Models for the field-based toxicity of copper and zinc salts to wheat in eleven Australian soils and comparison to laboratory-based models

Michael StJ Warne[a], Diane Heemsbergen[a], Mike McLaughlin[a], Mike Bell[b], Kris Broos[a], Mark Whatmuff[a,c], Glenn Barry[d], David Nash[e], Deb Pritchard[f] and Nancy Penney[g].

[a]Centre for Environmental Contaminants, CSIRO Land and Water, PMB 2, Glen Osmond, Adelaide, SA. 5064, Australia.
[b]Department of Primary Industries, Kingaroy Qld 4610, Australia.
[c]NSW Department of Primary Industries, Locked Bag 4 Richmond NSW 2753, Australia.
[d]Department of Natural Resources and Mines, Indooroopilly Qld 4068, Australia.
[e]Department of Primary Industries, Ellinbank VIC 3821, Australia.
[f]Curtin University of Technology, Muresk Institute, Northam WA 6401, Australia.
[g]Water Corporation of Western Australia, Leederville WA 6001, Australia.

michael.warne@csiro.au
diane.heemsbergen@csiro.au
mike.mclaughlin@csiro.au
mark.whatmuff@csiro.au
kris.broos@csiro.au
Glenn.Barry@nrw.qld.gov.au
Mike.Bell@dpi.qld.gov.au
David.Nash@dpi.vic.gov.au
D.Pritchard@curtin.edu.au
Nancy.Penney@WaterCorporation.com.au

Corresponding author: Michael Warne, CSIRO Land and Water, PMB 2 Glen Osmond, South Australia 5064, Australia. Tel. +61 8 8303 8533; Fax. +61 8 8303 8565; e-mail: michael.warne@csiro.au

Capsule

Field-based toxicity of Cu and Zn to wheat can be modelled using soil properties. Laboratory based models should not be used to estimate toxicity in the field.

ABSTRACT

Laboratory-based relationships that model the phytotoxicity of metals using soil properties have been developed. This paper presents the first field-based phytotoxicity relationships. Wheat (*Triticum aestivum* L.) was grown at eleven Australian field sites at which soil was spiked with copper (Cu) and zinc (Zn) salts. Toxicity was measured as inhibition of plant growth at 8 weeks and grain yield at harvest. The added Cu and Zn EC10 values for both endpoints ranged from approximately 3 to 4760 mg/kg. There were no relationships between field-based 8 week biomass and grain yield toxicity values for either metal. Cu toxicity was best modelled using pH and organic carbon content while Zn toxicity was best modelled using pH and the cation exchange capacity. The best relationships estimated toxicity within a factor of two of measured values. Laboratory-based phytotoxicity relationships could not accurately predict field-based phytotoxicity responses.

Key words – Zinc, Copper, Wheat, Phytotoxicity, Soil Properties

INTRODUCTION

It has long been recognised that soil physicochemical properties are important modifiers of the toxicity of contaminants (e.g. De Vries and Tiller, 1978; Lexmond, 1980; Alloway, 1995) yet this knowledge has not widely been incorporated into ecological risk assessments or the derivation of soil quality guidelines. As a result very conservative values have tended to be adopted in order to protect all or most terrestrial ecosystems.

Recently, a number of studies have converted this qualitative knowledge into quantitative relationships. Thus, linear relationships, termed normalisation relationships, have been developed that explain the variation in toxicity of As, Cu, Zn and Ni to micro-organisms, plants and invertebrates (Rooney et al., 2006; Smolders et al., 2003; Smolders et al., 2004; Oorts et al 2006; Broos et al., 2007; EU, 2006; Song et al., 2006) and the uptake of Cd, Cu, Pb and Zn by plants (Nan et al., 2002; Li et al., 2003; McLaughlin et al., 2006). These relationships permit the normalisation of terrestrial toxicity and uptake data across soil types, and allow soil quality guidelines to be site or soil specific, thus providing one consistent level of protection at each site. This is a significant improvement on the traditional approach, which applies a single guideline and therefore different levels of protection to each site. The new approach should greatly reduce unnecessary remediation or decontamination of sites and permit regulators and proponents to concentrate on those sites that pose the greatest risk.

The authors are not aware of any field-based relationships for phytotoxicity responses. It is worth noting that many have questioned the appropriateness of

laboratory phytotoxicity data for field situations. For example, Davies (1992) found that pot experiments may not predict the toxicity of heavy metals to crops grown in field conditions, while De Vries and Tiller (1978) found that data from glasshouse-grown plants gave 'completely erroneous' indications of metal uptake by field-grown plants.

Thus, while phytotoxicity normalisation relationships represent a significant advance in environmental management, their reliance on laboratory-based toxicity means that they may not be applicable to field situations. As a result, it is important that predictions of laboratory-based normalisation relationships be compared to field data.

This study reports findings from the Australian National Biosolids Research Program (NBRP). The NBRP was established to quantify the potential human and environmental benefits and risks of applying biosolids to agricultural land and to develop soil and biosolids quality guidelines for metals (i.e. Cd, Cu and Zn) and nutrients. The program is predominantly field-based, with 17 field sites in major agricultural regions of Australia. Models of the toxicity of Cu and Zn to micro-organisms (Broos et al., 2007) and risks of soil-grain transfer of biosolid Cd (McLaughlin et al., 2006) have already been published. The aims of this paper were to develop relationships based on soil physicochemical properties able to model the field-based toxicity of Cu and Zn to bread wheat (*Triticum aestivum* L.) and to determine how well laboratory-based relationships predict phytotoxicity in the field.

MATERIALS AND METHODS

Eleven field sites from major agricultural regions of Australia were selected to provide a range of soil physicochemical properties (Table 1), with separate toxicity tests for Cu and Zn conducted for each site. Each test consisted of 12 duplicated treatments - 11 application rates of either $CuSO_4$ or $ZnSO_4$ and a control plot which received no metal addition. The concentrations of Cu and Zn added to the plots (minimum of 3m x 4m) were based on the results of laboratory-based toxicity tests (Warne et al., in press) and selected so that the effects on wheat at each site would range from no effect through to 100% lethality.

The metal salts were immediately incorporated into the soil to a depth of 10 cm using a rotary hoe or discs. Fertilizer was then applied to all plots according to local farmer practice.

Soil and crop data were collected from the sites for 3-4 years depending on the site. The crops grown were consistent with farmer practice in that region, resulting in 14 different crops being grown, but this paper reports only data for the most prevalent species - bread wheat (*Triticum aestivum* L.). Details of the sites and years when wheat was grown are shown in Table 2.

Plant sampling and preparation

Plant samples were collected during each cropping season at 8 weeks post-sowing and at harvest. The 8-week sampling was used to assess early plant growth responses when plants might be more sensitive to metal toxicity, but also to

provide a sampling at a growth stage that was relatively similar to that used in laboratory-based phytotoxicity assays.

At each sampling, the outer two rows of crops on each side of a plot were discarded to avoid edge effects. At the eight week sampling, 20 wheat plants were randomly selected from the central rows of each plot and the entire plant removed. At harvest, two one-metre long rows from each plot were collected by cutting the plants off at the soil surface and combined.

All plant samples were washed in a 0.1% solution of a phosphorus and metal-free detergent in reverse osmosis water and then dried at 40^{o}C. For the harvest samples, the grain was then separated from the stalks. The stalks were ground to < 2 mm. Stalk and grain samples were separately stored in sealed plastic vials prior to analysis.

Total biomass of the sampled plants (g) was determined at the 8-week sampling, while at harvest shoot yield and grain yield were calculated (t/ha) and grain size was determined by measuring the mass of 100 dry grains. Grain protein content was determined by ashing whole grains in a LECO C, N and S analyzer (Leco Corporation, St. Joseph, Michigan, USA) at approximately 1200 ºC and analyzing for nitrogen. The percent protein in wheat grains was calculated by:

$$\text{Grain Protein (\%)} = \text{grain nitrogen (\%)} \times 5.7 \qquad (1)$$

Soil sampling and preparation

Within two weeks of metal salt application, four composite top soil samples (0-10 cm depth) were collected using a 5 cm diameter auger from each plot. In addition soil samples were also collected from directly beneath the sampled plants at each harvest. All soil samples were air dried at 40ºC, ground, sieved < 2 mm and stored in air-tight containers at room temperature prior to chemical analysis.

Soil properties

The soil pH (0.01M $CaCl_2$) was measured in 50 mL aerated deionised water (1:5 soil/solution ratio) after shaking for 1 h and settling for 20 minutes then adding 1mL of 0.5M $CaCl_2$, shaking for 30 seconds and settling for another 20 minutes (Rayment and Higginson, 1992). The cation exchange capacity (CEC, $cmol_c\ kg^{-1}$) was measured in alcoholic 1M NH_4Cl at pH 8.5 for Avon, Spalding, Tintinara and Cecil Plains and at pH 7.0 for all other soils (Rayment and Higginson, 1992). Organic carbon (OC) was determined as the difference between total carbon and carbonate carbon. Total carbon concentration was measured by ignition with a Leco CNS elemental analyser (Leco, St. Joseph, MI, USA) and the carbonate carbon was determined from pressure increases after addition of HCl to the soil in closed containers (Sherrod et al., 2002). Clay content was determined using the USDA particle size analysis method (USDA, 1996).

Metal analysis

Total soil digests. A 0.5 g sample of soil was digested in reverse *aqua regia*. Quality control was maintained by digesting certified reference samples (two standard soils in duplicate), and four blank samples. After digestion, samples

were vortexed and passed through a Whatman No. 42 filter paper, discarding the first few mL of filtrate (Whatman International, Maidstone, Kent, UK).

Soil solution extraction. A 20 to 30 g sample of soil was wetted to -5 kPa in a syringe and allowed to equilibrate for 16 h before removal of pore water by centrifugation (McLaughlin et al, 1997). Samples were centrifuged at 4,000 *g* for 45 min and then at 30,000 *g* for 45 min and the supernatant passed through a 0.45 µm filter. Electrical conductivity and pH were measured immediately after filtration. Soil solutions were then diluted to 10 mL and acidified with 1 mL of concentrated HNO_3 and stored at 4 ºC until analyzed.

$CaCl_2$ extraction. A 25 mL aliquot of 0.01 M $CaCl_2$ was added to 5 g dry soil and shaken end over end for 4 h. The pH of the soil was measured and then the tubes were centrifuged at 1,300 *g* for 20 min and the supernatant passed through a 0.45 µm filter. Samples were stored at 4ºC until analyzed.

All aqueous extracts of soil and digest solutions were analyzed for concentrations of Cu and Zn using ICP–OES.

Calculations and Statistical analyses

Background concentrations of Cu and Zn at each site were calculated using the method of Hamon et al. (2004). All statistics were calculated with Sigmastat Version 3.1.1 (Systat Software, Point Richmond, VA, USA).

Added metal concentrations (i.e. measured total metal concentration of each soil sample minus the average metal concentration of the controls for that site) were used to determine toxicity threshold values. The concentrations and the 95% confidence intervals (95% CI) that caused a 10, 20 and 50% reduction (i.e. EC10, EC20 and EC50) in plant biomass at eight weeks, shoot yield, grain yield, grain protein content and 100 grain weight at harvest were calculated by fitting a logistic distribution to the data (Barnes et al., 2003).

The toxicity data obtained from each site at successive sampling times are not statistically independent. To overcome this, only the toxicity data for the first wheat crop grown at each site (Table 2) were used to develop relationships between phytotoxicity and soil physicochemical properties.

Normality of the toxicity data and soil properties was tested using the Kolmogorov-Smirnov test with a significance level of 0.05. Parameters that were not normally distributed were log transformed. This was sufficient to achieve normality in all cases. The parameters used in the models were: log EC50 (grain yield), log EC20 (grain yield), log EC10 (grain yield), pH ($CaCl_2$), log CEC ($cmol_c.kg^{-1}$), clay content (%), log OC (%), background Cu concentration and background Zn concentration. The degree of collinearity of the soil properties was determined using the Pearson Correlation Coefficient test. Only properties that were not significantly ($p>0.05$) correlated were combined in multiple linear regressions (MLRs). Toxicity values were related to soil properties using simple and multiple linear regression analysis. Constant variance of the toxicity data about the regression plane was tested by determining the Spearman rank

correlation between the absolute values of the residuals and the observed values of the dependent variable. Whether any particular datum exerted an unduly large influence on the relationships was tested using Cooks distance test and the test for leverage.

RESULTS AND DISCUSSION

Soil physico-chemical properties in control soils

Selected soil properties of the sites are presented in Table 1. Soil pH ($CaCl_2$) ranged from 4.0 to 7.6 with 8 of the soils having a pH below 5.5. The OC content ranged from 0.9 – 5.6%, with one markedly higher value. Clay content ranged from 4 to 66 % and CEC ranged from 3 to 61 $cmol_c/kg$, with one site having a markedly higher value for both parameters.

Correlation between soil physicochemical properties

The only significant ($p \leq 0.05$) correlations were between clay content and log CEC and between clay content and background metal concentrations. These combinations of properties were not used in the MLR analysis.

Toxicity data

Grain yield and 8-week plant biomass toxicity data are presented in this paper and used to derive normalisation relationships. These were selected, as grain protein content and 100 grain weight were not significantly ($p > 0.05$) affected by either Cu or Zn (data not shown) and for shoot yield there was only data for 7 sites.

Toxicity data (EC10, EC20 and EC50 for each endpoint) were expressed using total added, soil solution and $CaCl_2$-extractable metal concentrations in soil. These sets of toxicity data for each combination of endpoint and metal concentration measure were compared to determine which could best explain toxicity across the sites. The measure of metal concentration that has the smallest variability in toxicity values across the sites is deemed to be the best measure of the bioavailable fraction (McLaughlin et al., 2000; Broos et al., 2007) and therefore is the most appropriate to use in developing normalisation relationships. As an example of these comparisons, the plots of Cu and Zn log EC50 values are presented (Figures 1 and 2 respectively). There was a wide range of ECx values across soils, irrespective of the concentration measure used. Overall, the range of the EC50 values expressed as total metal concentrations were similar or smaller than EC50 values expressed as soil solution or $CaCl_2$-extractable metal concentrations. The same occurred for the EC10 and EC20 analysis (data not shown).

A number of authors (Smolders et al., 2003; Smolders et al., 2004; Oorts et al., 2006; Zhao et al., 2006; Broos et al., 2007) have also found extractable or soil solution measurements were not useful predictors of plant and microbial toxicity in soils and thus used added total metal concentrations to develop normalisation relationships.

Given the results of the above comparison from the present study, combined with results from other research (Smolders et al., 2003; Smolders et al., 2004; Oorts et al., 2006; Zhao et al., 2006; Broos et al., 2007), that there is considerably more

total metal concentration data available and there is regulatory acceptance and understanding of this concentration measure, we used toxicity data based on total added metal concentrations.

The EC10, 20 and 50 (grain yield) values for Cu ranged from approximately 130 to 1130, 155 to 1560 and 210 to 5700 mg/kg, respectively (Table 3). The corresponding Zn toxicity values ranged from approximately 90 to 4760, 130 to 4775 and 260 to 4790 mg/kg, respectively (Table 4). The EC10, 20 and 50 (8 week plant biomass) values for Cu ranged from approximately 3 to 1100, 36 to 1130 and 220 to 2070 mg Cu/kg respectively (Table 3). The corresponding Zn toxicity values varied from approximately 17 to 2765, 75 to 3 120 and 160 to 12 900 mg Zn/kg, respectively (Table 4).

The differences between the highest and lowest toxicity value for each measure of toxicity and endpoint were highly variable, varying between 5 to 330 fold for Cu and 17 to 160 fold for Zn. These differences in toxicity across the sites could have been due in part to different sensitivities of the various cultivars of wheat used (Table 2), spatial and temporal differences in climatic conditions, differences in soil properties and temporal variation in the bioavailability of the metal (i.e. aging). Laboratory-based toxicity tests (unpublished data) have shown that there were no significant ($p \leq 0.05$) differences in the sensitivity to either Cu or Zn of three wheat cultivars (cv. Callingiri, cv. Dollarbird and cv. Frame) used in the current study, so this factor is unlikely to be important in the variation in toxicity values between sites. On the other hand, laboratory-based toxicity tests exposing *T. aestivum* cv. Frame to Cu and Zn in the same soils as the present study found

differences in soil properties explained the majority of the variation in phytotoxicity (Warne et al., in press). It is therefore likely that soil properties, modified by effects of climatic conditions and variable metal aging, were the major factors causing the wide variation in toxicity across sites.

Eight week plant biomass EC10, EC20 and EC50 values for both Cu and Zn were not significantly ($p>0.05$) correlated to the corresponding grain yield toxicity values (based on results of both Pearson correlation coefficient and Spearman rank order correlation tests), with the exception of the Zn EC50 value. This has potential implications as many phytotoxicity studies use short-term growth endpoints similar to the 8 week plant biomass used in the present study in hazard and risk assessments, and they have been used to derive normalisation relationships between phytotoxicity and soil physicochemical properties (e.g. Rooney et al., 2006; Warne et al., in press). Thus, if this finding applies to other datasets then such data may have little relevance to long-term plant responses.

Development of predictive toxicity models

All the significant ($p\leq0.05$) and valid linear relationships for each combination of metal and toxicity measure are presented in Table 5.

Only one significant ($p\leq0.05$) relationship (eqn 1, Table 5) could be developed for the Cu 8 week plant biomass data – for Cu log EC50. This was based on soil pH and explained 54% of the variation in toxicity data. Normally relationships that explain such a low proportion of the data variability would not be used, but as no better relationships were available it could be used as an interim measure.

Four significant ($p \leq 0.05$) relationships were developed for the Cu grain yield phytotoxicity data (eqns 3 to 6, Table 5). No statistically significant ($p \leq 0.05$) relationships could be derived for Cu log EC50. For Cu log EC20 and log EC10 data, soil pH formed significant ($p \leq 0.05$) but relatively poor quality positive relationships that are not reliable enough to use in predicting toxicity. The soil properties that formed significant ($p \leq 0.05$) MLRs with Cu log EC20 and log EC10 data were pH and log OC (eqns 4 and 6, Table 5). These relationships explained 76% and 80%, respectively, of the variation in these endpoints (Table 5).

The relationships for Cu developed in the present study and those developed for *T. aestivum* (20 d shoot growth) laboratory assays by Warne et al. (in press) all contained soil pH but had different second parameters in the MLRs. The laboratory-based relationships had either CEC or electrical conductivity as the second parameter, while those from the present study had OC. The highest quality laboratory-based relationship that involved log OC was only able to account for approximately 60% of the variability (Warne et al., in press). Possible causes for the increased importance of log OC in the field-based relationships are explored later.

Only one significant ($p \leq 0.05$) relationship (eqn 2, Table 5) could be developed for the Zn 8 week plant biomass data, and that was for Zn log EC50. This was based on soil pH and it could explain 85% of the variation in toxicity data.

In contrast, 13 significant ($p \leq 0.05$) relationships (eqns 7 to 19, Table 5) were developed for the Zn grain yield toxicity data. Positive simple linear relationships (SLRs) for Zn were based on pH or log CEC (Table 5). The SLRs based on log CEC (eqns 8, 13 and 17, Table 5) explained less than 50% of the variation in toxicity data and thus were not suitable for predicting grain yield toxicity data. The SLRs based on pH (eqns 7, 12 and 16, Table 5) explained between 53 and 61% of the variation in toxicity data. The addition of log OC or log CEC as an additional parameter to pH led to an additional 6 and 13% of the variation in the toxicity data being accounted for, respectively. It should be noted that the probability associated with the log CEC parameter in the log EC10 relationship was 0.068 and thus strictly this relationship should not be used. However, when models are based on small datasets (in this case 10 values) it is appropriate to use higher levels of probability as the acceptance level for statistical significance (Bailey, 1979). Relationships for Zn that explained more than 60% of the observed variability (i.e. eqns 7, 10, 11, 14, 15 and 19, Table 5) can be used to predict toxicity values with reasonable confidence.

The addition of background Cu and Zn concentrations to SLRs only increased the variability accounted for in the Zn log EC50 relationships (Eqns 9 and 10, Table 5). This suggests that the sensitivity of the wheat was not greatly affected by background concentrations of Cu or Zn. This result is in direct contrast to the findings of McLaughlin et al. (2001) who found that the sensitivity of micro-organisms to Zn was inversely related to background Zn concentrations and Broos et al (2007) who found that Zn background concentrations helped explain Zn toxicity to microbial substrate induced respiration.

None of the above relationships had data points which influenced or leveraged the relationships, based on the results of influence and Cook's distance statistical tests. However, the OC, clay content and CEC data each contained one site which was markedly different to the others. By including these data to develop relationships it is assumed that data points lying between the extreme value and the remainder of the data conform to the same relationships that were developed. However, we have no data to support or disprove this assumption. It is also possible that while the extreme values do not influence the relationships their inclusion could lead to changes in the ability of the relationships to accurately estimate phytotoxicity. From a regulatory point of view, under-estimation of toxicity is of greater concern than over-estimation. Therefore the effect of including and excluding the extreme values on the extent of under-estimation of toxicity at the 11 sites was determined for the best relationships developed in the present study. This was not necessary for the 8 week plant biomass relationships as they were based on pH which had no extreme values. However, for the grain yield the five best relationships contained either CEC or OC which had extreme values. The average percent of underestimation by the relationships never exceeded 25% and for three of the five relationships the underestimation was less when the extreme sites were excluded. However, exclusion of the extreme values would severely limit the range of soils to which the relationships could be applied. Given the above and that a key intended use of these relationships is to normalise toxicity data as part of a process of deriving soil and soil amendment quality guidelines, it was decided to use relationships that included the extreme values.

Some or all of the soil properties (i.e. pH and OC for Cu and pH and log CEC for Zn) featuring in the best statistical models of the present study also occur in the laboratory-based phytotoxicity relationships developed in other studies for Cu and Zn effects on plants and micro-organisms (Table 6). This suggests that there may be a common set of easily-measured soil properties that can be used to predict Cu and Zn toxicity to a diverse range of terrestrial organisms.

The relationships for Cu developed from the field experiments agree with well-known reactions of this metal with soil. Increasing soil pH markedly increases Cu sorption in soil to both mineral and organic matter phases (e.g. Smith, 1994; Alloway, 1995), as well as increasing the movement of Cu from labile to non-labile forms, often termed aging or fixation (Ma et al. 2006a, b). Organic carbon forms organo-metallic complexes with Cu which decreases the bioavailability of Cu to plants (e.g. Alloway, 1995). Thus, increasing soil pH and OC content would be expected to decrease the toxicity of Cu and this was observed (Table 5).

The relationships predicting the toxicity of Cu to wheat developed by Warne et al. (in press) from laboratory-based studies did not contain OC and it was argued that this may be due to the limited range of OC values for soils that were used (i.e. 0.95 – 3.45% with one value of 5.7%). This was supported by Broos et al. (2007) who also found OC was not a significant ($p>0.05$) modifier of Cu toxicity to microbial substrate induced nitrification and respiration in fourteen Australian soils that included all those used in the present study. Additionally Broos et al. (2007) found that reanalysis of data from Smolders et al. (2004) and Oorts et al (2006), after removing soils with OC concentrations greater than those found in

the Australian soils, resulted in OC no longer being an important factor explaining the toxicity of Zn and Cu to nitrifying micro-organisms. While the field soils used in the current study have essentially the same range and distribution of OC values as those used by Warne et al. (in press) and Broos et al. (2007), OC was found to be an important modifier of field-based Cu toxicity. A possible explanation for this is that OC is involved with water retention and in field situations this is likely to play a greater role than in the laboratory where optimal soil moisture is generally maintained.

The significant ($p \leq 0.05$) relationships for Zn which include CEC are consistent with well established mechanisms of Zn retention and/or fixation in soil (Crout et al. 2006) as noted above for Cu. The use of CEC has a number of strengths and limitations discussed in Warne et al (in press). If relationships that include log CEC data are to be used to derive soil and soil amendment quality guidelines then (i) a CEC method must be specified and only this method used to measure CEC and implement the guidelines, or (ii) other CEC methods can be used providing they have been calibrated against the recommended CEC method.

The quality of the field-based relationships developed in the present study are similar to, or better than, the best laboratory-based relationships for Cu and Zn to plants (Smolders et al., 2003; Rooney et al., 2006; Warne et al., in press); to micro-organisms (Smolders et al., 2004); and Ni to plants, invertebrates and micro-organisms (EU, 2006). It thus appears possible to develop high quality relationships using field-based phytotoxicity values despite the increased variability that is expected in such data compared to laboratory-based studies.

Predictive accuracy of the relationships

In order to maximise the number of sites used to develop the relationships we did not validate the relationships using independent datasets. Rather, we determined the accuracy of the highest quality (based on adj r^2 values) relationships by plotting measured field-based Cu EC10 and EC20 values against the corresponding values calculated by the relationships for Cu and the corresponding EC10, EC20 and EC50 values for Zn (Figures 3 & 4 respectively). The vast majority (47 out of 55) of the predicted values were within a twofold margin of error from the measured values, and in only 3 cases was the toxicity underestimated by a factor greater than two. Thus, the developed field-based relationships were relatively reliable predictors of the toxicity of Cu and Zn to wheat in these field studies. This was unexpected given that the field-based models were derived from phytotoxicity data from nine cultivars of *T. aestivum* that were grown over 4 different seasons in very different climatic conditions.

The ability of the laboratory-based normalisation relationships developed by Warne et al. (in press) to predict the toxicity of Cu and Zn to *T. aestivum* in the field was tested by plotting EC50 and 10 values derived from the field experiments against the corresponding values predicted by the laboratory-based relationships. This was done for both 8 week plant yield and grain yield at harvest. The laboratory-based relationships predicted the field-based 8 week plant biomass data (Figures 5 and 6 for Cu and Zn respectively) considerably better than they predicted grain yield data (data not shown). This most probably reflects these

endpoints having a more similar exposure duration and growth period (i.e. 21 and 56 days) compared to 6 months for the grain yield data.

The most important point to note from Figures 5 and 6 is that the range in toxicity values for Cu and Zn were much greater under field conditions than in the laboratory. In many instances the laboratory-based models significantly underestimated Cu and Zn toxicity in the field. Points to the right of the lower 2 fold error line (Figures 5 and 6) show that the frequency of instances in which laboratory-based models underestimated toxicity in the field increased as the severity of the toxic effect being modelled decreased (i.e. EC10 > EC50). These results indicate that the laboratory-based relationships for Cu and Zn toxicity to wheat are clearly not suitable for predicting phytotoxicity responses for the same plant species in the field.

The reasons for the much wider range of toxicity values found under field conditions than predicted by the laboratory model are unknown. It may be that effects of soil physicochemical conditions in modifying metal toxicity are expressed less in controlled laboratory conditions, or that multiple stressors (e.g. heat, moisture, disease, etc.) accentuate the effect of soil physicochemical conditions in modifying toxicity. Irrespective of the reasons for the greater range in toxicity found in the field soils than predicted by the laboratory models, in relation to regulatory use of the information, overestimation of toxicity using laboratory-based relationships is protective and therefore of little concern. However, any underestimation of toxicity by the laboratory models is of concern. Our data indicate that it is important to benchmark laboratory-based normalisation

relationships with field data to validate or recalibrate the relationships in situations where they will be applied.

Normalisation relationships, such as those developed in the present study, do have a number of limitations (Warne et al., in press) but can still play a significant role in risk and hazard assessment by providing estimates of the toxicity of chemicals when experimentally derived values are lacking. Through their ability to normalise toxicity data they can also be used to derive site or soil specific SQGs.

CONCLUSION

Field-based phytotoxicity values based on total added metal concentrations were either more accurate, or at least as accurate, a measure of bioavailability as those based on soil solution and calcium chloride extractable metal concentrations. Early growth toxicity data (e.g. 8 week plant biomass) may not be a suitable surrogate for effects on grain yield at harvest. The variation in toxicity values across the sites, irrespective of the measure of toxicity, was 5 - 330 fold for Cu and 17 - 160 fold for Zn, indicating that soil properties strongly influenced phytotoxicity. Multiple linear regression relationships incorporating soil properties as variables were able to explain between 57 and 87% of the variation in field-based toxicity of Cu and Zn. Copper toxicity could be modelled using soil pH and organic carbon content while Zn toxicity could be modelled using the soil pH and the cation exchange capacity. The addition of background zinc concentrations proved useful for explaining additional variation in Zn EC50. The best relationships for EC50, 20 and 10 for each metal all contained the same soil parameters and were consistent with the known behaviour of Cu and Zn in soil,

which strengthens the case for using such relationships in predicting the risks of metal contamination in different soils. Relationships between soil properties and phytotoxicity data derived from laboratory studies could not accurately predict field-based phytotoxicity responses. We recommend that relationships based on field derived data be used to predict phytotoxicity risks in the field, rather than using predictions based on laboratory-derived relationships.

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1 Table 1: Details of the field trials and selected soil properties (dry weight basis).

Field site	Location[a]	Soil type	pH (0.01 M $CaCl_2$)	Organic carbon (%)	Clay content (%)	CEC[c] ($cmol_c\ kg^{-1}$)
Avon	SA	calcarasol	7.6	1.1	12.4	10
Brennans	WA	tenosols	5.4	0.9	4.0	3.2
Cecil Plains	Qld	vertosol	7.3	1.4	66	61
Dookie	Vic	dermosol	4.9	2.0	23	13.0
Dutson Downs	Vic	sodosol	4.0	5.6	5.0	11.6
Flat Paddock	NSW	chromosol	4.4	1.2	17	7.8
Kingaroy	Qld	ferrosol	5.0	1.8	41	16.5
Night Paddock	NSW	chromosol	5.1	3.4	24	17.4
Spalding	SA	chromosol	6.3	1.9	27	17.7
Tintinara	SA	sodosol	6.3	1.8	10	10.3
Wilsons	WA	tenosols	4.8	2.6	6.0	5.0

2 [a] SA = South Australia, NSW = New South Wales, QLD = Queensland, VIC = Victoria, WA = Western Australia. [b] B = biosolids, M = metal salts. [c] CEC = cation exchange

3 capacity. [d] these sites were not used to grow wheat for this study.

Table 2: Cropping seasons when wheat (*T. aestivum* L.) was grown in National Biosolid Research Program sites and the cultivars that were grown.

Site	Cropping seasons when wheat (including cultivar) was grown			
	1	2	3	4
Avon	Aroona	Y-C[a]		
Brennans	Callingiri		Callingiri	
Cecil Plains			Kennedy	
Dookie		Whistle		
Dutson Downs		C-D[b]		
Flat Paddock		DB[c]		
Kingaroy				Hartog
Night Paddock		DB		
Spalding	Aroona	Y-C		
Tintinara		Frame		
Wilsons		Callingiri		

[a] Yitpee-Crickoff cultivar; [b] Chara-Drysdale cultivar [c] Dollarbird cultivar.

Table 3: The concentrations (mg/kg) of added Cu that reduced wheat grain yield and 8 week plant biomass by 50, 20 and 10% (EC50, EC20 and EC10) and 95% confidence limits in brackets for each site.

Field trial	Grain yield		
	EC50	EC20	EC10
Avon	1 147 (60 – 21 700)	1 138 (152 – 8 500)	1 133 (261 – 4 900)
Brennans	286 (196 – 420)	175 (94 – 330)	132 (55 – 317)
Cecil Plains	5 705 (640 – 51 200)	1 560 (577 – 4 200)	731 (300 – 1 780)
Dookie	476 (270 – 840)	228 (120 – 433)	148 (64 – 350)
Dutson Downs	649 (390 – 1 080)	385 (180 – 805)	284 (100 – 800)
Flat Paddock	212 (133 – 340)	156 (89 – 274)	130 (58 – 294)
Kingaroy	310 (230 – 413)	241 (152 – 382)	209 (112 – 390)
Night Paddock	3 170 (61 – 166 000)	1 316 (61 – 28 500)	787 (39 – 15 700)
Spalding	632 (93 – 4310)	603 (19 – 1200)	586 (0.8 – 434 000)
Tintinara	1 040 (634 – 1710)	752 (345 – 1 640)	622 (207– 1 870)
Wilsons	1 760 (795 – 3900)	768 (290 – 2020)	473 (132– 1 700)
		8 week plant biomass	
	EC50	EC20	EC10
Avon	2 070 (234 – 18 300)	35.7 (0.42 – 3 030)	3.32 (0.003 – 4 130)
Brennans	375 (141 – 996)	360 (0.5 – 256 000)	351 (0.02 – 6×10^6)
Cecil Plains	1 154 (905 – 1 470)	792 (595 – 1 060)	635 (400 – 1 010)
Flat Paddock	315 (140 – 715)	168 (72 – 396)	117 (37 – 370)
Kingaroy	272 (227 – 328)	220 (170 – 283)	193 (137 – 272)
Night Paddock	526 (335 – 830)	232 (135 – 400)	144 (70 – 300)
Spalding	223 (133 – 375)	74.7 (31 – 182)	40 (11 – 136)
Tintinara	1 183 (1 100 – 1 250)	1 130 (1 000 – 1 265)	1 100 (900 – 1 330)
Wilsons	330 (170 – 650)	102 (38 – 275)	52 (14 – 190)

Table 4: The concentrations (mg/kg) of added Zn that reduced wheat grain yield and 8 week plant biomass by 50, 20 and 10% (EC50, EC20 and EC10) and 95% confidence limits in brackets for each site.

Field trial	Grain yield		
	EC50	EC20	EC10
Avon	4 789 (na)[a]	4 773 (na)	4 760 (na)
Brennans	263 (170 – 410)	134 (42 – 437)	91 (16 – 515)
Cecil Plains	4 560 (1 120 – 18 600)	3 000 (1 500 – 6 000)	2 351 (1 520 – 3 600)
Dookie	833 (555 – 1 250)	548 (300 – 1 000)	428 (205 – 900)
Dutson Downs	702 (236 – 2 100)	370 (110 – 1 260)	255 (50 – 1 280)
Flat Paddock	632 (330 – 1200)	453 (190 – 1 080)	374 (111 – 1 260)
Kingaroy	389 (246 – 615)	303 (190 – 480)	262 (15 – 655)
Night Paddock	1 320 (na)	1 315 (na)	1 312 (na)
Spalding	1 780 (1 100 – 2 780)	1 400 (920 – 2 100)	1 217 (626 – 2 360)
Tintinara	1 350 (515 – 3 500)	883 (190 – 4 100)	670 (95 – 5 000)
Wilsons	363 (220 – 600)	163 (82 – 325)	102 (39 – 268)
		8 week plant biomass	
	EC50	EC20	EC10
Avon	12 900 (1 000 – 170 000)	198 (4.7 – 8 260)	17.2 (0.04 – 7 580)
Brennans	270 (207 – 354)	195 (91 – 420)	161 (54 – 490)
Cecil Plains	3 900 (137 – 108 000)	3 120 (345 – 28 400)	2 765 (540 – 14 200)
Flat Paddock	182 (75 – 445)	75 (21 – 267)	45 (8.5 – 240)
Kingaroy	377 (307 – 463)	287 (223 – 369)	244 (163 – 366)
Night Paddock	1 120 (795 – 1 580)	785 (510 – 1 210)	640 (370 – 1 110)
Spalding	1 875 (1 180 – 2 990)	967 (483 – 1 935)	657 (267 – 1 615)
Tintinara	2 123 (865 – 5 220)	1 490 (600 – 3 720)	1 210 (390 – 3 750)
Wilsons	159 (110 – 229)	79 (38 – 165)	52 (19 – 143)

[a] na – not available

Table 5: Statistically significant ($p \leq 0.05$) linear regressions of toxicity for grain yield and 8 week plant biomass (8 wk pb) and selected soil properties for Cu and Zn values based on added metal concentrations (mg kg^{-1}). Standard errors of the coefficients are presented in parentheses. All equations had 10 degrees of freedom.

Equation no.	**Metal**	**Toxicity endpoint**	**Toxicity measure**	**Regression Equations[a]**	**Adjusted r^2**	**Prob**
1	Cu	8 wk pb	log EC50	1.38 (0.43) + 0.23 (0.07) * pH	0.54	0.015
2	Zn			-0.16 (0.46) + 0.54 (0.08) * pH	0.85	<0.0001
3	Cu	grain yield	log EC20	1.61 (0.45) + 0.20 (0.08) * pH	0.34	0.036
4				0.63 (0.37) + 0.32 (0.057) * pH + 1.17 (0.29) * log OC	0.76	0.001
5			log EC10	1.44 (0.40) + 0.20 (0.070) * pH	0.42	0.0108
6				0.56 (0.31) + 0.31 (0.048) * pH + 1.05 (0.24) * log OC	0.80	<0.001
7	Zn		log EC50	1.39 (0.40) + 0.29 (0.07) * pH	0.61	0.003
8				2.07 (0.35) + 0.86 (0.31) * log CEC	0.40	0.021
9				2.14 (0.29) + 1.06 (0.28) * log CEC - 0.005 (0.002) * CB Zn	0.57	0.014
10				1.32 (0.25) + 0.20 (0.05) * pH + 0.72 (0.17) * log CEC - 0.004 (0.001) * CB Zn	0.87	< 0.001
11				1.17 (0.34) + 0.23 (0.063) * pH + 0.53 (0.22) * log CEC	0.74	0.002
12			log EC20	1.00 (0.50) + 0.33 (0.088) * pH	0.56	0.005

13			1.73 (0.41) + 1.04 (0.37) * log CEC	0.41	0.02
14			0.32 (0.61) + 0.41 (0.094) pH + 0.81 (0.48) log OC	0.64	0.007
15			0.73 (0.43) + 0.26 (0.080) * pH + 0.64 (0.28) * log CEC	0.70	0.003
16		log EC10	0.77 (0.57) + 0.35 (0.10) * pH	0.53	0.007
17			1.53 (0.45) + 1.14 (0.41) * log CEC	0.40	0.021
18			0.069 (0.71) + 0.44 (0.11) pH + 0.84 (0.56) log OC	0.59	0.012
19			[b]0.48 (0.51) + 0.27 (0.094) * pH + 0.70 (0.33)* log CEC	0.66	0.005

1 [a] OC = organic carbon content; CEC = cation exchange capacity; CB Zn = background zinc concentration. [b] the probability associated with the

2 log CEC parameter is 0.068.

3

1 Table 6. Details of the test organism and soil physicochemical properties used in laboratory-based microbial and plant toxicity normalisation
2 relationships.

Metal	Organism	Parameters	Reference
Cu	Tomato and Barley	1st. Ca or CEC. 2nd. Fe oxide, OC, clay content or pH	Rooney et al., 2006
	Microbial (substrate inducted nitrification, SIN)	1st. pH. 2nd clay content or log CEC	Broos et al., 2007
	Microbial (substrate induced respiration, SIR)	Clay content	Broos et al., 2007
Zn	Bread wheat	1st. pH. 2nd log CEC	Smolders et al., 2003
	Microbial (SIR)	Background Zn conc	Broos et al., 2007
	Microbial (potential nitrification rate, PNR)	1st pH. 2nd OC	Smolders et al., 2004
	Microbial (SIR)	1st pH. 2nd background Zn and log CEC	Smolders et al., 2004
	Microbial (maize residue respiration, MRR)	log CEC	Smolders et al.,2004

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Figure 1: Box and whisker plots of the log EC50 values for total added (TA) Cu, calcium chloride (CC) extractable Cu and soil-solution (SS) Cu on grain yield, shoot yield and 8 week plant biomass (8 wk). The box spans the interquartile range of the log EC50 values, with the vertical line within the box indicating the median. Horizontal lines extending beyond the boxes indicate the 10^{th} and 90^{th} percentiles and dots are log EC50 values outside the 10^{th} and 90^{th} percentiles.

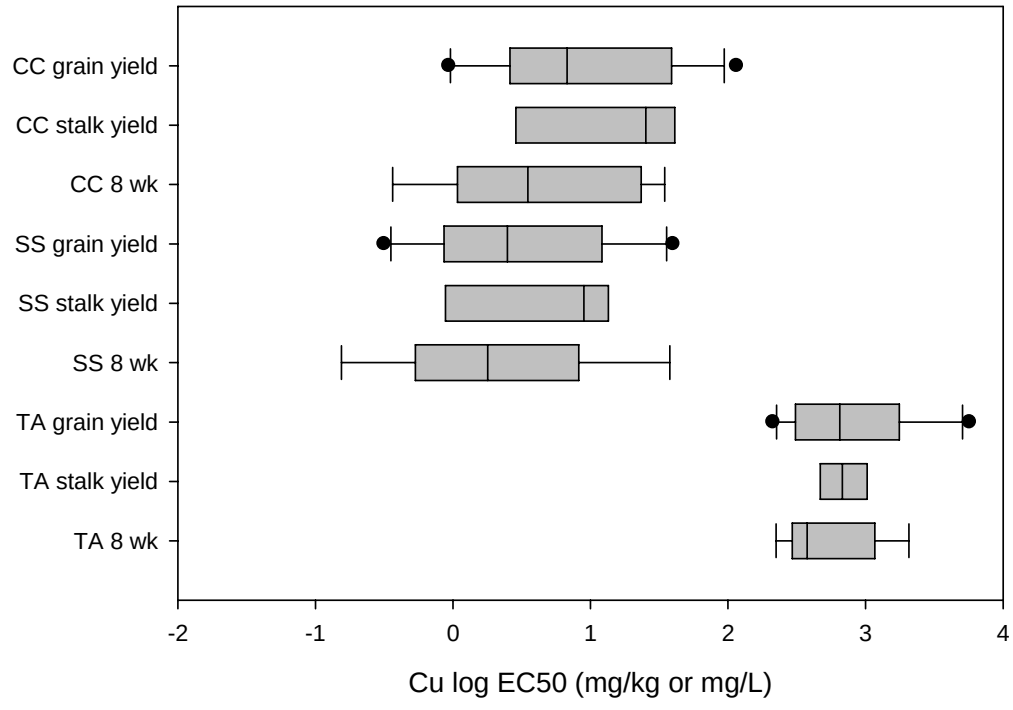


Figure 2: Box and whisker plots of the log EC50 values for total added (TA) Zn, calcium chloride (CC) extractable Zn and soil-solution (SS) Zn on grain yield, shoot yield and 8 week plant biomass. The box spans the interquartile range of the log EC50 values, with the vertical line within the box indicating the median. Horizontal lines extending beyond the boxes indicate the 10^{th} and 90^{th} percentiles and dots are log EC50 values outside the 10^{th} and 90^{th} percentiles.

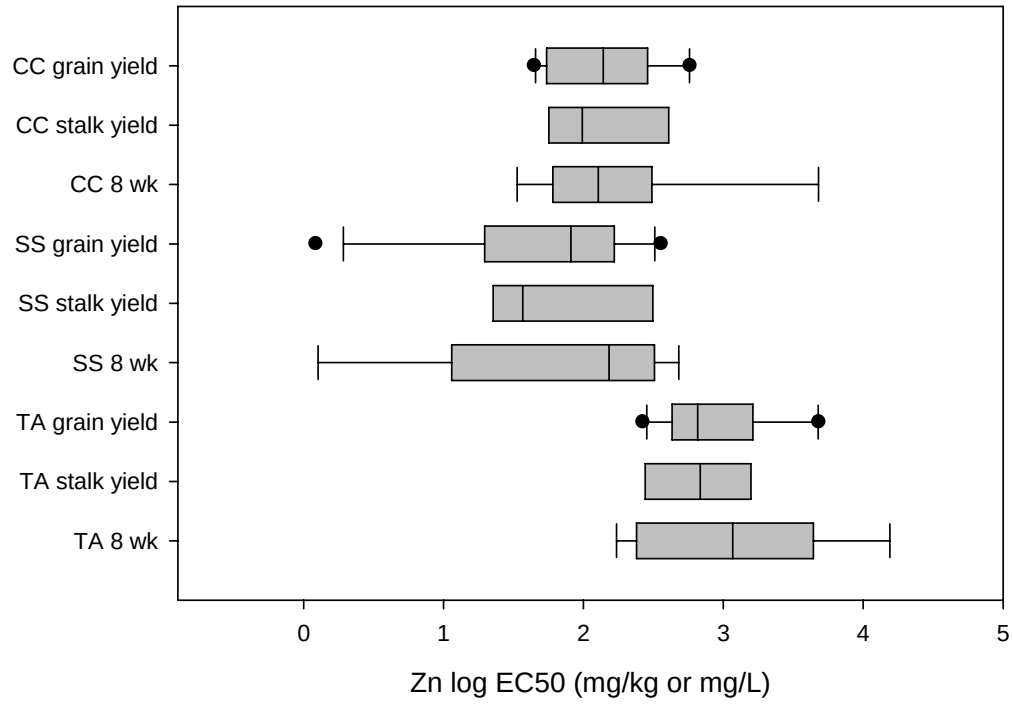
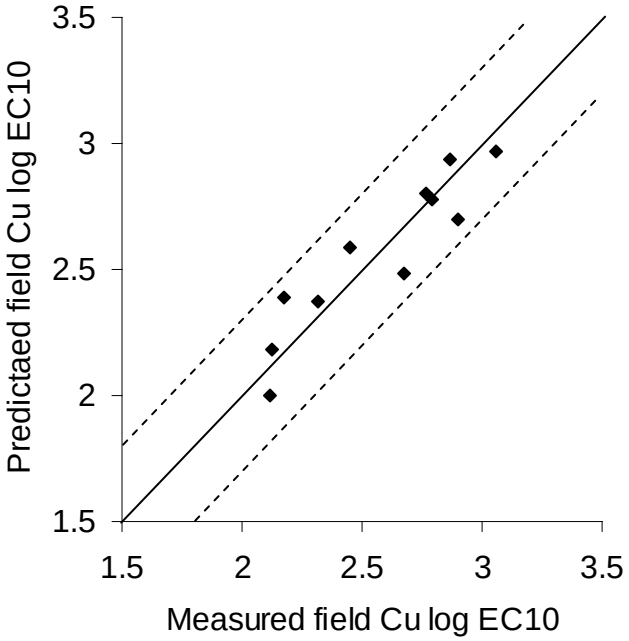


Figure 3: Measured field-based Cu toxicity data (wheat grain yield) versus predicted values using selected field-based relationships (a) log EC10 and (b) log EC20. The solid line is a 1:1 slope and the dashed lines indicate a two fold error from the solid line.

(a)



(b)

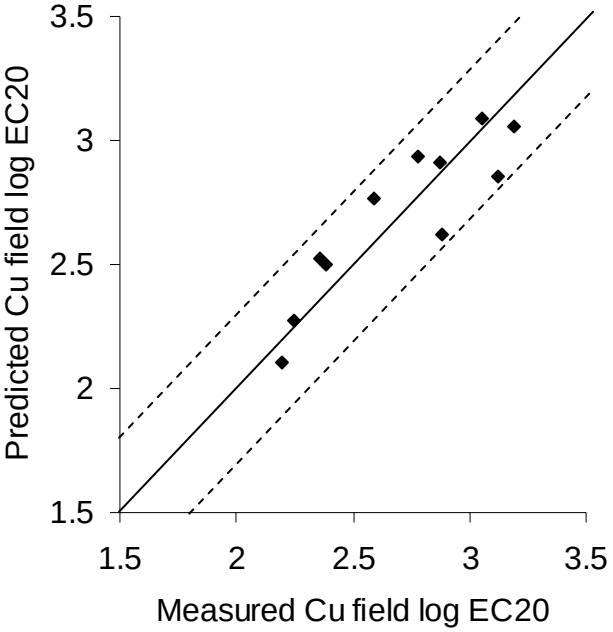
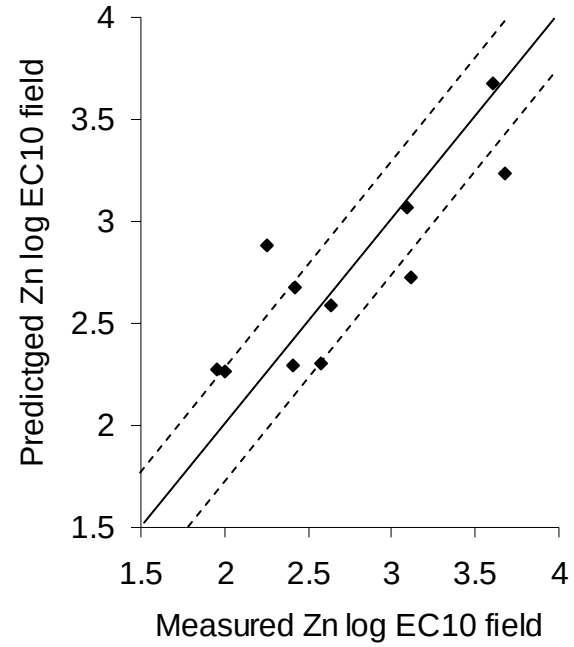
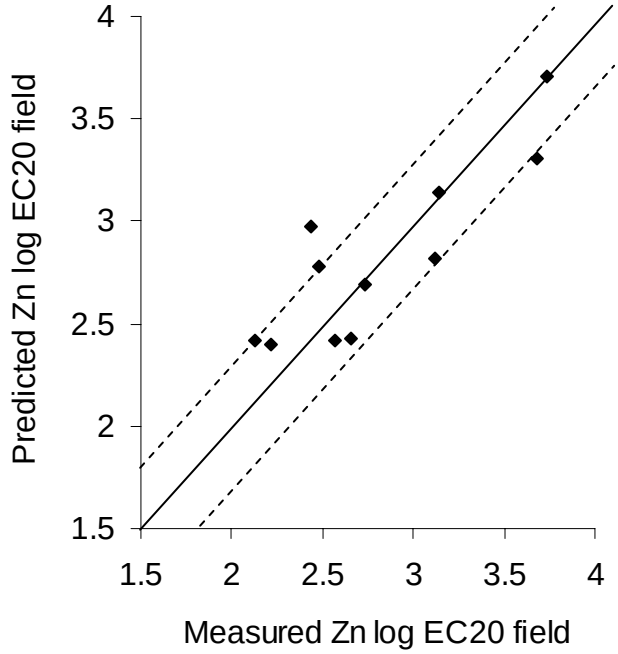


Figure 4: Measured field-based Zn toxicity data for wheat grain yield versus predicted values using selected field-based relationships (a) log EC10, (b) log EC20 and (c) log EC50. The solid line is a 1:1 slope and the dashed lines indicate a two fold error from the solid line.

(a)



(b)



(c)

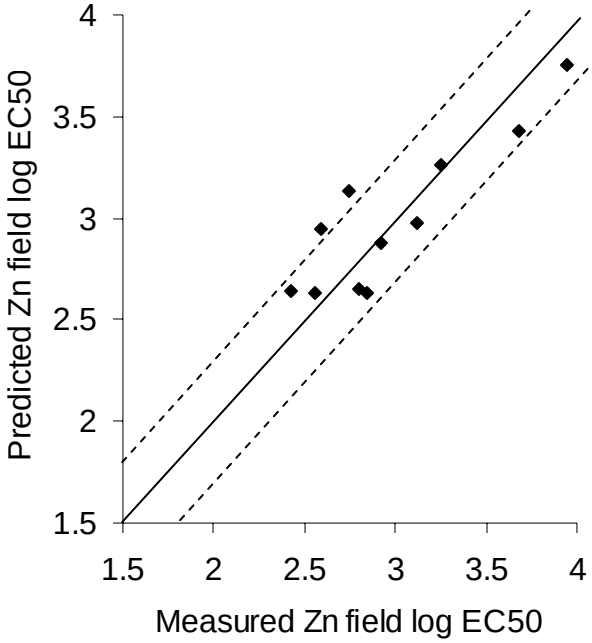

Predicted Zn field log EC50
Measured Zn field log EC50
4
3.5
3
2.5
2
1.5
1.5
2
2.5
3
3.5
4

Figure 5: Measured Cu toxicity values (8 week yield) for wheat grain yield versus predicted values using selected laboratory-based relationships based on 20 day plant growth for log EC50 (diamonds) and (b) log EC10 (squares). The solid line is a 1:1 slope and the dashed lines indicate a two fold error from the solid line.

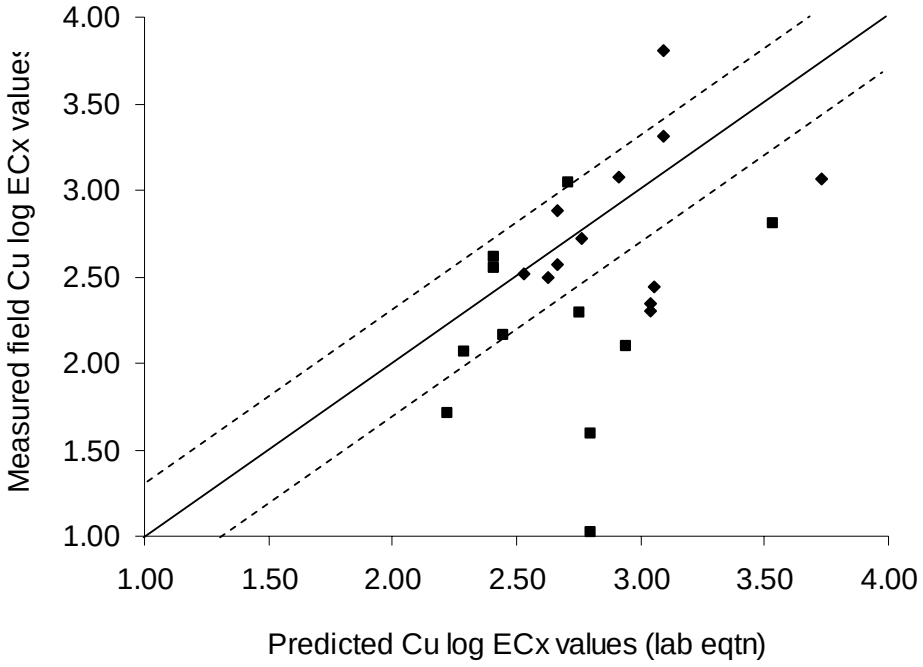


Figure 6: Measured field-based Zn toxicity values (8 week yield) for wheat grain yield versus predicted values using selected laboratory-based relationships based on 20 day plant growth for log EC50 (diamonds) and (b) log EC10 (squares). The solid line is a 1:1 slope and the dashed lines indicate a two fold error from the solid line.