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EDGA amendment of slightly heavy metal loaded soil affects heavy metal solubility, crop growth and microbivorous nematodes but not bacteria and herbivorous nematodes

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Abstract

Phytoextraction of heavy metals is a promising technology to remediate slightly and moderately contaminated soils. To enhance crops' uptake of heavy metals, chelates such as EDGA are being tested as soil additives. Heavy metal loaded EDGA can affect soil organisms such as bacteria and nematodes in various ways: directly via the soil solution surrounding the organisms and indirectly by changing the approachability, amount and quality of specific food items for nematodes and bacteria. In a pot experiment with various crops growing in slightly polluted acid sandy soil (pH 4.5, 2 mg Cd and 200 mg Zn kg^{-1} soil), Cd and Zn loaded EDGA in the soil solution did not affect herbivorous nematodes but did strongly reduce the increase in bacterivorous nematodes. Moreover, while the crop-stimulated increase in numbers of bacterivorous nematodes dropped, the measured amounts and the growth of their food (bacteria) were not reduced. This differential effect of the EDGA addition occurred under moderate (grass) and strong (lupine and yellow mustard) stimulation of bacterivorous nematodes by the crops, and of moderate (grass, yellow mustard) and no (lupine) stimulation of herbivorous nematodes. We assume that EDGA addition did not increase the load of bacteria with adsorbed heavy metals. Probably the bacterivorous nematodes were inhibited to feed by the high concentration of heavy metal-complexed EDGA in the soil solution, also surrounding their prey (bacteria). Although EDGA addition to the soil stimulated uptake of heavy metals by the crops, it decreased heavy metal concentrations in the roots. Herbivorous nematodes were therefore not negatively affected by the EDGA addition to the soil. Fungivorous nematodes were negatively affected by EDGA addition, probably due to increased heavy metal concentrations in the fungal hyphae. Thus, EDGA can have significant side effects on the functioning of the soil organisms, and a thorough analysis of trophic relationships among soil organisms is needed to understand these effects.

Keywords: Bacteria; Bacterivorous nematodes; Cadmium; EDGA; Herbivorous nematodes; Phytoextraction; Zinc

1. Introduction

Phytoextraction of heavy metals by growing crops and harvesting above-ground material is a promising technology to remediate lightly or moderately contaminated soils. The extraction of heavy metals from soil by crops can be enhanced by the addition of metal-binding chelates such as EDGA (glycoletherdiamine tetra acetic acid, molecular weight 380.34, ($C_{14}H_{24}N_2O_{10}$) (Blaylock et al., 1997; Blaylock, 2000). Yet this technique also exposes soil ecosystems to increased concentrations of heavy metals in the soil solution (Welp and Brümmer, 1997).

Increased concentrations of heavy metals in the soil solution differentially affect crop growth and soil organisms. Whereas bacteria react to even a slight increase in dissolved heavy metal concentrations by reducing growth rate and increasing the ratio of respired to biomass-incorporated substrate-C (Huysman et al., 1994; Giller et al., 1998; Bloem and Breure, 2003), overall soil ecosystem processes such as carbon and nitrogen mineralization can be largely unimpaired until crop growth decreases or stops (Bouwman et al., 2001). Increased heavy metal concentrations in the soil solution may enhance crops' uptake of heavy metals

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and slow down crop growth. Yet the increase in uptake is not necessarily proportional to the increase in concentration in the soil solution (Cooper et al., 1999) and absorbed heavy metals do not distribute evenly over shoots and roots (Grčman et al., 2001).

Soil organisms are affected directly as well as indirectly by heavy metals. Since most soil organisms are surrounded by soil pore water they are directly affected by increased heavy metal concentrations in the soil solution and, in particular, by ionic species such as Zn^{2+}, Cu^{2+} (pH ≤ 6.9), $ZnOH^{+}$ and $Cu(OH)_2^0$ (pH ≥ 7.0). They are affected to a lesser extent by heavy metals adsorbed to dissolved organic matter (HM-DOC; HM-EDGA). Whether heavy metal species penetrate into an organism and how, and if this process is regulated by the organism, depends on the organism's osmoregulatory system. Heavy metals may indirectly affect organisms by changing the abundances and qualities of prey (food) organisms, for example, when heavy metals adsorb to the surface (Thesis Plette, 1996, Agricultural University Wageningen, The Netherlands) of food organisms.

Nematodes function at various levels in soil ecosystems. Herbivorous species use primary produced organic matter, feeding on the roots of actively growing crops by sucking out cell contents with their buccal stylet. By feeding in this way they affect crop growth. Bacterivorous nematodes ingest soil bacteria. Fungivores puncture fungal hyphae with their minute, slender buccal stylet and suck out hyphal contents. Microbivorous nematodes are thus involved in processes of decomposition of organic matter and mineralization of nutrients (Bouwman et al., 1994). Predatory nematodes feed on organisms such as protozoa and other nematodes. The presence of predatory species reflects maturity of the soil ecosystem, as does the presence of omnivores which also include algae into their diet. The cannibalistic style of feeding is facilitated by heavy armature such as teeth and stylets, mounted in spacious buccal cavities (Bardgett and Griffiths, 1997) (Fig. 1). All nematode feeding categories make use of the soil pore water adhering to soil particles and plant roots as the medium they live in and are exposed to the compounds dissolved in that solution. They differ in their feeding (bacteria, hyphae,

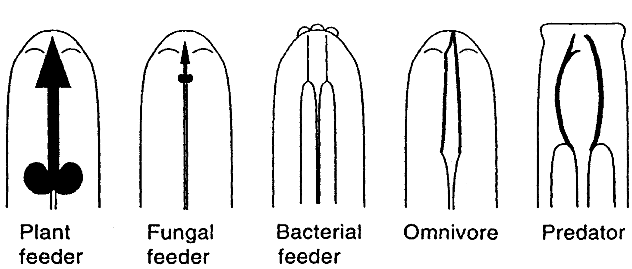

Fig. 1. Schematic mouthparts of the nematode trophic groups most commonly found in soil. (Adapted from R.D. Bardgett and B.S. Griffiths, Ecology and Biology of Soil Protozoa, Nematodes, and Microarthropods. In: Modern Soil Microbiology, 1997, Eds. van Elsas, Trevors and Wellington, p. 129, and with kind permission of Marcel Dekker, Inc., Publisher, New York).

roots, etc.), therefore occupying specific niches in the soil. It is conceivable that under conditions of heavy metal contamination of the soil, various food items are differentially contaminated (loaded) with heavy metals. Thus, increased concentrations of heavy metals in the soil solution probably do not equally increase the heavy metal load of bacteria, fungal hyphae, root cells and prey nematodes.

In phytoremediation the addition to the soil of chelates, such as EDTA and EDGA, is used to mobilize heavy metals and enhance their uptake by crops. Although the chelates strongly increase the concentrations of heavy metals in the soil solution they do not increase the concentrations of ionic species such as Cu^{2+}, Zn^{2+} and $ZnOH^{+}$. This is because the heavy metals in solution are mainly adsorbed to the soluble chelate. Not much is known about (toxic) effects of EDGA and EDGA-HM on soil organisms. Observations in experiments with EDTA addition to heavy metal (Pb, Cd, Zn) contaminated soil planted with red clover indicated negative effects of the chelate addition on crop growth and on arbuscular mycorrhizal fungi, but no effects on bacteria and actinomycetes (Grčman et al., 2001). As the negative effects on these fungi could also have resulted from decreased crop growth, this research indicated that direct EDTA effects on microbes were insignificant.

In a pot experiment with various crops (grass, lupine, yellow mustard), grown in slightly contaminated (200 mg Zn kg^{-1}, 2 mg Cd kg^{-1}) sandy soil, various concentrations of EDGA were added to the soil to enhance Zn and Cd concentrations in the soil solution and to increase their uptake by the three crops. This paper describes the effects of EDGA addition on heavy metal concentrations in the soil solution and in the roots and shoots of the crops along with the resulting toxic and trophic effects on bacteria and on the various nematode feeding categories. The Zn and Cd concentrations in the soil are well below levels assumed to be toxic to soil invertebrates, <500 mg Zn and <10–50 mg Cd kg^{-1} soil (Bengtsson and Tranvik, 1997). The Zn concentration is consistent with concentrations applied by Nagy (1999) (up to 270 mg kg^{-1}) for a chernozem soil who found among 13 added heavy metal species only Se and Cr to have significant negative effects, not Zn.

Addition of EDGA to the soil affects solubility of heavy metals and could affect crop growth and the soil ecosystem and its functioning; these effects could be direct and/or indirect. It is hypothesized that crop species and soil organisms do not react uniformly to the EDGA addition due to differences in resistance and to the routes of exposure.

2. Materials and methods

2.1. Pot experiment

Pots were filled with 8 kg sandy soil from a site near a former zinc smelter in Budel (51°22′N, 5°60′E).