Do soil enzyme activities generate good endpoints for assessing heavy metal toxicity in soils?

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Abstract
Three contrasting field soils were amended with increasing concentrations of Cu and Zn and an assessment made over two years (three annual samplings) of the efficacy of soil phosphatase, sulphatase and urease activities as biological endpoints to measure heavy metal toxicity. Generally, EC_{50} values generated from these relatively simple assays were similar to those from soil biological and plant growth properties that are usually regarded as more relevant indicators of soil health and function. In addition, for the enzyme activity results there were fewer data sets that, either did not fit the sigmoidal dose-response model used to produce EC_{50} values, or fitted poorly with low R^2 values and/or large 95% confidence intervals, than were found from the data of the other methods used. All results suggest that the soil limit concentrations in Australasian guidelines for Cu and Zn are likely to be protective of these soil properties.

Key Words
Soil biochemical properties, heavy metals, dose-response, EC_{50}, biosolids, guidelines

Introduction

Despite soil heavy metal limits being an integral part of rules and guidelines for biosolids re-use, regulators, the farming industry and the wider community remain cautious about land application, partly because the metals, once in the soil, are there in perpetuity. This is especially true in New Zealand – whose economy is strongly dependent on the export of agricultural produce – where there is a fear of damaging our so-called "clean green" reputation. This situation is not helped by there being no clear origin for the actual heavy metal soil limit concentrations in the various guidelines that were developed from the EC Directive of 1986 (CEC, 1986; e.g., NZDoH, 1992; NZWWA, 2003; EPA Victoria, 2004).

In recent times, attempts have been made to derive EC_{20} and EC_{50} values (concentrations causing a 20% or a 50% decline of microbial numbers or activity) for heavy metal effects on microbial and biochemical properties of soils exposed to biosolids (Speir *et al*., 2007). Soil enzyme activities were included among the endpoints for this research because they are regarded as sensitive indicators of soil health and biological functioning and because they are easy to measure. Conclusions were that definitive EC values could not be calculated because the data sets were too variable and the maximum heavy metal concentrations were not high enough. The variability issue arises because biosolids enhances soil biological activity though its nutrient and organic matter contents and because it is almost impossible to make an heterogeneous mix of biosolids and soil. The metal concentration issue arises because biosolids is not sufficiently contaminated to raise soil metal concentrations enough to severely inhibit biological activities. Many applications of biosolids would be required to obtain enough metal to determine definitive EC values.

As a result of the problems encountered using biosolids, we established field trials where soils were amended with heavy metal salts (Cu and Zn), duplicating the range of concentrations used by the researchers in the Australian National Biosolids Research Program. A range of soil biochemical properties were measured, including the activities of phosphatase, sulphatase and urease. Effects were related to metal concentration and EC_{50} were determined using a sigmiodal dose-response model. The usefulness of the enzyme activity-derived EC values was assessed in comparison to those from other biological measures of soil function.

Materials and Methods

Field trials

Three field trials were established on contrasting soils in different regions of New Zealand; on Waihou silt loam near Hamilton, on Tahunanui sand near Nelson and on Templeton fine sandy loam near Lincoln. The soils were all under pasture. Each trial comprised 30 randomly-assigned plots (1 m x 1 m) receiving $CuSO_4$ at seven dosages, $ZnSO_4$ at seven dosages, and an undosed control, all duplicated. The amounts of Cu applied were sufficient to raise soil concentrations by 5 – 2000 mg kg^{-1}, and of Zn to raise soil concentrations by 10 – 3000 mg kg^{-1}. The soils were amended by removing the top 10 cm from each plot,

coarsely sieving to remove herbage, roots and stones, mixing with the appropriate amount of salt using a concrete mixer, and finally returning to the same location in the field. Following amendment the plots were left for two weeks prior to first sampling. After sampling, the plots were sown with ryegrass/clover seed.

Sampling and analysis
Approximately 1 kg soil was taken from each plot (25 – 30 soil cores, 0-10 cm depth and 2 cm diameter) two weeks after amendment and 1 year and 2 years later. Soil was sieved on returning to the laboratory and a subsample air-dried for heavy metal analyses and for other chemical properties. The remainder was maintained at 4° C for biochemical analyses, which were carried out as soon as possible.

Total Cu and Zn concentrations were determined by XRF of pressed discs of finely ground soil. Phosphatase and sulphatase enzyme activities were measured as described by Speir *et al*. (2007), based on the methods of Tabatabai and Bremner (1969, 1970). Urease activity was measured as described by Searle and Speir (1976), except that the colorimetric analysis was done manually. The other biological and biochemical assays included microbial biomass C (MBC) (Vance *et al*., 1987) and respiration (Sparling and Zhu, 1993), substrate-induced nitrification (SIN) (OECD, 2000) and above- and below-ground wheat seedling biomass in a plant germination test (Smart *et al*., 2004).

Data analysis
Mean biological data from each treatment in each trial were related to mean total metal concentration using the sigmoidal dose-response program developed by CSIRO, Australia (Barnes *et al*., 2003), based on the model developed by Haanstra *et al*. (1983). Values of EC_{50} were calculated, along with their 95% confidence intervals.

Table 1. EC_{50} values for enzyme activities and total soil Cu.

Property	Soil	Sampling	EC_{50} (mg kg–1)	95% confidence interval (mg kg–1)	Slope	R2
Phosphatase	Waihou	Two weeks	2880	1190 – 6980	-1.63	0.93
		One year	1090	630 – 1890	-2.57	0.96
		Two years	1380	930 – 2040	-3.80	0.95
	Tahunanui	Two weeks	920	400 – 2130	-2.11	0.93
		One year	580	280 – 1200	-3.13	0.93
		Two years	590	390 – 900	-2.72	0.98
	Templeton	Two weeks	1220	330 – 4450	-1.45	0.91
		One year	670	610 – 740	-3.40	0.998
		Two years	630	490 – 820	-2.57	0.99
Sulphatase	Waihou	Two weeks	13300	3410 – 52400	-1.55	0.93
		One year	2140	1400 – 3270	-3.00	0.95
		Two years	1860	1430 – 2400	-4.68	0.96
	Tahunanui	Two weeks	720	290 – 1780	-1.84	0.96
		One year	310	180 – 530	-2.79	0.98
		Two years	280	100 – 770	-2.29	0.97
	Templeton	Two weeks	390	60 – 2320	-1.67	0.94
		One year	210	50 – 830	-1.98	0.96
		Two years	310	170 – 550	-2.52	0.99
Urease	Waihou	Two weeks	4850	3350 – 7030	-2.74	0.98
		One year	2950	1680 – 5200	-3.10	0.91
		Two years	2670	1740 – 4090	-4.76	0.91
	Tahunanui	Two weeks	410	40 – 4370	-1.88	0.88
		One year	360	20 – 6470	-2.16	0.87
		Two years	730	380 – 1380	-6.22	0.86
	Templeton	Two weeks	1130	750 – 1690	-3.24	0.97
		One year	720	500 – 1050	-4.99	0.96
		Two years	870	740 – 1030	-6.11	0.99

Results

The soil enzyme EC_{50} values for Cu are shown in Table 1. In all but one instance (urease at Tahunanui) EC_{50} values declined markedly between the two week and one year samplings and the slope of the sigmoidal curve always increased over this period. Increased slope, coupled with a high R^2 value, always results in a smaller 95% confidence interval and generally the smallest confidence intervals occurred after two years. The soil enzyme EC_{50} values for Zn are shown in Table 2. The temporal trends are less distinct for Zn, but again, slopes are usually less initially. Overall, for Zn, R^2 values are lower than those for Cu, indicating a poorer fit of the data and this is reflected in larger 95% confidence intervals.

Table 2. EC_{50} values for enzyme activities and total soil Zn.

Property	Soil	Sampling	EC_{50} (mg kg−1)	95% confidence interval (mg kg−1)	Slope	R2
Phosphatase	Waihou	Two weeks	20000	100 – 5 x 106	-1.75	0.42
		One year	2730	1720 – 4320	-2.27	0.96
		Two years	2180	1450 – 3290	-3.34	0.94
	Tahunanui	Two weeks	2190	1180 – 4080	-6.07	0.73
		One year	2490	150 – 42000	-5.68	0.34
		Two years	2800	160 – 48000	-3.44	0.43
	Templeton	Two weeks	1330	600 – 2960	-2.21	0.95
		One year	1180	760 – 1820	-3.52	0.93
		Two years	1480	920 – 2380	-3.16	0.92
Sulphatase	Waihou	Two weeks	15800	1600 – 156000	-1.88	0.83
		One year	3090	1630 – 5850	-3.43	0.86
		Two years	2160	1420 – 3270	-3.38	0.93
	Tahunanui	Two weeks	3700	990 – 13800	-1.58	0.85
		One year	1100	510 – 2370	-3.01	0.88
		Two years	860	220 – 3420	-2.74	0.74
	Templeton	Two weeks	1880	650 – 5480	-2.14	0.88
		One year	760	530 – 1100	-6.09	0.93
		Two years	1020	850 – 1220	-8.25	0.97
Urease	Waihou	Two weeks	26000	300 – 2.2 x 106	-1.59	0.71
		One year	3820	2670 – 5460	-2.61	0.97
		Two years	2620	1760 – 3900	-3.94	0.93
	Tahunanui	Two weeks		No fit to model	-22	0.07
		One year		No fit to model	-6.04	0.08
		Two years	1360	380 – 4940	-6.39	0.33
	Templeton	Two weeks	1240	70 – 23000	-1.36	0.82
		One year	1020	280 – 3730	-2.19	0.91
		Two years	1370	30 – 56000	-1.38	0.71

Discussion

The greatest advantages of enzyme activity assays over other biological measures of soil health and function are simplicity of the methods and ease of analysis. The assay methods used here require little soil, short incubation periods, few chemicals and simple instrumentation. In most instances, the enzymes have proved to be sensitive to increasing soil metal burden and the data have fitted the sigmoidal dose-response model extremely well. Even when the three data sets for each enzyme were grouped (data not shown), R^2 values remained similar to those shown and EC_{50} values were similar to one year and two year values.

The enzyme data compared very favourably with results gained using the more time-consuming assays – MBC, basal respiration, SIN, and seedling shoot and root biomass (data not shown). For Cu, phosphatase EC_{50} values were usually similar to those generated from MBC and SIN, although the SIN curves had steeper slopes and, consequently, smaller confidence intervals. Except in the Waihou soil, sulphatase activity was more sensitive (lower EC_{50} values) than the other enzymes and MBC and SIN. Seedling shoot dry weight gave inconsistent results, whereas root dry weight gave similar EC_{50} values to those for phosphatase in Waihou and Tahunanui soils, but closer to those for sulphatase in the Templeton soil.

For Zn, MBC, respiration and SIN gave inconsistent results (greater variability and some very large 95% confidence intervals), but acceptable EC_{50} values were similar to those for phosphatase or sulphatase. Seedling shoot and root dry weights also gave inconsistent EC_{50} values.

Overall, our results suggest that soil enzyme activities can be used to generate useful endpoints for assessing heavy metal toxicity to soil health and function and that their EC_{50} values are not dissimilar to those from more direct measures of soil microbial function and plant performance. It is also clear that these properties are unlikely to be seriously adversely affected in these soils at the maximum Cu and Zn concentrations recommended in Australasian guidelines (100 mg kg^{-1} for Cu and 200-300 mg kg^{-1} for Zn). Our data also highlight the need for appropriate controls when conducting experiments of this type, since all soil biological and biochemical properties can be influenced by factors other than heavy metals (e.g., soil pH, fertiliser application).

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