What Happens After the First Shock? Temporal Changes in Microbial ECx-values in Metal Contaminated Field Trials

Kris Broos, Michael Warne, Mike McLaughlin

Centre for Environmental Contaminants Research, CSIRO Land and Water, PMB 2, Adelaide, SA 5064, AUSTRALIA (kris.broos@csiro.au)

INTRODUCTION

Elevated metal concentrations in soils can disturb soil microbial processes. Several studies have concluded that soil microorganisms are more sensitive to elevated metal concentrations than other terrestrial organisms (e.g. Giller *et al.*, 1998). Due to their essential role in soil nutrient cycling, it is argued that microorganisms should be protected. Nevertheless, maximum permitted concentrations of contaminants in soil are often derived without considering soil microbial endpoints, or, when they are considered, the data are often based on laboratory experiments where soils are spiked with metal salts. Due to salt-induced changes in metal chemistry, some negative ecotoxic effects can be measured immediately, but adverse effects may disappear fairly rapidly over time under field conditions. Other effects, such as death and extinction of specific microbial species (and therefore loss of specific soil functions), may occur slowly in the field, and are not detected during short term laboratory experiments (Giller *et al.*, 1999).

METHODS

Soils from 8 experimental field sites of the Australian National Biosolids Research Program (NBRP) were used to analyse the effect of Cu and Zn salts on both Substrate Induced Nitrification (SIN), that assesses specific microbial functionality, and Substrate Induced Respiration (SIR), that assesses total microbial population activity. Soil samples were taken immediately after addition of metal salts (T0) and after one cropping season, approximately 8 months later (T1).

To measure SIN, a source of NH_4 was added to the soils and the quantity of nitrate formed after 28 days was measured (OECD, 2000). Results were expressed as the % of substrate (NH_4-N) utilized after 28 days. The SIR rate was measured based on the OECD guidelines (OECD, 2000) with some modifications. The respiration rate (CO_2-C/g soil/h) during a 6-hour incubation experiment was measured after addition of ^{14}C-labelled glucose (5 mg C/g dry soil).

RESULTS AND DISCUSSION

Result show that the SIR EC50 values increased with time or were not significantly different at T1 compared to T0 for both Cu and Zn (Table 1). In more than half of the fields, it is not possible to detect an EC50 value one cropping season after metal addition.

In the majority of cases the SIN EC50 values for Cu and Zn exhibited the same trend as the SIR data (Table 2). However, in 2 of the 8 fields the toxicity of Cu significantly increased (lower EC50).

The values presented in Tables 1 - 2 are based on total metal concentrations, and only show EC50 values, but analyses are also being conducted on added metal concentrations, for both EC50 and EC20 values and will be reported during the conference. Chemical measurements of metal availability (soil solution metals and $CaCl_2$ extractable metals) will be taken into account to verify if 1) the exposure of the microbes to the metals changes over

time or 2) microbial populations develop metal tolerance. Furthermore, the influence of acidification and increasing ionic strength due to metal salt addition will be discussed.

This dataset links short term metal toxicity at T0 (analogous to short term laboratory experiments) with toxicity in field conditions (T1) and may potentially provide some factors for extrapolating thresholds derived by standardized tests into more environmentally relevant thresholds in the field.

Table 1. EC50-values of substrate induced respiration (SIR) for Cu and Zn immediately after application (T0) and after one cropping season (T1).

Field Site	Total Cu (mg/kg)			Total Zn (mg/kg)		
	T0	T1	significance	T0	T1	significance
Tintinara	877	2742	*[2]	2910	2155	ns
Spalding	413	766	ns[3]	2090	14439	ns
Avon	1207	>mc[1]	*	2759	>mc	*
Cecil Plains	2341	>mc	*	4946	>mc	*
Kingaroy	2176	1839	ns	>	>mc	ns
Bundaberg	699	>mc	*	798	no effect	*
Flat Paddock	465	1537	ns	991	>mc	*
Night Paddock	1402	>mc	*	2982	no effect	*

[1] calculated EC50 value greater than maximum concentration
[2] EC50 values significantly different (non overlapping 95% confidence intervals)
[3] EC50 values not significantly different

Table 2. EC50-values of substrate induced nitrification (SIN) for Cu and Zn immediately after application (T0) and after one cropping season (T1).

Field Site	Total Cu (mg/kg)			Total Zn (mg/kg)		
	T0	T1	significance	T0	T1	significance
Tintinara	726	623	ns[3]	528	2994	ns
Spalding	*1245*	*836*	*[2]	1011	1027	ns
Avon	no effect	no effect	ns	no effect	no effect	ns
Cecil Plains	1972	no effect	*	1256	no effect	*
Kingaroy	744	1646	*	936	>mc[1]	*
Bundaberg	212	479	*	201	426	ns
Flat Paddock	171	341	*	221	476	*
Night Paddock	*985*	*445*	*	1621	736	ns

[1] calculated EC50 value greater than maximum concentration
[2] EC50 values significantly different (non overlapping 95% confidence intervals)
[3] EC50 values not significantly different

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