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Review article

THE INFLUENCE OF HEAVY METALS ON THE DEVELOPMENT AND ACTIVITY OF SOIL MICROORGANISMS

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ABSTRACT: Heavy metal contents in agricultural soils and their relationships with soil microbiological characteristics were studied which were with polluted heavy metals. Most investigations about influence of heavy metals on the development and activity of soil microorganisms have focused on effects where loss of microbial function can be observed and yet such studies may mask underlying effects on biodiversity within microbial populations and communities.in this reviewThesensitivity of differentmeasurements of heavy metals is discussed, and data compiled to compare relativetoxicity of different metals.

Keywords: heavy metals; soil microorganisms; microorganism's biodiversity; detritus catalysis

INTRODUCTION

The contamination of soils by heavy metals is significantproblem, which leads tonegative influenceon soil characteristics and limitation of productiveand environmental functions. The soil microbialcommunity has a fundamental role in the processof organic matter degradation and mineralization, which allows the recycling of nutrients [1]. Heavy metals affect the number, diversity and microbial activity of soil microorganisms. They can cause slow down speed of growth andreproduction of microorganisms, in the soil thenprevail slower growingmicroorganisms with lowerdiversity and higher resistance to heavy metals, but decreased biological activity [2].Concern about heavy metals in soil derivesnotonly for their toxicity to living organisms inhabitingsoil but also for theirimmobilization withindifferent organic and inorganic colloids, in theimmobilized form they can persist for long timebefore being again available to living organismsincluding plants[3].Monitoring methods characterizing microbiologicaland biochemical soil properties are successfullyused to evaluate the intensity of the soilcontamination. They are more sensitive and theirreaction to soil contamination is faster in comparisonwith the monitoring of the chemical andphysical properties of soil which are manifestedafter a long time[3].there are differencesbetween many studies. Some of them confirmthe negative influence of heavy metals on thesoil microbiological activities, the others showthat there is no evidence of correlationbetweenmicrobiological soil properties and increasing heavy metal pollution [1]. Thedifferences between studies are also causedby the fact that some of them work with the artificialcontamination of soil prepared in laboratory; the others use the soil from the real contaminatedareas. Each method has its pros and cons. Howeverthe microbiological characteristics bring the importantinformation about the effect of pollutantson the soil ecosystem and it is necessary to takeinto account that single microbial characteristic cannot be used universally for monitoring of the soil pollution [4].

Bioavailability of Heavy Metals

What is meant by ``bioavailability'' is usually vague and is rarely quantified, particularly in microbial investigations. In reality, bioavailability cannot be measured, because it can only be prognosticate bythe growth of the organism of interest and anevaluation of the uptake or toxicity of a metal after the fact [5]. A wide range of soil properties such as pH, organic matter content, clay content, iron oxide content and E_h all alter the effectsof given metal loadings on soil microbes [6]. Of these, soil pH is often found to have the largest influence, due to its strong effects on solvability and speciation of metals both in the soil as a whole and particularly in the soil solution. Thus, each unit decrease in pH results inapproximately twofold increases in the concentrationsof metals such as Zn, Ni and Cd in the soil solution [7].

Even the metals present in the soil solution will not all bebioavailable because of chelation by organic molecules and the occurrence of chemical forms whichcannot be taken up directly. It is generally assumedthat the free ion is the chemical species which istaken up and causes toxicity when present in excess[5,9,10]. However, this has seldom been represented and there is almost no letter on this point in relevance to soil microbes. Some procedures are now available to measure free ion activities in soil solution at realistically low concentrations of metals[11,12].

Plant root exudates affect metal availability bothdirectly (e.g. Fe^{3+}) or through the effectsexudateshave on microbial activity and resulting rhizosphere chemistry. As bacteria are present within colonies insoil or protected by clays[13,14] theymay often not bedisplayed to the equilibrium solution activity of heavy metals. Metals may becomebound to bacterial or fungal cell walls or on extra cellular polysaccharides of bacteria and the ingestion of such bacteria by protozoa or nematodes will result in vastly different ostentatious to metals in the predators than would result simply from ostentatious to the metals present in the soil solution.Microorganisms may also alter metal availability in their juxtaposition due to localized acidification of the environment, or production of compounds which complex metals. For example, iron oxidising bacteria, which reduce iron pyrites to $FeSO_4$ andH_2SO_4, can cause extreme acidification causingincreases in metal availability.The presence of interfering ions on metal uptakeand therefore toxicity has, again, been studied inplants but little work has been done with soil microbes. It should also be noted that interactionsmay be due to effects on soil chemical equilibria, rather than uptake per se. An example here is theeffect of the formation of chloro-complexes with Cd. It was found that chloride in solution efficiently desorbs Cd from the soil solids into solution, leading to greater Cd uptake by potatoes than in soilswhich contain little chloride [14].Such interactions may also affect microbes, butthere is no information to date.Differences in bioavailability at a limited area of contaminated sites can be large, due to the chemical forms in which metals occur. It is known that soils with very large concentrations of total metals may have small bioavailable deduction, due to the presence of insoluble mineralforms[15]. When wastes are applied to soils, the metals stock may be in a range of forms (e.g. elemental, oxides, carbonates, sulphates, nitrates) varying in solubility, which may also alter on weathering. To emulate these conditions in laboratory and field studies, metal salts are added, often the more soluble ones Added metal ions then work together with the soil (through processes of precipitation, ion exchange, complexation, oxidation, reduction)and finally come to equipoise with the dominant chemical situations but the kinetics of this is largely unknown. Reactions such as ion exchange are acutely fast, but uniform mixing may be a problem in the context of display of soil microorganisms to heavy metals. If the metals are added only on the top of soil (soil surface), they will remain in the top layer (surface centimetres) of soil, and normal microbial activity may happen under this zone.some studies shows higher influence of heavy metals in light-textured soils than heavy-textured soils (e.g.highclay) or soils with organic matter content in microbial processes. [16,17,18,19,20],although most studies was not showed positive relationship between the soil properties (such as pH, clay or organic matter content) and measured intensity of side effects in contrasting soils [22,18,23,24].

Differences in the Resistance of Microorganisms to Heavy Metals

Species of microorganisms [24], strains of the same species[25] and also activities of thesame microbial species[26,27,28]can all show noticeable differences in their resistance to toxicity of heavy metals. Because mostbioassays are based on measurement of size or activity of diverse microbialcommunities, soils withinnate differences in community structure may showdifferences in sensitivity to metal toxicity.van Beelen et.al[29] found that the sensitivity to toxicants of the microbial community responsible forthe mineralization of acetate in soils with no historyof exposure to elevated metal concentrations, differed by many orders of magnitude between soils ofsimilar physical and chemical properties. This suggests that differences in communitystructurebetween soils which vary in sensitivity to metal toxicity could be an important factor in explaining discrepancies between studies.

Microbial Numbers and Biomass

The influence of heavy metals on the size of microbial communities changes,depending upon which group of microorganisms is being discussed, on the metal involved, and on the particular environment. For instance, on the phylloplane, bacteria appear to be more sensitive to metal pollutionthan fungi.Bewley (1980)[30]indicated that contaminated oak leaves had fewer bacteria on them than unpolluted controls, and a highly negativecorrelation was found between numbers of bacteria and the concentration of Pb on hawthorn leaves [31]. On the otherhand, the abundance of fungi appeared to be unaffected irrespective ofwhether perennial rye grass [30], oak, or hawthorn leaves wereconsidered.

However, this particular environment is highly susceptible toother forms of pollution, such as sulfur dioxide, and these may contributeto the observed effects, making it difficult to draw any firm results about the true importance of heavy metals in this type of environment.In contrast to the condition found on living leaves, polluted leaf litter taken from Agrostistenuis growing on Pb-Zn mine waste contained significantly lower numbers of fungi than litter from a control pasture site, but there were no differences in the abundance of bacteria and actionmycetes [32]. There was also a marked reduction inthe numbers of mites, although springtails were more numerous. Strojan(1978) [33]recorded lower numbers of arthropods, particularlymites, in leaf litter at polluted sites adjacent to a Zn smelter. However, no such differences were found when polluted and clean litter werecompared during incubation studies at uncontaminated sites, an observationlater confirmed by Freedman and Hutchinson [34]. They alsorecorded consistently lower numbers of fungi in soils very close to a Cu Nismelter, but these values were not significantly different from those atmore distant sites. Neither could Nordgren (1983)[35] find a decreasein fungal viable counts along a heavy metal gradient, although, using adirect microscopic method, they did detect a reduction in total fungalbiomass with increasing metal concentrations, particularly that of Cu.Bisessar(1982)[36] also found that soil Cu concentrations correlated withchanges only in bacterial numbers, whereas the concentrations of Pb and Cd showed a negative correlation with the abundance not only of bacteria, but also actinomycetes, fungi, nematodes, and earthworms. The overallimpression gained from these reports is that heavy metals reduce theabundance of microorganisms, but this is not invariably so. In a Zn-pollutedregion of Dublin Bay, 15 times more bacteria per gram of dry sedimentwere found than at control sites[37].Such static figures unfortunately provide no information as to howsuch population changes might have occurred or whether changes werestill occurring at the time of sampling. Of interest in this regard is thereport by Vaccaro (1977) [38] that the addition of Cu (0.01 and 0.05ppm) to two enclosed marine ecosystems caused an increase in the relativenumbers of heterotrophic bacteria. This increase was thought to haveoccurred as a result of organic carbon having been released from Cu-sensitiveorganisms. It was also indicated that the bacteria that survived the dominant Cu concentration expanded tolerance to the metal over a period of time and in turn provided a source of inorganic nutrients thatcould then be used by later phytoplankton communities. Cycling of phytoplankton communities that showed some similarities to this wasobserved by Effler (1980)[39].When Cu was applied to a lake ecosystemon three occasions at about monthly intervals, there were substantialreductions in productivity initially, but after 5 or 6 days there appearedto be a recovery of activity. One definite conclusion that can be drawnfrom such data is that heavy metals display a differential toxic action, oneof the consequences of which will be the alteration of the qualitative compositionof microbial communities.

Microorganisms Biodiversity

Microorganisms communities in soil are seems to be extremely diverse, with estimates of as many as 13,000 species of bacteria present in a single gram of soil [40] and an unknown diversity of soil fungi and algae. Gross measurements of microbial diversity have been used to estimate environmental stress[41], but such studies have been barricade by problems of sampling, extraction and culturing leading to bias towards certain groups within mixed microbial communities as indicated above. Pollution affect on reduce in microbial diversity both in terms of species richness due to the extinction of species which absence sufficient endurance to the stress imposed, and can potentially lead to the enrichment of special species which survive well in the stress condition [41]. Genetic diversity is always present within species and may be crucial in determining the response of a population to changing conditions [42]. Highly stable, uniform environments with abundant resources allow the dominance of particularly competitive species [44,45], whereas moderate stresses may decrease the likelihood of competitive exclusion. Humpbacked relationships between species diversity and disturbance, or diversity and productivity appear to be the norm for animal and plant communities[46,44]; and such models may enforce for microbial responses to environmental gradients [46]. We have evidence to suggest that a unimodal, humpbacked relationship holds between genetic diversity within R.leguminosarum bv. trifolii populations and heavy metal stress in soils from the Braunschweig experiments. If such a model maintain true more widely, then a moderate rise in metal loading may lead to an increase or a decrease in apparent diversity, depending on the initial state of the system. In the long-time research at Ultuna, Sweden clover rhizobia separated from sewage sludge treated plots represented a marked lag time in nodulation when assayed under metal-free situations which is hard to describe unless a limited range of strains were surviving[47]. Kinkle et al. (1987)[48]discover no effects of heavy metals on the abundance of different serotypes of B. japonicum but as stated earlier the metal concentrations in the soils studied were small. Until now only few studies have tried to examine more subtle effects of heavy metal pollution on the structure of microbial communities or on the genetic diversity of particular groups of organisms.

Reber (1992)[49] applied a physiological approach in which the ability of the bacterial microbial community to utilize a variety of substrates was assayed to collate the relative activities of different groups of microorganisms and this ability has been related to metal tolerance. These studies have caused to highlight that subtle effects of heavy metals on the diversity of microorganisms in soils are happening which may disturb the potential response of the soil microbial community to new stresses (i.e. it may decrease the resilience of the soil ecosystem). Evidence from the field indicates that under longtime metal tension there is a change in the genetic structure of the soil microbial community, without there as a result being an increase in metal tolerance. A decrease in the total soil microbial biomass under persistent metal stress has been perceived in many field studies, but is perhaps to be preceded by changes in community structure. A decreased size of the microbial biomass can likely at least partially be described by physiological reasons such as a decrease in the microbial substrate utilization efficiency and an increased preservation energy requirement. A decrease in the number of substrates which can be utilized and thus a reduction in the efficient exploitation of all ecological niches may also describe the decrease in the size of the biomass [50,51].

Detritus Catalysis

The ultimate effect of reduced microbial activity will be decreased detritus catalysis, either measured as a decrease in weight loss of detritus or an increase inthe detritus layer, due to undecomposed detritus remaining on the soil. Watson(1977)[51]found out an increase in the two layer near a primary lead smelter in Missouri, USA, and Coughtrey et al. (1979)[53] detected an increased detritus layer around Avon mouth in England. Freedman andHutchinson (1980)[34] found indications of an increasein the detritus layer at approximately 10 to 15 km from the emission source at Sudbury(emitting mainly Ni and Ca). Dry weight loss of 3 types of tree leaves was evaluated within the 8 km. At that distance, the dry weight loss was only 79 to 83% of that in control areas after 851 days catalysis. Strojan (1978)[34] realized that the dry weight loss of oak and Sassafras leaves was only 22 to 26% after 1 yr in an area with 256 µg Cd g-1 soil, 172 µg Cu g^{-1},971 µg Pb g^{-1} and 14600 µg Zn g^{-1}, compared to 37 to 39% in control areaswith 8.8 µg Cd g^{-1}, 47 µg Cu g^{-1}, 258 µg Pb g^{-1} and 676 µg Zn g^{-1}. To detectchanges in detritus accumulation an even higher pollution level was needed. Friedland *et al.* (1986) [52] reviewed metal effects on microbial activity and concludedthat the current metal concentrations in the forest floor of an area in Vermont,USA, were not capable of significantly reducing catalysis of organic matter.The heavy metal concentrations were about 10 µg Ni g^{-1} and 2 µg Cd g^{-1},100 to 200 µg Pb g^{-1} soil, 100 µg Zn g^{-1}, 10-20 µg Cu g^{-1}, 10 µg Ni g^{-1} and 2 µg Cd g^{-1}. At the Gusum area (Cu and Zn pollution), a decrease in pine needle catalysiswas evident within 1 km of the mill (where the humus layer contained about 700µg Zn g^{-1} and 400 µg Cu g^{-1}) B. Berg and B. Soderstrom and H. Staaf (1980) [53], they discovered an effect up to a distance of 10 km from the smelter, when unpolluted pine needles were studied. If local, polluted needleswere used, the catalysis rate was even slower near the mill and a negativeeffect was found at greater distances from the smelter. This indicates that bothsoil and litter quality variables are responsible for the suppression of detritus catalysis in metal polluted areas. However, Freedman and Hutchinson(1980)[34] found no effect of detritus quality on catalysis rate around the Sudburysmelter. The results of Strojan (1978)[33] indicated that differences in catalysis ratedue to heavy metal pollution increased with time. A similar tendency was foundby Inman and Parker (1978)[54].Berg and Staaf (1980)[53]suggested that the detritus catalysis rate in a later stage was determined bythe catalysis rate of lignin. If lignin degradation is especially sensitive toheavy metal toxicity, one would expect pollution effects to be more easily detectedin later stages of detritus catalysis.

Methanogenesis

In marine sediments sulfate reduction is regarded as being much more important than methanogenesis, whereas in freshwater sedimentsgrowing evidence suggests the opposite to be the case[55].Nickel, Co, and Mo are essential elements for certain methanogens, andconsequently some recent studies have been made on the effects of thesemetals on methane production in natural environments. J. G. Jones. (1982) [55]could find no stimulation of methanogenesis in fresh water sediment slurries amended with 0.06 ppm Ni or Co or 0.096 ppm Mo,although slight stimulation was observed with some surface sedimentsamples. In sulfate-limited sediment samples, however, the addition ofabout 1900 ppm Mo resulted in a 60% or 80% decrease in methane productionwhen the incubation atmosphere was hydrogen and carbon dioxideor nitrogen and carbon dioxide, respectively. Contrary to this, whenadequate sulfate was present with nitrogen and carbon dioxide atmosphere, stimulation of methanogenesis was observed. Enhanced methane production from salt marsh sediments after the addition of 1000 ppm sodium molybdate was also recorded by Capone *et al.* (1983)[56]. They suggestedthat the stimulation by sodium molybdate could be attributed tothe inhibition of sulfate-respirers. J. G. Jones. (1982)[55] had earliercome to a similar conclusion, but in addition proposed that bacteria otherthan sulfate-reducers might also be involved.In an investigation of the effects of various pollutants on methanogenesis in fresh water sediments.

Pedersen and Sayler (1981)[57] found that 1and 10 ppm of Hg Cb had no significant effects on the process. Capone *et al.* (1983)[56] similarly observed no effect with 10 and 100 ppm $HgCl_2$ Theyalso included several other metals in their study and found that the effectswere variable and depended not only on the metal itself, but also on itsform. At concentrations of 1000 ppm methanogenesis was inhibited byCH_3HgCl, whereas $HgCl_2$, $PbCl_2$, and $KCrO_7$ caused an initial inhibition, followed by a period of stimulation. The chlorides of Ni, Cd, and Cu, as well as $ZnS0_4$,PbS, and HgS, caused short-term inhibition, but displayedno significant long-term effects.

Respiration

Working with respiration rates of soils polluted to varying degreesaround a Ni-Cu smelter, Freedman and Hutchinson[34] recordedlower rates of carbon dioxide efflux at more contaminated sites, and statisticalanalysis revealed that Cu had a greater influence than equalamounts ofNi. Copper was also thought to be the most important heavymetal around the town of Gusum, South Sweden[35],where again decreased soil respiration was characteristic ofthe more pollutedsoils, particularly when Cu was present in excess of 1000 ppm. The addition of Pb[58] and of Cr, Cd,Cu, Zn, and Mn[59] to soil samples also causeda decrease in respiration rates. Doelman and Haanstra [58] showed that sandy soils exhibited about a 15% decrease in respiration whenamended with 375 ppm Pb, the lowest concentration used, whereas a claysoil required 1500 ppm Pb to achieve the same inhibition. A peat soilshowed no effects even at the highest concentration of Pb (7500 ppm).

Dinitrogen Fixation

Although in water and sediments almost all heavy metal studies havebeen directed toward various aspects of carbon cycling, in the case of soilthe effects of heavy metals on nitrogen transformations have alsoreceived a considerable amount of attention. Rother*etal.*[26]could find little or no effect of various metals on nitrogenase activity (acetylene reduction) in a number of polluted and uncontaminatedsoils. In fact they concluded that in theirinvestigation soil moisturewas probably more limiting than heavy metal toxicity. The rates of dinitrogen fixation they recorded were low (0.6-8.0 g N/ha per day), butwere within ranges recorded for similar habitats [60]. The metals researched were Zn, Pb, and Cd, of which the first two were present in particularly high concentrations in some soil, samples (8000 and 26,000ppm, respectively). Rother *et al*[26] suggested that the reason for thelack of effect was because the pollution was long-standing and only relativelysmall amounts of free metal might be present. In addition, the pHrange (6.5-6.8) of the soils was such that there would have been very littlePb or Zn available, since these metals were present mostly in the form of carbonates and sulfides, which would be insoluble. Takakuwa and Wall (1981)[61] have observed that, in general,1000 ppm of Hg, Pb, Zn, Cr, Mo, and Cd all caused inhibition ofacetylene reduction activity in saltmarsh sediments, whereas Ni caused astimulation at concentrations of 100 and 1000 ppm. They suggested thatthis enhancement may have been the result of a Nirequirement that hasbeen found to be necessary for certain hydrogenases.

Most studies of the inhibitory effects of heavy metals on symbioticdi nitrogen-fixing associations have placed greater emphasis on the wholeplant response rather than on the process of dinitrogen fixation or the interactions with the microorganisms involved. In the alfalfa symbiotic dinitrogen-fixing system, Porter and Sheridan (1981)[62] showed that Cd, Cu,and Zn were highly toxic. Lead, on the other hand, appeared to have littleor no effect when included in nutrient solutions at concentrations up to100 ppm. Earlier, Wickliff *et al.* (1980)[63] had observed that $CdCl_2$ innutrient solutions in which *Alnusrubra* (red alder) was growing could affect dinitrogen fixation. The presence of up to 15 ppm Cd decreased nitrogenase activity by up to 89%. Inhibition of the enzyme was found tooccur when root nodules contained in excess of 3.4 ppm Cd. At the lowerend of the concentration range (0.01-0.1 ppm), although nitrogenase activity was decreased, plant growth was not altered. In addition, when nodulated plants were exposed to 0.01-0.1 ppm Cd there was increasednodulation as the Cd concentration increased. They suggested that atthese low concentrations more nodules were formed to compensate forthe reduced enzyme activity. Because of the high negative correlationbetween acetylene-reduction activity and Cd concentration in the nodules, Wickliff and Evans (1980)[63] suggested that one way the metal exertedits effect on the enzyme system was by causing increased resorption, or lysis, of the endophyte. However, Wickliff *et al.* (1980) [64] also suggested that the inhibition may have arisen as a result of a reduction in available photosynthatesince, at high Cu concentrations, chlorosis of the plant leavesindicated that chlorophyll biosynthesis and therefore photosynthesiswere being impaired. Recently, Porter (1983)[65] came to a similar conclusionwith regard to the alfalfa system.Earlier hint was made of the essentiality of several of the heavymetals for many biological processes. Consequently, the beneficial effectsof many metals on dinitrogen fixation have been the subject of a fewrecent studies. Yatazawa *et al.* (1980)[66] recorded unfavorable effects on the*Azalia-Anabaena* symbiosis when certain heavy metals were deficient.They found that the threshold levels ofMn and Mo for growth were 0.02and 0.0003 ppm and for dinitrogen fixation were 0.01 and 0.001 ppm,respectively.

Molybdenum was also studied by Gault and Brock well (1980) [67], but on legume dinitrogen-fixing systems. The survival ofluceme and clover rhizobia was adversely affected by sodium molybdate, but not by molybdic acid, ammonium molybdate, or molybdenum disulfide, andthis was reflected in poorer nodulation of host plants. Even so, dinitrogen fixation, as measured by foliage nitrogen content, was always higher inMo treatments when compared to treatments that did not incoporate Mo. Skukla and Yadav (1982)[68]**,** working with chick peas *(Cicerarietinum),*observed that as Zn concentrations were increased up to 19 ppm thenumber of nodules, their dry weight and leg hemoglobin contents, and the amount of nitrogen fixed increased, but beyond this concentration therewas a decline in these parameters. However, the presence of 25-50 ppmphosphorus could counteract the effects of 40-100 ppm Zn and maximum nodulation and dinitrogen fixation was observed when 25-50 ppmphosphorus was combined with 5-10 ppm Zn. The observation of theseeffects with Zn over a relatively narrow range oflow concentrations couldwell be a reflection of their choice of a Zn-deficient loamy sand as experimentalmaterial. Rhoden and Allen (1982)[69] also studied the effects of Znalong with Mn on dinitrogen fixation, but quantitative comparison withother studies was made difficult by their choice of units of addition. Theyamended a Norfolk sandy loam with Mn and Zn at levels of 0, 5, 10, and20 kg/ha, with pH regimes of 5.5, 6.0, and 6.5, and measured theresponses of various cultivars of *Vignaunguiculata.* They found that the effects of Mn and Zn on nodulation and dinitrogen fixation depended on the cultivar and soil pH, but, in general, maximum nodulation wasobserved with 5 kg Mn/ha or 20 kg Zn/ha. Dinitrogen fixation rates responded similarly, except that only 10 kg Zn/ha was required for optimumactivity.

Enzyme Activity

Different evaluations of enzyme activities have been doing in relation to heavy metal contamination in soil. These range from very unspecific enzymes, likedehydrogenase, to those that are involved in more specific reactions, like urease.Acid phosphatase activity seems to be a good indicator of pollution. Tyler (1974)[72] obtained lower activity levels around the Gusum smelter with Cu and Zn levels only 3 to 5 times the concentration found in samples from background sites, and Tyler and Westman (1979)[70] found lower values within 30 km of Ronnskairsverken, where a mean of 35 µg Cu g^{-1} soil, 71 µg Zn g^{-1}, 78 µg Pb g^{-1}, 1.6 µg Cd $g^{-1}$and 53 µg As g^{-1} were found in the humus. Low levels of acid phosphatase activity around the Sudbury smelter have also been announced [34]. Urease seems in many cases to be equally or even more sensitive to heavymetal pollution as acid phosphatase [72,74]. Mathur et al. (1980)[75] investigated several hydrolytic enzymes in relation to Cu content of cupriferous bogs. Although variance existed in the degree of inhibition, most of the enzymes were affected in a similar way. Thus, there seems to be great correlation between changes in activities of different enzymes in connection to heavy metal pollution. The only significant exception to this is beta-glucosidase activity, which was inefficient by Cu + Zn concentrations in soil, where phosphatase activity was halved contrast to control samples [71]. Low enzymatic activity in soil may be because of low concentrations of the enzyme, or metal inhibition of the enzyme by covering of active groups, by protein denaturation, by other influences on enzyme configuration or by competition with activating metal ions[73]. By appending a chelating agent (Na-EDTA), some of the acid phosphatase and urease activity could be recycled in Cu and Zn contaminated forest humus, showing that metal inhibition partly described the decrease in enzyme activities in soil with high metal contents.A decrease in enzyme concentrations was, however, still evident. Enzyme synthesis has been demonstrated to decrease severely in response to heavy metal addition. Addition of 2000 µg Pb g^{-1} soil to starch or maltose amendedsoil decreased the synthesis of amylase and alfa-glucosidase by 75% and 50%,respectively [74]. In the case of starch amendment a decrease in amylase producing bacteria was also found. Cole (1977) [74] showed that amylase activity have fewer sensitivity to Pb inhibition than enzyme synthesis. Thus, decrement in enzyme activities found in several investigations is probably mainly an effect of a decreased enzyme synthesis associated with inhibited microbial growth than to direct enzyme inhibition by the metals.

Mycorrhizae and plant Growth

The effects of heavy metals on mycorrhizae and on the interplay between fungi and plants differ somewhat between different types of mycorrhizae VA mycorrhiza can elevate plant uptake of Zn and Cu, when these metals are available at low concentrations in the soil[73,74,75,76,77]. In certain cases an increased mycorrhizal infection rate can also be foundafter addition of low levels of metals (e.g. Zn)[78]. However, Graham *et al.* (1986)[79] reported that the root colonization of citrusseedlings by a VA mycorrhizal fungus was reduced logarithmically with soil Cuconcentration. Minimum toxic amounts of Cu ranged from 19 to 34 µg g^{-1} soil.Reports on the effects of VA mycorrhizae on plant growth in heavy metal polluted soil are contradictory. Killham and Firestone (1983)[80] challenged perennial bunchgrasswith three levels of a heavy metal mixture (Cu, Ni, Pb, Fe, Co) at three levelsof water acidity.

The presence of VA mycorrhiza enhanced shoot uptake of Cuand Zn especially, but to some extent also Pb and Zn. This resulted in reduced growth of mycorrhizal plants compared to non-mycorrhizal ones. These effects weremost prominent at higher levels of acidity. The increased metal uptake at lowerpH could be due to a greater availability of the metals in the soil, but the factthat the fungus, *Glomusfasciculatus,* performs better at low pH [81], could also be of importanceOn the other hand, Gild on and Tinker (1983)[82] found that VA mycorrhiza appeared to give some protection to clover in soils with added Zn, Cu and Cd. Lower levelsof metals in the shoot were found in mycorrhizal compared to non-mycorrhizalplants. The mitigating effect was found despite the infection rate being reducedby heavy metal addition. Dueck *et aI.* (1986)[83] also found a slightly higher biomassof grasses in Zn-polluted soils (460 µg Zn g^{-1} soil) if the grass roots were mycorrhizal. However, they reported that the shoot: root ratio of Zn content was higher inmycorrhizal than in non-mycorrhizal plants.The presence of ericaceous mycorrhizae appears to be important for plant survivalin metal polluted areas [84]. Mycorrhizal *Calluna vulgaris* plants had a high degree of resistance to Cd and Zn, while non-mycorrhizal plant shad almost no tolerance. The increased resistance was coupled with a lower internalconcentration of metals in the shoots of the plants with mycorrhiza. The mycorrhizal effect was especially prominent with a *Calluna* race from a polluted site comparedwith a race from an unpolluted area.An increased toleranceto metals in polluted soils has also been found in greenhouse experiments. Brownand Wilkins (1985)[85] reported that the presence of *Amanita muscarina* and *Paxillus* in volutes increased the tolerance to Zn of both tolerant and non-tolerant races of *Betula.* A reduced uptake of Zn to the shoots, and an accumulation of Zn in them ycorrhiza was also found. Both fungi were equally effective in increasing themetal tolerance of the tree, although P. *involutus* was less tolerant to Zn than A. *muscaria* in pure culture studies [85].Dixon (1988)[86] also found that mycorrhizae mitigated the effect of heavy metals(Cd, Ni, Pb) on growth of *Quercusrubra.* Mycorrhizal seedlings had comparatively lower metal content in the shoot and higher in the root compared to non-mycorrhizalcontrols. High soil concentrations of the metals decreased ectomycorrhizal development and thus also the protective effect of the mycorrhiza. Jones *et al.* (1986)[87]found that the presence of *Scleroderma flavidum* reduced Ni toxicity to birch, andattributed this to a reduced transport of Ni to the stem. However, three of them ycorrhizal fungi had no effect, and none of the four mycobionts induced tolerance to enhanced levels of Cu in the growth mixture. Neither Ni nor Cu addition affected the mycorrhizal infection rate. The mechanisms behind the increased tolerance of ectomycorrhizal plants to heavy metal toxicity have not been elucidated. However, the accumulation of metals inmycorrhizal roots indicate that the fungal hyphae might bind metals, thus renderingthem unavailable to the plant. Fungi can bind metals in the cell wall [88],thereby lowering the concentration of the metal in the soil solution. Denny andWilkins (1987)[89] reported that most Zn bound to the fungal cell wall was found in the extrametrical hyphae and not in the mantle. More Zn was also bound tohyphae growing in association with *Betula,* than to fungal hyphae growing in aroot free environment. Jones and Hutchinson (1986)[87] suggested that the morphology of *Scleroderma flavidum* mycorrhiza was important in providing Ni tolerance tothe host plant. It might be that fungi which develop large clusters of mycorrhizalroots with thick mantles are more effective in reducing metal toxicity to the plant, as suggested by Colpaert and Van Assche (1987)[90].The production of organic acids, such as oxalic acid, by ectomycorrhizal fungi[91,92] could also be of importancein determining metal toxicity to the plant, since these acids can bind heavy metals. Morselt *et al.* (1986)[93] demonstrated the presence of metallothionein-like proteins in several ectomycorrhizal fungi using histochemical staining. This might explain why ectomycorrhizal fungi can increase plant uptake at low metal concentrations[77], but reduce it at toxic levels of themetals.Bell *et al.* (1988)[94] took field samples and found decreased incidence of ectomycorrhizal root tips in soils naturally enriched with Cu, Pb and Zn. The organic and A horizonsin metal enriched sites had soil metal contents 3.5 to 6.4, 1.5 to 5.9 and 2.1 to4.1 times higher than control sites for Cu, Pb and Zn, respectively.

Microorganisms Biomass of soil

Microorganisms are diverse from their vulnerability to metal toxicity and sufficient metal disposal will result in immediate death of cells due to disruption of essential functions, and to more gradual changes inpopulation sizes due to changes in viability or competitive ability. What is perhaps more surprising isthat soil microorganisms subject to long term metal stress, even at modest levels of exposure, are notable to maintain the same overall biomass as inunpolluted soils. Development of tolerance andshifts in community structure could be expected tocompensate for loss of more sensitive populations.Instead, results from laboratory ecotoxicological studies suggest that changes in community structurego hand in hand with a decrease in the soil microbial biomass [95].There is now a considerable amount of evidencedocumenting a decrease in the soil microbial biomass as a result of longterm exposure to heavymetal contamination from past applications of sewage sludge as reviewed by McGrath (1994)[96].

Analysis of soils contaminated with heavy metals from other sources such asCu and Zn in animal manures [97], run-off from timber treatment plants[98,99], past applications of Cu-containing fungicides[100,101] and analysis of soils in thevicinity of metal-contaminated army disposal sites[102] confirm that adecrease in the microbial biomass occurs at a relatively modest, and sometimes even at a surprisinglylow [103] metal loading. The widespread occurrence of this effect of metal toxicitysuggests that there may be a common physiologicalexplanation.

CONCLUSION

Toxicity levels for soil microbial processes are difficult to estimate accurately fromliterature data. Methodological differences between different studiesin sampling strategies, sampling intensity, measurement standardization etc. withoutdoubt also explain part of the variation. The relative toxicity of the different metals, however, is fairly constant. The following degree of toxicity appears to be the mostcommonly found Cd > Cu > Zn >Pb. This was irrespectiveof the soils having high or low organic matter content, although the decreasingtoxicity in soils with high organic matter content was clearly seen.

The present review has pointed to several areas, where research is needed. Forexample, in a natural pollution situation there is almost never one single metalthat is found in increasing amounts in the soil. Instead combinations of severalelements, often together with other pollutants such as SO2, are emitted. We havetoo little data on how the soil biota responds to such combinations of pollutantsto be able to predict effects, although pure culture studies have indicated that synnergistic or antagonistic effects can be found. We also need more quantitativedata on the modifying capacity of different abiotic soil properties. More studiesinvolving natural soil, which attempt to produce mathematical descriptions of theimportance of for example pH, CEC and organic soil components on the toxicityof different metals to different microbiological variables are therefore needed. The modifying effect of the microbial community itself by adaptation, selectionof tolerant organisms etc. also lacks quantitative data, especially from field studiesor at least studies involving natural soil. For example, we do not know whichtime scales are relevant in a natural pollution situation for the development ofa more metal tolerant microbial community. We also do not know to what degreesuch a community can compensate for toxic effects, for example during conditionsof an additional stress, like low temperatures or drought.

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