRATIONS IN WOBURN MARLETT GARDEN SOIL ACROSS  
A FIFTEEN METRE TRANSECT

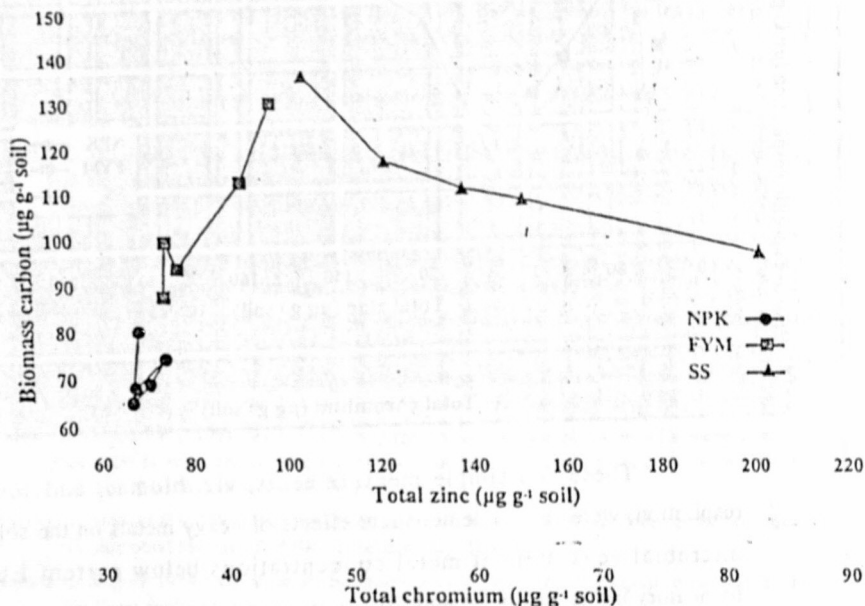


As with other work (e.g. Bardgett et al. 1994) the concentrations of Cr, Cu, Cd and Zn were closely correlated, (e.g.  $r = 0.991$  between Cr and Zn), so it is impossible to assign biological effects to any single metal. Zn and Cr only are shown in Figs. 2-3. However, there was, initially, a clear linear increase in biomass followed by an equally clear decline, beginning at approximately  $44 \mu\text{g Cr}$ ,  $33 \mu\text{g Cu}$ ,

123  $\mu\text{g Zn}$  and 2  $\mu\text{g Cd g}^{-1}$  soil (Fig. 2). The initial apparent increase in biomass with increasing metal concentration was in fact due to increasing soil organic C concentrations over this metal range (B. K. Tiwari personal communication) as discussed above.

FIGURE 2

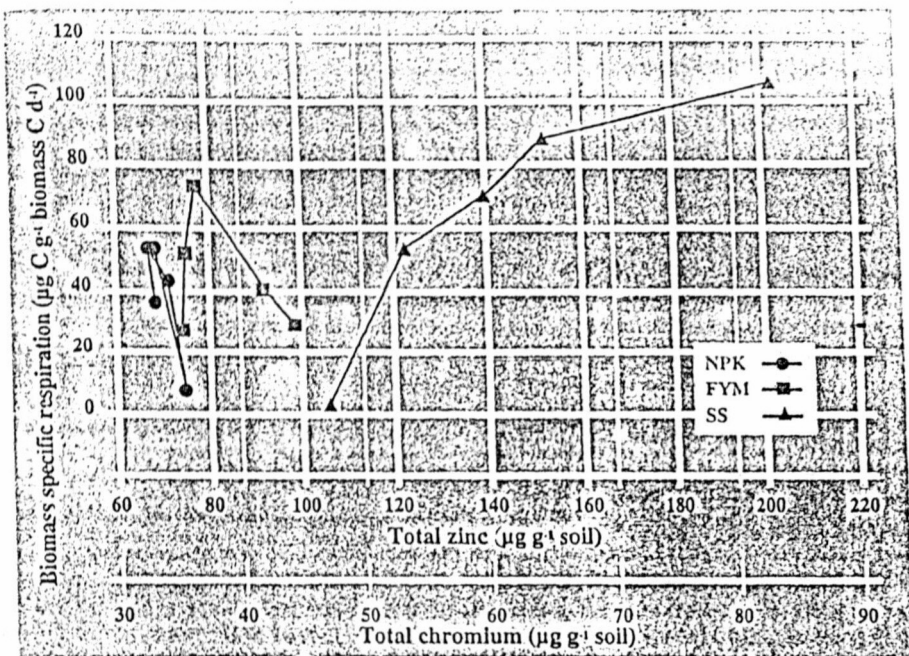
SOIL MICROBIAL BIOMASS CARBON IN NPK, FYM AND METAL CONTAMINATED SEWAGE SLUDGE TREATED PLOTS ALONG BOTH Zn AND Cr SOIL GRADIENTS (SEE TEXT)



Biomass specific respiration varied erratically at lower metal concentrations. However, at metal concentrations equivalent to about 123  $\mu\text{g Zn}$  or 44  $\mu\text{g Cr g}^{-1}$  soil it began to regularly increase in response to increasing metal concentration, again as discussed above (Fig. 3).

FIGURE 3

BIOMASS SPECIFIC RESPIRATION IN NPK, FYM AND METAL CONTAMINATED SEWAGE SLUDGE TREATED PLOTS ALONG BOTH Zn AND Cr SOIL GRADIENTS (SEE TEXT)



These two simple measurements, viz. biomass and soil respiration, were able to demonstrate effects of heavy metals on the soil microbial ecosystem at metal concentrations below current EU mandatory limits for Zn, Cu and Cd.

It must be stressed that we currently have no evidence whatsoever that Cr, either alone or acting synergistically with the other metals, is causing these effects. Conversely, of course, we have no evidence to support the opposite argument. The results are presented to show that we can use microbial parameters such as biomass and microbial activity to determine the microbiological effects of Cr in soil. We suggest that this work should now be done.

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