Modelling the toxicity of copper and zinc to wheat and other crops and incorporation of the results into a proposed framework to derive biosolids guidelines

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Introduction

The application of biosolids to land can have both beneficial and detrimental environmental effects. The positive effects can arise from nutrients and organic carbon in the biosolids while the negative effects generally arise because of the contamination associated with biosolids (e.g. Broos *et al*., 2006; Heemsbergen *et al*., 2006; McLaughlin *et al*., 2006) but can also arise due to excessive ammonia (Whatmuff *et al*., 2006). Therefore, any regulatory guidelines developed to manage the land application of biosolids must be able to enhance the beneficial effects and minimise the deleterious effects.

Guidelines developed overseas are generally not considered appropriate for Australia due to our soils, climatic conditions and agricultural practices being quite different (Whatmuff, 1996). Therefore Australia has developed biosolids guidelines at both a national and state level (NSW EPA, 1997; SA EPA 1997; DPIWE, 1999; WA DEP, 1997a, 1997b, 2002; EPA Victoria, 2004; and NRMMC, 2004). The parts of these guidelines related to contaminants are based predominantly on the results of field based experiments conducted by the NSW Department of Agriculture NSW at a single site - Glenfield.

The results from this study were adopted by NSW as this site represents a worst-case scenario of the soils to which biosolids from Sydney would be applied. However, given the wide variety of soil and climatic types within Australia the guidelines may be both over- and under-protective of agricultural and environmental systems at any given location. It was from this limitation of the current biosolids guidelines in Australia that the National Biosolids Research Program (NBRP), of which the subject of this report is part, arose.

Since the NSW biosolids guidelines were developed a number of significant advances have occurred in the methods used to derive environmental quality guidelines. These include the incorporation of the concepts of risk, site specific guidelines and the use of species sensitivity distributions (e.g. ANZECC and ARMCANZ, 2000; Warne, 2001). Given the discussion above, we consider it appropriate that the scientific basis of biosolids guidelines needs to be expanded to include all typical Australian soil, climatic and agricultural practices and that the

latest methods for deriving guidelines should be used. This paper proposes a method of how the above issues can be addressed.

Methods

Laboratory-based phytotoxicity tests
Control soils from 12 sites that received copper (Cu) and zinc (Zn) metal salts (i.e. Avon-SA, Brennans-WA, Bundaberg-Qld, Cecil Plains-Qld, Dookie-Vic, Dutson Downs-Vic, Flat Paddock-NSW, Kingaroy-Qld, Night Paddock-NSW, Spalding-SA, Tintinara-SA and Wilsons-WA) were collected, air dried and sieved (2mm) prior to use. Each toxicity test consisted of eleven treatments: a control, a fertilizer control and nine increasing metal concentrations each conducted in duplicate.

Aqueous solutions of the metals salts ($CuSO_4$ and $ZnSO_4$) were sprayed onto the soil and mixed in order to obtain the desired metal concentration. These tests used freshly spiked soils and thus yielded the toxicity of the metals at time zero (T0). The measures of toxicity were percentage of germinating wheat seeds and wheat seedling growth. Details of the plant toxicity tests are provided in Smart *et al.* (2004). At the time of writing, not all the phytotoxicity tests had been completed.

Field-based phytotoxicity tests
A total of 17 field sites were established throughout Australia as part of the NBRP. Sixteen sites received biosolids while 12 received metal salts. There were 11 sites that received both biosolids and metal salts. Each metal salt site contained 12 treatments: a control and 11 rates of either added Cu or Zn with each treatment duplicated at each trial. All treatments received the type and rate of fertilizer typically applied in that area. The metal concentrations were based on the results of the laboratory-based phytotoxicity tests so that the effects on wheat would range from no effect through to 100% lethality.

At the first harvest (T1) a composite of four top soil samples collected directly under the sampled plant was collected (to 10 cm depth). All soil samples were air dried at 40^{o}C, ground, sieved to <2 mm and stored in airtight containers under room conditions prior to analysis. The soil samples were analysed for pH, electrical conductivity, total, calcium chloride extractable and soil solution metal concentrations.

The crops grown at each site were those grown by the farmer. Plants samples were collected at 8 weeks post-sowing or a maximum biomass and at harvest. At eight weeks/maximum biomass the total biomass of the sampled plants (g) and average plant biomass (g/plant) were determined. At harvest stalk and grain yields (tonnes/ha), 100 grain weight (g), protein content (%), and grain and stalk concentrations of metals and nutrients (g/kg) were determined. All measurements were conducted following the methods set out in Smart et al. (2005).

Statistics
The concentrations that caused a 50%, 20% and 10% inhibition of each of the plant endpoints (EC50, EC20 and EC10 respectively) and their 95% confidence intervals were determined for both the laboratory and field-based phytotoxicity tests using the method of Barnes *et al*. (2003).

Proposed method for modelling the phytotoxicity of copper and zinc and incorporation into guidelines for biosolids

At the time of writing, not all the laboratory-based phytotoxicity tests have been completed and thus the remainder of the manuscript will describe the proposed method and examples of the methods that will be used. Completed calculations will be presented at the conference.

Phytotoxicity of copper and zinc

There was considerable variation in each of the various field-based phytotoxicity measures and endpoints measured in this study (EC10, EC20 and EC50 for yield at 8 weeks, and grain and stalk yield at harvest across the sites, irrespective of the measure of metal concentration used (i.e. total, $CaCl_2$ and soil solution extracts). Table 1 presents this data for Cu EC50 values at T1 (i.e. after the first harvest).

The better a chemical measure is of the bioavailable fraction the smaller will the variation in toxicity values across sites. The total Cu concentrations in soil and Zn concentrations in soil extracted with $CaCl_2$ had the least variation and thus were the best measures of the bioavailable fraction (Table 2). Despite this it is likely that we will use total added metal concentrations for both Cu and Zn as it has been found to be the best measure of bioavailability for Australian microbial toxicity (Broos *et al*., *in prep*) and is the form that has been used in similar European studies (Oorts *et al*, 2006; Smolders *et al*., 2004).

Table 1. The toxicity values (EC50) for various measures of copper concentration (i.e. total, calcium chloride and soil solution) in soils at the field sites for the first (T1) harvest.

Site	**Cu EC50 values for grain yield (t/ha) at T1 harvest**		
	Total	**Soil Solution**	**$CaCl_2$**
Avon	1962	0.56	1.1
Brennans	no effect	25.2	58
Bundaberg	no effect	no effect	no effect
Cecil Plains	1834	50.1	10
Dookie	475	5.2	10.7
Dutson Downs	250	7.8	10.9
Kingaroy	576	0.59	6.6
Spalding	632	2.49	6.8
Tintinara	820	9.57	3.6
Wilsons	3820	41.4	97

Table 2. Variation in the range of EC values (max / min) for EC50, EC20 and EC10 values for grain yield at harvest measured using total, soil solution and calcium chloride metal concentrations in soils from the field sites.

Metal	Type of metal concentration data	Range of EC50 values	Range of EC20 values	Range of Ec10 values
Copper	Total	8.03	6.46	128.59
	Soil solution	89.68	189.54	947.53
	$CaCl_2$	91.03	153.16	809.56
Zinc	Total	42.01	249.39	1237.9
	Soil solution	40.55	246.56	3142.2
	$CaCl_2$	15.73	78.9	490.4

Note: The measure of metal concentration with the smallest range of values for each metal is the best measure of bioavailability.

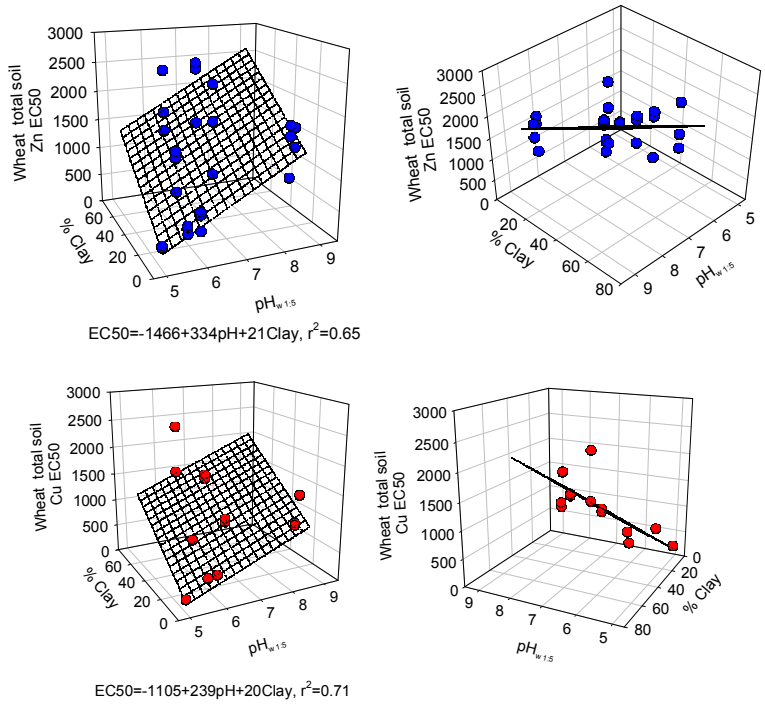
Derivation of models of phytotoxicity

Forward and backward step-wise linear regression will be used to determine which soil physicochemical properties or combinations of properties explain the most variation in phytotoxicity data from laboratory-based tests using field soils freshly spiked with Cu and Zn metal salts (T0) and field soils collected after the third harvest (T3). However, it is expected that the toxicity will be greatest at T0 and therefore the models developed in this study will be based on that data. The soil physicochemical properties used in the models will be: pH, CEC (cmolc/kg), clay content (%), organic C (%), background Zn (mg/kg) and background Cu (mg/kg).

At this stage we have some incomplete phytotoxicity models (i.e. they use laboratory- based wheat phytotoxicity data for 7 of 12 sites) for both Cu and Zn (Figure 1), that were developed earlier in the NBRP. It should be noted, that the addition of metal salts can decrease soil pH (e.g. Speir *et al*. 1999), ideally the soil pH at the EC50 should be used in the models, or the pH of the metal salt treatment closest to the EC50.

It is expected that the final model derived by the NBRP for wheat phytotoxicity will have the same parameters as those presented in Figure 1, as these parameters have been found to be the most important by several studies (Smolders *et al*., 2004; Broos *et al*., 2006; Heemsbergen *et al*., 2006; Oorts *et al*., 2006). However, the models are expected to have different numerators and y-intercepts, as we believe it is more appropriate to use EC10 or EC20 values to derive biosolids guidelines.

Figure 1. Three dimensional plots of the relationships modeling the toxicity (median effect concentration - EC50) of (a) zinc and (b) copper to soil clay content and pH. .



It will then be assumed that the relationship developed for wheat is valid for the other species used in the T1 field-based phytotoxicity tests (i.e. canola, sugar cane, millet and triticale) and from the literature. This assumption is necessary as there are essentially no comparable studies for other plant species. The EC values will then be normalised to combinations of different soil pHs (i.e. 4 to 8) and clay contents (i.e. 5 to 60%) using the developed phytotoxicity models.

The resulting normalized EC values will then be manipulated using the rules adopted from Van de Plassche *et al*. (1993) and used in the Australian and New Zealand water quality guidelines (ANZECC & ARMCANZ, 2000), so that a single value is obtained for every plant species for which there is toxicity data available, at each of the earlier determined combinations of clay content and pH .

The resulting data will be entered into the BurrliOZ species sensitivity distribution software (Campbell *et al*., 2000) and the concentration of Cu and Zn that should protect 99, 95 and 90% of plant species at a variety of combinations of soil clay content and pH values, will be calculated. Figure 2 presents the graphical output of the BurrliOZ software for the freshwater

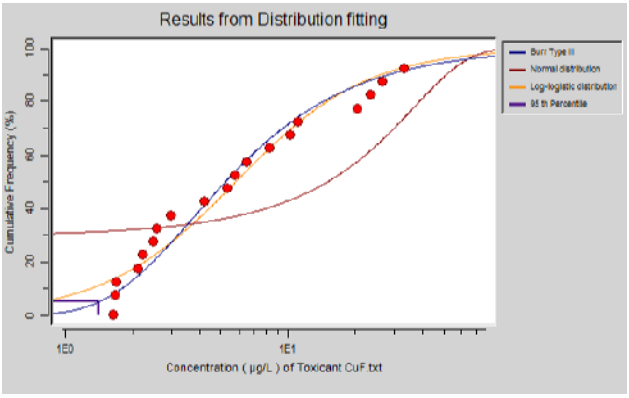
Cu toxicity data used to derive the Australia and New Zealand water quality guideline, as an illustration of how this software works.

Most environmental quality guidelines have adopted 95% of species as the standard level of protection (e.g. ANZECC & ARMCANZ, 2000) and this will be the values we recommend.

Derivation of soil specific biosolids guidelines

As a result of the above we will have a series of soil specific concentrations (at various combinations of pH and clay content) that should protect 95% of plant species. Heemsbergen *et al*. (2006) has shown that the uptake of Cu and Zn from soils amended with biosolids and with metal salts, to plants is similar. Therefore, the methods set out in the various existing biosolids guidelines could be used to convert these site specific soil protection values into soil specific guidelines for biosolids.

Figure 2. Graphical output of BurrliOZ using the freshwater toxicity data from the Australian and New Zealand water quality guidelines. The blue horizontal and vertical line near the origin of the graph indicates the concentration that should protect 95% of species.



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