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Citation classics

Heavy metals and soil microbes

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ABSTRACT

The discovery in the early 1980s that soil microorganisms, and in particular the symbiotic bacteria *Rhizobium*, were highly sensitive to heavy metals initiated a new line of research. This has given us important insights into a range of topics: ecotoxicology, bioavailability of heavy metals, the role of soil biodiversity, and the existence of 'keystone' organisms. Concurrently, and particularly in Europe, the research led to new approaches to the protection of soils from pollution that take into account the many effects on soil microorganisms. To date these key findings have largely been ignored in the USA, although our results caused considerable controversy there. In the past decade there have been many advances in the ecotoxicological assessment of metals and their effects on soil organisms but major gaps in knowledge and theory remain with regard to how microorganisms are exposed and respond to metals in soils. In this brief review we emphasise the need for long-term experiments and basic research to forge this understanding and improve environmental protection policies.

1. Introduction

It all began in May 1984 when Steve McGrath arrived excited in Ken Giller's office at Rothamsted clutching some rather sickly, yellow clover plants. He was just back from conducting field research on a long-term experiment at Woburn, where he had been surprised to see striking differences between treatments: yellow and stunted clover plants on plots that had received annual amendments of sewage sludge from 1942 only until the early 1960s, and dark green clover with prolific growth on the plots that had received farmyard manure over a similar period[1]. We got to work and our subsequent experiments confirmed Steve's suspicions that the poor clover growth was due to the lack of N_2-fixation (McGrath et al., 1988; Giller et al., 1989). At the same time these experiments spawned a new line of research that eventually led to a change in the rules for the protection of agricultural soils in the UK (DoE, 1996).

In the 1990s there was much controversy regarding the relevance of the results of the Woburn Market Garden Experiment (Smith and Giller, 1992; Smith, 1996). The main argument was that these soils were contaminated with sewage sludge that had concentrations of Cd way above what was possible if contemporary sludges were applied. Articles in *New Scientist* (Giller and McGrath, 1989) and other news sources (e.g. Farmers' Weekly, national newspapers) describing our results on the effects of heavy metals on *Rhizobium* led to a retort entitled 'Science backs sludge' (Blake, 1991) quoting Steve Smith from the Water Research Centre, UK, a public body responsible for research to advise UK water authorities on disposal of sewage sludge. Publication of our results from the Braunschweig field experiments in Germany, which showed adverse effects at low (<3 mg kg^{-1} total) soil Cd concentrations (Chaudri et al., 1993a), and a series of laboratory based studies that indicated the toxicity of Zn to rhizobia at relatively low concentrations (Chaudri et al., 1992a,b, 1993b) helped to dispel the criticism. The evidence was later augmented by the strong effects seen in Zn treated soils that contained low Cd concentrations in a long-term experiment at Gleadthorpe, UK (Chaudri et al., 2000).

When our initial findings were published Ernst Witter initiated research on the effects of heavy metals on soil microorganisms in long-term experiments in Sweden (e.g. Mårtensson and Witter, 1990; Witter and Dahlin, 1995; Dahlin et al., 1997). His research contributed to review of the guidelines for environmental protection in Sweden (Witter, 1992), and we collaborated together with a wider group of scientists in European projects on similar topics during the 1990s.

The 'Citation Classic' we have been asked to reflect on in this short article was a detailed 25-page review of research on the effects of heavy metals on soil microorganisms (Giller et al., 1998).

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[1] A colour photograph of these plants was included in our 1998 review on page 1400.

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Our review focused on two aspects: firstly a synthesis and analysis of how a wide body of – seemingly disparate – experimental observations on the sensitivity of microorganisms to heavy metals in soil could be reconciled, and secondly what the relevance of this knowledge was (and remains) for environmental protection. We use the opportunity here to reflect on further advances in our understanding since the publication of the 1998 review and to comment on the wider relevance of this field of research for understanding the functions of soil biodiversity and the functioning of biological processes in soil. Finally, we discuss the implications for legislation.

2. Reconciling contradictory evidence – new developments since 1998

A main question our review article attempted to address was the wide variability in results of studies that had tried to establish the toxicity of metals to soil microorganisms and microbial processes. In 1998 we stated that, in principle, there are only two factors that contribute to this disparity: differences in "bioavailability" and variability in sensitivity of the microorganisms. During the last ten years there has been considerable progress in defining bioavailability (Smolders et al., 2009) and in taking account of differences in bioavailability and sensitivity of soil organisms in EU Risk Assessment Research (e.g. EC, 2008). This was done in the main using the methods outlined in the EU Technical Guidance Document (EC, 2003) on environmental risk assessment. However, we also raised the question whether toxicity effects seen in short-term laboratory tests were relevant to effects likely to be found in the field.

2.1. Laboratory to field differences

It is now widely-recognised that confusion arises when the toxicity of metals is compared between results from short-term laboratory studies and those from long-term exposures that are often obtained through monitoring of field experiments. The evidence suggested that the microbial responses produced in short-term assays (*acute toxicity* or disturbance) were unpredictable and bore little resemblance to the long-term (*chronic toxicity* or stress) effects observed in the field. A study by Renella et al. (2002) confirmed the assertion made in the 1998 review that short-term studies using metal salts cannot be used to infer effects of long-term field exposure to metal toxicity, at least as far as effects on the soil microbial biomass are concerned. We, therefore, called for more studies to increase our understanding of the mechanisms that lead to the long-term effects seen in the field, as these are most relevant for protection of the environment.

The study of indigenous soil microbes demands a different approach to that used in most toxicological studies. A common experimental approach is to take a (relatively) uncontaminated soil and add metals in the laboratory or field. In plant or invertebrate tests, exogenous organisms are then exposed to these soils, usually using a single genotype that has no time to adapt to the toxicity to which it is exposed. However, native soil microbes will probably be well-adapted, for example, to the concentration of metals present in the particular soil (McLaughlin and Smolders, 2001). As we pointed out, this is even more likely in the case of samples taken from long-term contaminated plots or field gradients where metals have been added slowly and have equilibrated with the soil colloids over several years. Unlike laboratory tests, adaptation or selection of specific microbial groups or (sub)populations that are metal resistant or tolerant is likely to occur. We know that adaptation occurs, for example, in the case of nitrifier populations (Rusk et al., 2004; Fait et al., 2006; Mertens et al., 2006). The original observations of metal toxicity to rhizobia in the Woburn Market Garden Experiment demonstrated that a metal-tolerant strain of *Rhizobium leguminosarum* bv. *trifolii* survived in the most contaminated plots at that site, that nodulated white clover but was ineffective in nitrogen fixation (Giller et al., 1989; Hirsch et al., 1993).

In recent experiments, the same bioassays for toxicity to plants, invertebrates and soil microbial processes were performed either on soils from planned field experiments, or zinc or copper gradients that had existed in the field for some years, in several countries (Smolders et al., 2004; Oorts et al., 2006b, 2007). If such field resources did not exist, soils were amended with Ni or Co salts and "aged" under outdoor conditions for 12–18 months before toxicity assays. This enabled the calculation of a ratio between, for example, the concentration which elicited a 50% reduction in biological response (EC50) in the field and in the laboratory (Smolders et al., 2004; Oorts et al., 2006b, 2007). This ratio has been termed the "lab to field" or pollutant "ageing" factor. In this way, an attempt is made to account for laboratory-to-field differences which may make the risk assessment more realistic.

2.2. Bioavailability

"Total" soil metal concentrations are a poor indicator of the actual concentration in the soil solution to which soil microorganisms are exposed. Factors such as pH and soil texture, that can strongly influence metal bioavailability, are sometimes taken into account when establishing permissible limits for soil metal concentrations. Although the EU Technical Guidance Document (EC, 2003; Appendix VIII) emphasises the need to compare metal toxicity data at "similar levels of availability", there is still no universally acceptable method to assess bioavailable soil metal concentrations.

Published tests of the toxicity of metals in soil to microorganisms, plants and animals go back some 30–40 years. Many of the older studies are of limited use in defining permissible concentrations of metals in soil, because they lack a broad enough range to define a full dose-response, study only one or few soils, do not employ standard methods, or fail to report important properties of the soils studied. This makes it impossible to identify key factors affecting the expression of toxicity or to identify thresholds of toxicity. To address these shortcomings, a series of new studies has been performed for Zn, Cu, Ni and Co on microorganisms, invertebrates and plants using a wide range of soils and dose-responses. These provide full soil characterisation, including the soluble metal concentrations in the soil solution, and use standard methods for the bioassays (Smolders et al., 2004; Oorts et al., 2006a; Rooney et al., 2006, 2007; Criel et al., 2008; Li et al., 2009). The toxicity results were then analysed statistically to identify the strongest relationships or explanatory factors that reduce the variability seen when considering total metal concentrations. One variable, that explained the largest proportion of the variation in toxicity thresholds based on total metal concentrations for all these divalent cations between soils, was the effective cation exchange capacity (eCEC) at the natural pH of the soil. Metal toxicity decreased linearly with increasing eCEC. Interestingly, eCEC is a function of pH, clay and soil organic matter, all of which are key factors well known to affect metal bioavailability (Smolders et al., 2009). Furthermore, eCEC can be determined easily on bulk soil and is, therefore, a practical test for routine use. Either measured eCEC, or a calculated eCEC from measured pH, clay and organic matter, can be used to normalise metal concentrations to predict the bioavailability and toxicity in a specific soil. It is worth noting that eCEC explained toxicity to plants and invertebrates more consistently than to microbes (Smolders et al., 2009). This points to a difference between these types of organism and to the difficulty of predicting toxicity to microbes in soils in particular.