

# Response of *Pinus radiata* and soil microbial activity to increasing copper and zinc contamination in a soil treated with metal-amended biosolids

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## Abstract

The effects of elevated concentrations of copper (Cu) and zinc (Zn) in a soil treated with biosolids previously spiked with these metals on *Pinus radiata* were investigated in a 312 day glasshouse pot trial. The total soil metal concentrations in the treatments were 16, 48, 146 and 232 mg Cu/kg and 36, 141, 430 and 668 mg Zn/kg. Increased total soil Cu concentration increased the soil solution Cu concentration (0.03 to 0.54 mg/L) but had no effect on leaf and root dry matter contents. Increased total soil Zn concentration also increased the soil solution Zn concentration (0.9 to 362 mg/L). Decreased leaf and root dry matter were recorded above the second level of Zn. Neither Cu nor Zn had any effect on the mycorrhizal colony of *P. radiata*. A lower percentage of Cu in the soil exchangeable fraction (5–12%) and lower Cu<sup>2+</sup> concentration in soil solution (0.001–0.06 µM) relative to Zn (soil exchangeable fraction, 12–66%; soil solution Zn<sup>2+</sup> concentration, 4.5–4419 µM) indicated lower bioavailability of Cu. Soil dehydrogenase activity decreased with every successive level of Cu and Zn applied. It was reduced by 50% at the total solution–phase Cu and Zn concentrations of 0.3 and 38 mg/L, respectively, and solid–phase exchangeable Cu and Zn concentrations of 8 and 185 mg/kg, respectively.

## Key Words

Radiata pine, forest, speciation, soil metal fractions, EC<sub>50</sub>, rhizosphere.

## Introduction

Forestry has a very important place in the economy of New Zealand and contributes NZ\$ 3.2 billion to the export economy annually. Production forests comprise 7% of New Zealand's total land area (1.8 million ha) of which radiata pine contributes 89.2% of the national plantation forestry estate (NZFOA 2008/2009). Radiata pine forests have in place intensive management regimes and grow relatively quickly. Biosolids application to forestry land is permitted in New Zealand, and this represents a major nutrient and organic carbon input for radiata pine plantations. However, heavy metals accumulating in soil through biosolids application may present an environmental risk. Contaminants could potentially enter the food chain as a result of possible future land–use change. The environmental effects of biosolids must be evaluated. Part of this assessment includes quantification of the long-term capacity of forests to receive biosolids without significant degradation. The concentration of bioavailable rather than total metal is an accurate index to assess the biotoxicity of heavy metals. In order to develop such an index, data are required on the effect of variable concentrations of different metal species on soil microbiological activity and plant growth. This paper presents the results of a study that was conducted to determine the bioavailability of Cu and Zn in soils amended with biosolids containing different Cu and Zn concentrations, and the effect of these metals on the growth and level of metal uptake by radiata pine.

## Method

A glasshouse trial was conducted using one–year old radiata pine (*Pinus radiata*) in pots containing soil (13 kg) that was amended with biosolids previously spiked with 3 levels of Cu or Zn. The control treatment was soil amended with biosolids not spiked with metal (Jeyakumar *et al.* 2008 and 2009). Total metal concentrations (mg/kg soil) in the treatments were for Cu 16 (control), 48 (Cu1), 146 (Cu2), 232 (Cu3); for Zn 36 (control), 141 (Zn1), 430 (Zn2), 668 (Zn3). These metal concentration ranges cover the soil metal concentration limits (100 and 300 mg/kg for Cu and Zn, respectively) recommended by the NZWWA (2003). The 7 treatments were replicated three times and arranged in a Randomized Complete Block Design (RCBD). One–year old radiata clones (R96004) were transplanted, one per pot, and regularly watered to maintain soil moisture at 80% 'pot field capacity'. The plants were harvested 312 days after planting. The

following parameters were measured at harvest: metal fractions, soil–solution total metal concentration, pH, and dehydrogenase activity in rhizosphere and bulk soils, as well as radiata pine foliar and root dry matter (DM) yields, mycorrhizae hyphal density, and plant metal concentration.

## Results

### *Dry matter yield and metal availability to the plants*

Increased Cu levels had no significant effect on both leaf and root DM yield. Increasing the soil Zn concentration decreased leaf DM yields beyond Zn1 treatment (Table 1). In roots a much greater DM yield reduction was observed between the Zn1 and Zn2 treatment than was observed for leaves.

The leaf Cu concentration at all levels of Cu was higher than the critical Cu concentration of 2.1–2.3 mg/kg, generally considered the limit for Cu deficiency in juvenile radiata pine plants (Boardman *et al.* 1997). Plant leaves at the Zn2 treatment showed severe yellowing and withering. The Zn3 treatment plants died during the experiment. The concentration of Zn in leaves and roots for all Zn treatments was significantly increased with every successive level of Zn applied. The leaf Zn concentration above the Zn1 treatments was much higher than the phytotoxic level of 200 mg Zn/kg DM defined by Boardman *et al.* (1997). The bioconcentration factor (BCF) (Table 1) was much lower for Cu (0.03–0.26) than for Zn (0.79–1.5) probably due to lower plant availability of Cu in soils. The leaf:root concentration ratio was also lower for Cu than for Zn (Table 1) suggesting that the translocation of Cu from root to leaf was lower than that for Zn.

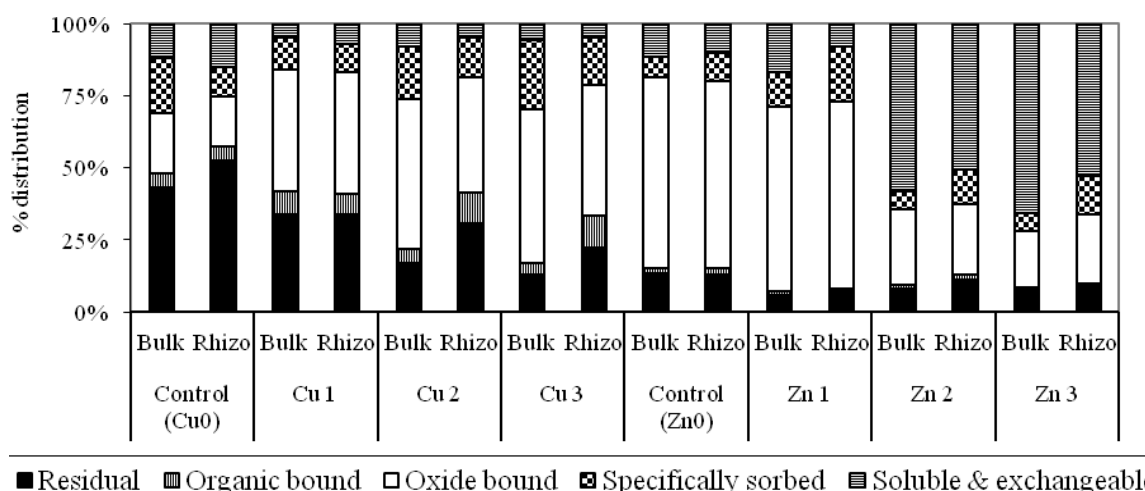
**Table 1. Effect of Cu and Zn on radiata leaf metal concentration and dry matter yield.**

Treatment	Soil solution Conc. (mg/L)	Dry matter (g)		Metal conc. (mg/kg)		Metal conc. Ratio (leaf:root)	BCF
		Leaf	Root	Leaf	Root		
Cu0	0.03	77.6 a	34.8 a	4.2 d	10 d	0.42	0.26
Cu1	0.27	67.5 a	32.7 a	5.3 c	65 c	0.08	0.11
Cu2	0.37	65.6 a	35.6 a	6.3 b	166 b	0.04	0.04
Cu3	0.54	65 a	39.6 a	7.3 a	267 a	0.03	0.03
Zn0	0.9	72.3 a	34.8 a	39 d	45 d	0.85	1.08
Zn1	4.4	68.1 a	33.7 a	205 c	550 c	0.37	1.45
Zn2	89	33.5 b	3.1 b	341 b	2464 b	0.14	0.79
Zn3	362	12.6 c	1.5 b	1000 a	5033 a	0.2	1.5

Values in columns followed by different letters are significantly different ( $p \leq 0.05$ )

### *Metal concentration in soil solution and solid phase*

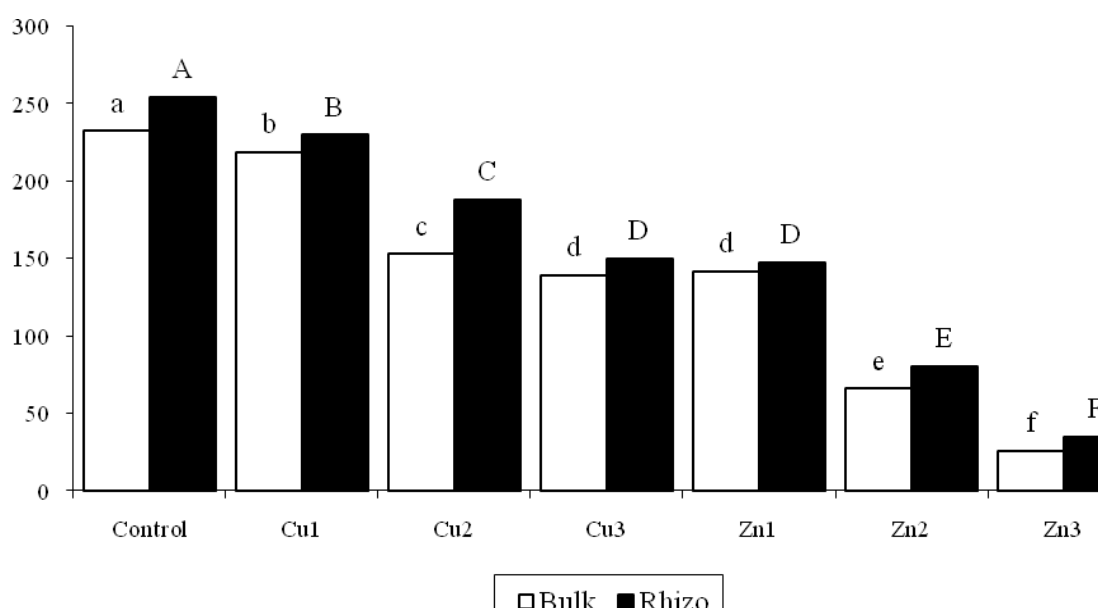
The total Cu concentrations (mg/L) in rhizosphere soil solution were 0.03, 0.27, 0.37 and 0.54 for the control, Cu1, Cu2 and Cu3 treatments, respectively. For Zn, the concentrations were 0.9, 4.4, 89 and 362 for the control, Zn1, Zn2 and Zn3 treatments, respectively. When the soil solution Cu species in the rhizosphere soils were investigated using the Windermere Humic Aqueous Model (WHAM) (Centre for Ecology and Hydrology 2002),  $\text{Cu}^{2+}$  concentrations ( $\mu\text{M}$ ) in soil solution were 0.001, 0.008, 0.04 and 0.06 for the control, Cu1, Cu2 and Cu3 treatments, respectively. These  $\text{Cu}^{2+}$  concentrations are lower than the critical  $\text{Cu}^{2+}$  concentration of 0.22  $\mu\text{M}$ , below which rhode grass growth was reported to be reduced (Sheldon and Menzies 2005). Though the  $\text{Cu}^{2+}$  concentrations are very low, the growth of radiata did not increase with an increase in the  $\text{Cu}^{2+}$  concentration probably because mycorrhizal association in the roots was assisting the plants to take up adequate Cu from the soil even at the lowest  $\text{Cu}^{2+}$  concentration. The low  $\text{Cu}^{2+}$  concentration is attributed to about 99% of Cu in soil solution being complexed to dissolved organic matter.  $\text{Zn}^{2+}$  concentrations ( $\mu\text{M}$ ) for the corresponding Zn treatments were 4.5, 41, 983 and 4419, respectively; and they contributed 61–80% of soil solution Zn. Copper, at all rates of addition, and Zn at the low rates of addition, was mainly associated with the oxide bound soil fraction (21–54% for Cu and 64% for Zn1). However, Zn at high rates of addition (Zn2 and Zn3) was mainly found in the bioavailable soluble & exchangeable soil fraction (58–66%) (Figure 1).



**Figure 1. Percentage distribution of Cu and Zn fractions in rhizosphere and bulk soils amended with biosolids under poplar**

#### *Mycorrhiza and dehydrogenase activity*

Both Cu and Zn at all levels of addition had no significant effect on mycorrhizal colonization of the roots. Although Cu was not found to be toxic to plants or mycorrhiza, it was found to decrease total microbial activity at all levels of Cu addition, as observed from the decrease in soil dehydrogenase activity with increasing Cu (Figure 2). As for Cu, increasing the rate of Zn also decreased soil dehydrogenase activity. The concentration of metal in the soluble+exchangeable solid phase and in the total solution phase that corresponded to a 50% reduction in dehydrogenase activity ( $EC_{50}$ ) was determined. The  $EC_{50}$  values for total solution phase Cu and Zn were 0.3 and 38 mg/L, respectively, and for solid-phase exchangeable Cu and Zn were 8 and 185 mg/kg, respectively.



**Figure 2. Dehydrogenase activity (TPF) in bulk and rhizosphere soils at different levels of Cu and Zn. Bars with different letters are significantly different ( $p \leq 0.05$ ). Simple letters indicate the variance among the bulk soils and capital letters for the rhizosphere soils.**

#### **Conclusion**

Biosolid-derived Cu in soils at rates of up to the highest level of 232 mg/kg tested in this study (total soil solution Cu concentration of 0.54 mg/L and  $Cu^{2+}$  concentration of 0.06  $\mu M$ ) had no effect on radiata leaf and root dry matter yield and root population of mycorrhiza but reduced total soil microbial activity as measured by dehydrogenase activity even at the lowest total soil Cu concentration of 48 mg/kg tested (total soil solution Cu concentration of 0.27 mg/L and  $Cu^{2+}$  concentration of 0.008  $\mu M$ ). Biosolid-derived Zn in soils at rates of 430 mg/kg (total solution Zn concentration of 89 mg/L) and above reduced radiata leaf and root dry

matter yield but had no effect at 141 mg/kg total soil Zn concentration. As for Cu, Zn at all rates of addition reduced dehydrogenase activity but had no effect on mycorrhiza. Therefore, applications of biosolids–derived Cu at rates of up to 232 mg/kg and Zn up to 141 mg/kg are not likely to pose any phytotoxic risk to radiata pine but can present a risk to microorganisms at much lower concentrations. Considering these results the current recommendation of soil Cu and Zn limits (100 mg/kg for Cu and 300 mg/kg for Zn) are high with respect to soil microbial activity but low for Cu with respect to radiata pine growth. In terms of bioavailable metal concentrations affecting microbial activity the Cu concentration limit is much lower than that of Zn. Cu is more toxic to microorganisms than Zn per unit total soil metal concentration.

### Acknowledgement

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