

## Technical Note: Rationale and methods for deriving the microbial toxicity based soil and biosolids quality guidelines

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### Introduction

A key aim of the Australian National Biosolids Research Program (NBRP) was to develop soil quality guidelines (SQGs) and quality guidelines (QGs) for amended soils (i.e. soils that have received amendments such as fertilisers, composts and biosolids). The recommended framework for doing this is presented in an overview by Heemsbergen et al. (*in prep a*) with examples provided based on phytotoxicity. A summary of the framework is provided in Figure 1. In deriving these guidelines it was decided that only Australian ecotoxicology data would be used. This decision was based on three factors. First Australian soils are quite different to those of Europe and North America being very old, nutrient deficient, low in organic carbon and having different clay mineralogies (Taylor, 1983). As soil properties can affect toxicity, this infers that the toxicity of chemicals could be different in Australian soils. Second, a comprehensive comparison of the relative sensitivity of Australasian and non-Australasian aquatic species to metals and metalloids (Hobbs et al., 2004, Hobbs, 2007) found there were significant ( $p \leq 0.05$ ) differences for approximately 35% and 47% of comparisons for freshwater and marine/estuarine ecosystems respectively. They also found that non-Australasian data would need to be divided by 7.1 and 2.2 in order to protect 95% of Australasian organisms in freshwater and marine/estuarine ecosystems respectively from 95% of the chemicals studied. Third it was found during the development of the normalisation relationships for plants (Warne et al., *in press*; Warne et al., *submitted*) that the Australian relationships did not apply to European phytotoxicity data and vice versa.

The framework for deriving soil and biosolids quality guidelines that arose from the NBRP (Heemsbergen et al., *in prep a*) recommends that separate limits are derived to

protect plant health (for copper (Cu) and zinc (Zn)), microbial health (for Cu and Zn), crop quality (for cadmium, Cd) and environmental health (for nutrients). Thus four separate sets of soil Trigger Values (soil TVs) and TVs for amended soil will be derived and the most restrictive (i.e. that permit the lowest amount of a chemical or soil amendment to be added) will be recommended as the TV. This step will offer a high degree of environmental and human health protection and is consistent with existing Australian national and state methods for determining biosolids application rates (NSW EPA, 1997; SA EPA, 1997; DPIWE, 1999; WA DEP, 2002; EPA Victoria, 2004; NRMMC 2004).

The recommended framework for deriving the TVs is presented in overview by Heemsbergen et al. (*in prep a*) with examples provided based on phytotoxicity. Details of how the TVs for plant quality were derived are provided in McLaughlin et al. (2006). As the data available for nutrients is quite different to that for metals a separate method will be used to derive TVs for nutrients (Bell et al., *in prep*). The aims of this report were to: provide the rationale behind the proposed method of deriving soil quality guidelines based on microbial toxicity; explain the proposed method and present the limit values.

### **Terminology and the Proposed Framework**

Within Australia soil quality guidelines (SQGs) and quality guidelines for amended soil are proposed to be generic terms used for the overall process of protecting soils. The numerical limits for toxicants are termed trigger values (TVs) because if they are exceeded further action is triggered. The terms used throughout the present study will be consistent with those used in Heemsbergen et al. (*in prep a*).

The proposed framework is suitable to derive TVs for both soil and amended soil (Figure 1). Steps 1 – 6 and 9 and 10 are common to the derivation of both sets of TVs but steps 7 & 8 are only required when deriving TVs for amended soil. In brief the steps are to (1) collate and assess the quality and appropriateness of toxicity data, (2) determine if temporal changes in toxicity and bioavailability occur, (3 - 4) derive and implement toxicity normalisation relationships, (5) use a species sensitivity distribution method to derive a concentration (termed added contaminant limit –ACL) that provides the desired level of protection to a standard soil, (6) derive a set of soil ACLs, (7)

derive a soil amendment (bio)availability factor (SAAF), (8) apply the SAAFs to derive a set of ACLs for amended soil, (9 and 10) determine and add the ambient background concentration (ABC) of the contaminant to the set of ACL values to calculate soil TVs or TVs for amended soil. Each of these steps is discussed in detail in Heemsbergen et al. (*in prep a*).

## Methods

The toxicity of Cu and Zn to microorganisms was measured using substrate induced respiration (SIR) and substrate induced nitrification (SIN) bioassays conducted in the laboratory using soils collected from the NBRP field-sites. Details of the methods and sites sampled are provided in Broos et al. (2007). The soil concentrations of Cu and Zn that caused a 10, 20 and 50% reduction SIN and SIR (EC10, EC20 and EC50) and their standard errors were calculated by fitting a logistic distribution to the added total metal concentrations data using the method of Barnes et al. (2003). Added total metal concentrations were determined as the measured total concentration of each soil sample minus the average total metal concentration of all the controls for that site. The toxicity of Cu and Zn to SIN and SIR was measured at each field-site within two weeks of the application of the metal salts and annually for two years. However for the reasons outlined in Heemsbergen et al. (*in prep b*) only the data from the first sampling event will be used in this paper. Normalisation relationships for the Cu and Zn toxicity data to SIN and SIR were developed using forward and reverse stepwise multiple linear regression (Broos et al., 2007).

Given, the decision to use only Australian terrestrial toxicity data we had toxicity data for only two microbial endpoints – SIN and SIR. It was therefore not possible to use species sensitivity distribution (SSD) methods in the usual manner to derive trigger values. Therefore it was decided to treat the two sets of toxicity data separately and derive separate limits for SIN and SIR. The resulting limits would then be compared to those derived for phytotoxicity, plant quality and nutrients to determine the most restrictive TVs.

The normalisation relationships developed by Broos et al. (2007) are presented in Table 1. In Broos et al. (2007) only those normalisation relationships for EC50 data were presented but in Table 1 relationships for EC10, EC20 and EC50 are presented. The

normalisation relationships for SIN data can explain between approximately 60 and 90% of the variation in SIN data. Given these are field-based toxicity tests this is remarkable. The normalisation relationships for SIN were therefore used to derive soil-specific ACLs.

The situation regarding normalisation relationships was not as good for SIR. Significant ( $p \leq 0.05$ ) normalisation relationships could only be developed for EC50 SIR data (Table 1). These could explain 77% of the variation in toxicity data for Zn but less than 50% of the variation for Cu. There were no statistically significant normalisation relationships ( $p > 0.05$ ) for the SIR EC10 and EC20 data of Cu and Zn.

There are a number of ways that this situation can be addressed.

1. apply the SIR EC50 normalisation relationships to the SIR EC20 or EC10 data;
2. apply the SIN normalisation relationships to the SIR EC20 or EC10 data;
3. compare the sensitivity of SIN and SIR endpoints to determine if limits based on SIN will be protective for SIR;
4. use an assessment factor (AF) method; and
5. enter the data into a species sensitivity distribution method.

*Option 1: Apply the SIR EC50 normalisation relationships to the SIR EC20 data*

It was decided not to apply this option because the SIN normalisation relationships for EC10, EC20 and EC50 toxicity data to both metals had markedly different gradients (or coefficients for the parameters) and y-intercepts. Therefore it is likely that the same would apply to the EC10, EC20 and EC50 relationships for SIR. Furthermore, if there are no relationships between SIR EC20 and soil properties it is not appropriate to ignore this and apply normalisation relationships to the data.

*Option 2: Apply the SIN normalisation relationships to the SIR EC20 data*

This was not adopted as the normalisation relationships for Zn SIN and Zn SIR are based on different soil physicochemical properties. Likewise the normalisation relationships for Cu SIN and Cu SIR are based on different soil properties. Therefore, it would be inappropriate to do this as it would lead to normalising SIR toxicity data according to parameters that do not affect it. This could lead to under- or over-estimation of the normalised toxicity. Furthermore, if there are no relationships between

SIR EC20 and soil properties it is not appropriate to ignore this and apply relationships to the data.

*Option 3: Compare the sensitivity of SIN and SIR endpoints to determine if limits based on SIN will be protective for SIR*

This option was not adopted because the concentration response relationships for SIN and SIR are quite different with the SIR concentration response relationships generally having a much shallower slope than the SIN relationships and thus they have lower EC10 and EC20 values. For example Zn SIR EC10 values are half to one fortieth the size of the SIN values at the same sites. Therefore deriving soil quality guidelines using the SIN normalisation relationships and SIN toxicity data could lead to serious underestimation of the concentrations that would prevent inhibition of SIR.

*Option 4: Use an assessment factor method*

In this method the single lowest toxicity value for a species or microbial function is divided by an assessment factor (AF). Typically the AFs are 10, 100 or 1000 with the magnitude of the AF being inversely related to the perceived environmental relevance of the toxicity data. The proposed framework of Heemsbergen et al. (*in prep a*) and other frameworks for deriving environmental QGs used by Australia (e.g. ANZECC and ARMCANZ, 2000); Denmark (Petersen and Pedersen, 1995), the OECD (1992), South African (Roux et al., 1996), the Netherlands (Van de Plassche et al., 1993) and the USA (USEPA, 1986) all prefer to use SSD methods to AF methods. The reasons for this are the fact that the AF method is not consistent with the risk paradigm and criticisms over the scientific validity of the method. For these reasons and to be consistent with current international practice this option will only be used if a SSD method can not be used.

*Option 5: Enter the data into a species sensitivity distribution method*

As stated in Heemsbergen et al. (*in prep a*) the proposed framework for deriving soil and biosolids guidelines prefers to normalise toxicity data to account for the known effects of soil properties. However, if normalisation relationships are not available then non-normalised data can be used in a SSD method. This will result in a single numerical limit, rather than a suite of soil specific limits (Heemsbergen et al., *in prep a*).

It was decided that option 5 was the most scientifically defensible and it was therefore adopted to derive limits for the SIR toxicity data.

Several of the state biosolids guidelines have a soil pH threshold below which they do not permit biosolids to be applied. These range between 4.5 and 5.5 (SA EPA, 1997; DPIWE, 1999; WA DEP, 2002; EPA Victoria, 2004) with 5 being the median threshold for soil pH measured in 0.01M CaCl<sub>2</sub>. A number of the NBRP sites have soil pH values below 5 (e.g. Broos et al., 2007). The inclusion of SIR data for such sites, where biosolids would not normally be permitted, in the SSD calculation could skew the distribution fitted to the data and affect the limit values determined. Therefore the SIR data for (1) all the sites and then (2) the SIR data for sites with a soil pH greater than or equal to 5 were entered into a SSD method. The method used was BurrliOZ (i.e. the SSD method developed and used to derive the Australian and New Zealand water quality guidelines – Shao, 2000; Campbell et al., 2000) and the 80<sup>th</sup> percentile of the sites calculated. The resulting values (soil ACLs) were converted to biosolids ACLs for both metals by multiplying by the biosolids availability factor (BAF). The BAF is the ratio of the bioavailability of each metal as a metal salt to each metal in biosolids. Heemsbergen et al. (*in prep c*) found that the relative bioavailability of Zn in metal salts and in biosolids was the same (i.e. BAF = 1). They also found that the bioavailability of Cu was lower than that of Cu salts with the difference being a function of soil pH – the higher the pH the larger the BAF. Details of the BAF values and how they were calculated are provided in Heemsbergen et al. (*in prep c*).

Due to the lower robustness as defined by Broos et al. (2005) of the SIR compared to the SIN in the control soils and the typically much steeper gradient of the concentration response curves for SIN (Broos et al., 2007), it was decided that EC20 data would be used to derive ACLs for SIR and EC10 data would be used to derive ACLs for SIN. The steeper gradient associated with the concentration response curves for SIN is most likely caused by the very limited number of species involved in the nitrification, whereas the SIR, a measure of soil respiration, is typically a measure of a broad range of organisms. Thus, there is a considerably smaller margin of safety between a concentration that caused a minor effect and a concentration that causes a major effect in the SIN versus the SIR.

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## Results and Discussion

The Cu EC10 normalisation relationship for SIN was used to estimate soil ACL values that ranged from 0 to 1500 mg/kg for soils with pH values ranging from 5 to 8 combined with CEC values ranging from 3 to 60 cmol<sub>e</sub>/kg (Table 2). The Zn EC10 normalisation relationship for SIN was used to derive soil ACL values that ranged from 160 to 13 300 mg/kg for soils with pH values ranging from 5 to 8.5 (Table 3).

For SIR, a single numerical value (soil ACL) was derived for Cu and Zn for each set of the SIR data (i.e. all sites and sites with a soil pH greater than or equal to 5). The Cu soil ACL values for both datasets were 115 mg/kg. The soil ACLs for Zn were similar being 320 and 295 mg/kg for all sites and sites with a soil pH greater than or equal to 5 respectively. Only the plots based on all the sites are presented (Figures 2 and 3 respectively) as the soil ACLs are so similar for both datasets. The soil ACL for Cu of 115 mg/kg was recommended as both methods yielded the same results. The two soil ACL values for Zn were from a pragmatic point of view essentially the same, but in order to be more conservative value of 295 mg/kg was rounded up to 300 mg/kg and is recommended.

Combining the soil ACL value for SIR with the soil ACL values derived using the SIN normalization relationship provides a single set of soil ACL values to protect microbial functionality for each metal (Tables 4 and 5). For Cu the SIN limited the soil ACL values at low pH and low CEC values while at high pH and/or CEC values SIR limited the ACL (Table 4). The SIR imposed a single maximum ACL of 115 mg/kg added Cu. For Zn, at soil pH values below 5.25 SIN limited the ACL values, while at higher soil pH values the SIR imposed a single maximum ACL of 300 mg/kg added Zn.

The Cu biosolids ACL values to protect microbial functionality are presented in Table 6. For soils with low pH and CEC values the SIN limited the ACL while at higher pH and CEC values SIR limited the ACL, however it did not impose a single limit as the BAF varies with soil pH. Thus, SIR imposes a different single limit for each unique soil pH (Table 6).

The ambient background concentration must then be determined and added to the soil and biosolids ACLs in order to produce the soil and biosolids TVs. The soil or biosolids

TVs, derived above, for microbial functionality then need to be compared to the corresponding values for phytotoxicity, plant quality and nutrients and the lowest selected.

### **Conclusions**

Derivation of site specific soil and biosolids trigger values to protect microbial functionality was possible. For Zn, substrate induced nitrification limited the added contaminant limits (ACLs) at low pH (i.e. < 5.25) while at higher pH substrate induced respiration limited the ACL and thereby the TVs. Similarly, for Cu the SIN limited the magnitude of the ACL values in low pH and CEC soils while at high pH and CEC values substrate induced respiration limited the ACL and thereby the TVs. The Cu soil ACL values for microbial functionality (i.e. SIN and SIR combined) ranged from 0 to 115 mg/kg added Cu. The Cu biosolids ACL values for microbial functionality ranged from 0 to 245 mg/kg added Cu. The Zn soil ACL and biosolids ACL values for microbial functionality were equal and ranged from 160 to 300 mg/kg added Zn.

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Table 1: The highest quality ( $r^2$ ) statistically significant simple and multiple linear relationships between selected soil properties and toxicity values (i.e. EC50, EC20 and EC10) for copper (Cu) and zinc (Zn) to substrate induced respiration (SIR) and substrate induced nitrification (SIN). Toxicity values are expressed as mg/kg of total added metal.

Regression equations <sup>a</sup>	n	r <sup>2</sup>	Pr > F
<b>Zn-spiked soils</b>			
<u>SIN</u>			
log EC50 = 0.93 + 0.34 pH	12	0.59	0.002
log EC20 = -0.01 + 0.47 pH	12	0.71	<0.001
log EC10 = -0.55 + 0.55 pH	12	0.74	<0.001
<u>SIR</u>			
EC50 = 586 + 49 Zn ABC <sup>b</sup>		0.77	0.006
EC20: no model	11		
EC10: no model	11		
<b>Cu-spiked soils</b>			
<u>SIN</u>			
EC50 = -1137 + 13 clay + 286 pH	10	0.87	<0.001
EC20 = -1544 + 581 log CEC + 270 pH	11	0.86	<0.001
EC10 = -1458 + 663 log CEC + 223 pH	11	0.83	<0.001
<u>SIR</u>			
EC50 = 641 + 22 clay	9	0.46	0.027
EC20: no model	11		
EC10: no model	11		

<sup>a</sup>ABC = Ambient background concentrations

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Table 2. The soil added contaminant limit (soil ACL, mg/kg) values for the toxicity of copper to substrate induced nitrification at different combinations of soil pH and cation exchange capacity (CEC, cmol/kg).

pH\CEC	3	10	20	60
5	0	320	520	835
5.5	85	430	630	950
6	195	545	745	1060
6.5	310	655	855	1170
7	420	765	965	1280
7.5	530	880	1075	1395
8	640	990	1190	1505

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Table 3. The soil added contaminant limit (soil ACL, mg/kg) values for the toxicity of zinc to substrate induced nitrification at different soil pH.

Soil pH	Soil ACL (mg/kg)
5	160
6	560
7	1995
8	7100
8.5	13 300

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Table 4. The soil added contaminant limit (soil ACL, mg/kg) values for the toxicity of copper to a combination of substrate induced nitrification and substrate induced respiration (SIR) at different combinations of soil pH and cation exchange capacity (CEC, cmol/kg). The value of 115 mg/kg is the concentration that should protect 80% of sites from experiencing a 20% inhibition of SIR.

pH\CEC	1	3	≥ 4.9
5.0	0	0	115
5.5	0	85	115
6.0	0	115	115
6.5	0	115	115
7.0	103	115	115
>7.1	115	115	115

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Table 5. The soil added contaminant limit (soil ACL, mg/kg) values for the toxicity of zinc to a combination of substrate induced nitrification and substrate induced respiration at different soil pH. As the biosolids availability factor for zinc is one the biosolids ACL values are the same as the soil ACL values. The value of 300 mg/kg is the concentration that should protect 80% of sites from experiencing a 20% inhibition of SIR.

Soil pH	Soil ACL (mg/kg)
5.0	160
$\geq 5.25$	300

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Table 6. The biosolids added contaminant limit (biosolids ACL, mg/kg) values for the toxicity of copper to a combination of substrate induced nitrification and substrate induced respiration at different combinations of soil pH and cation exchange capacity (CEC, cmol/kg). These values are the result of multiplying the soil ACL values (Table 4) by the biosolids availability factor.

pH\CEC	1	3	>4.9
5.0	0	0	165
5.5	0	130	175
6.0	0	190	190
6.5	0	200	200
7.0	195	215	215
7.5	230	230	230
8.0	245	245	245

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Figure 1. The framework for deriving soil trigger values (TVs) and TVs for amended soil proposed by Heemsbergen et al (*in prep a*).

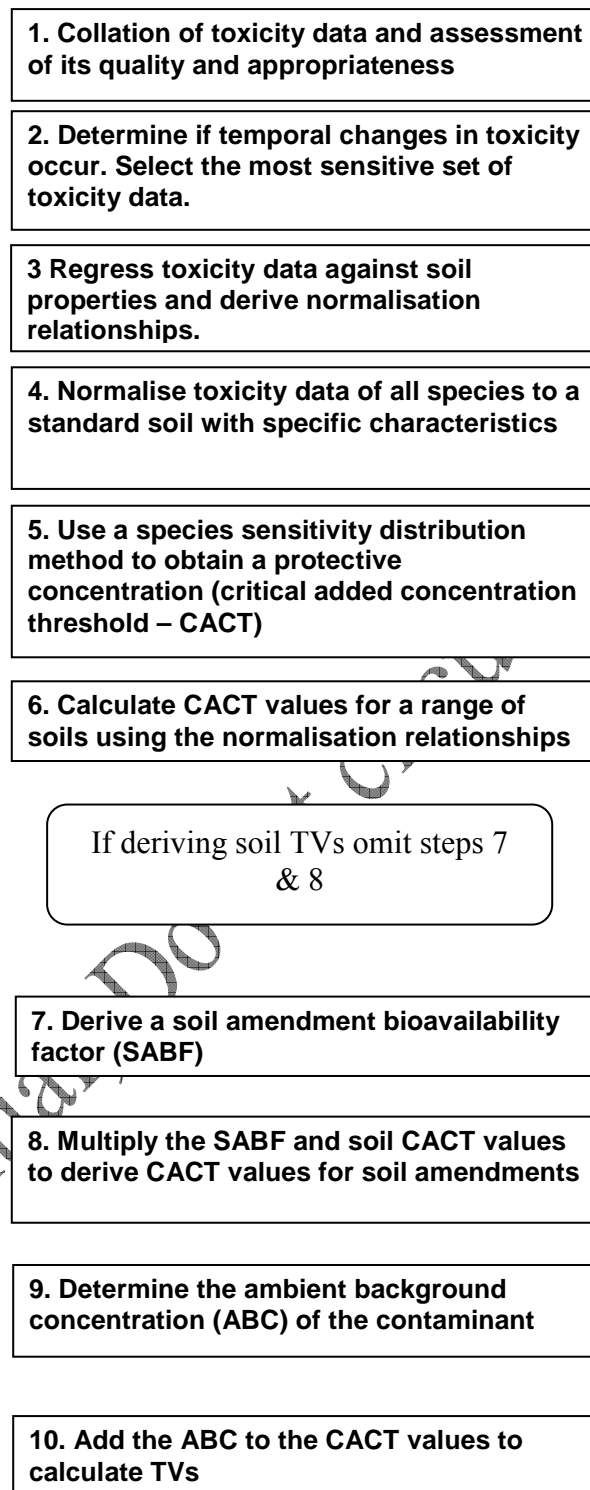
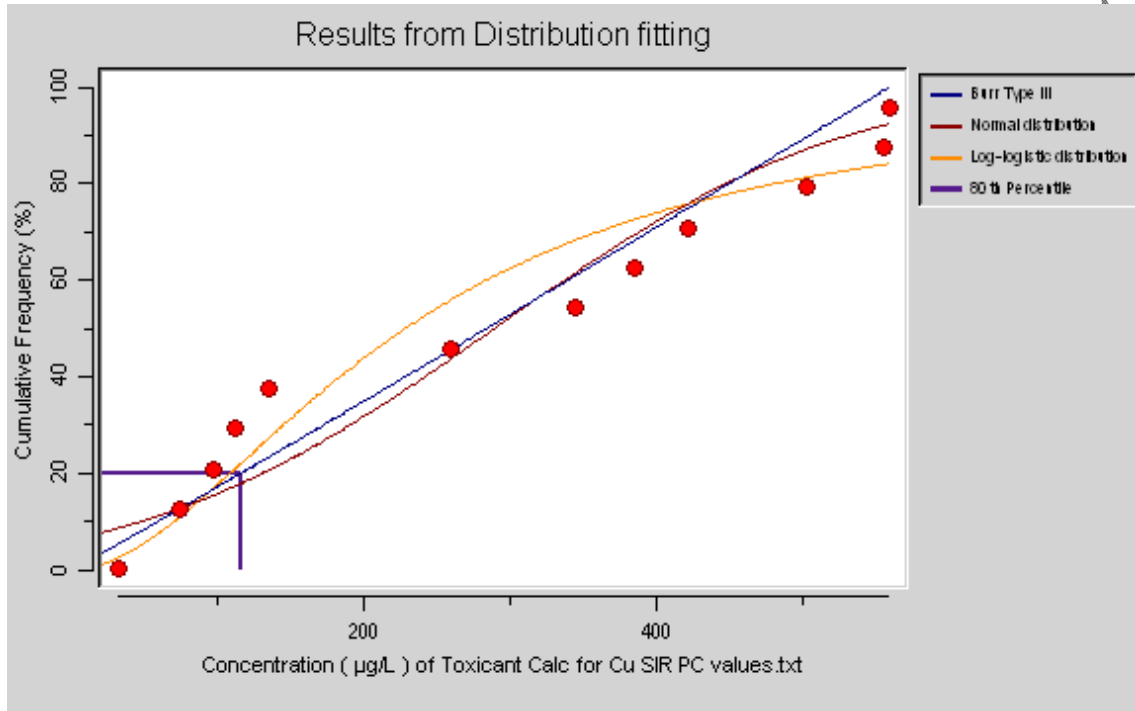
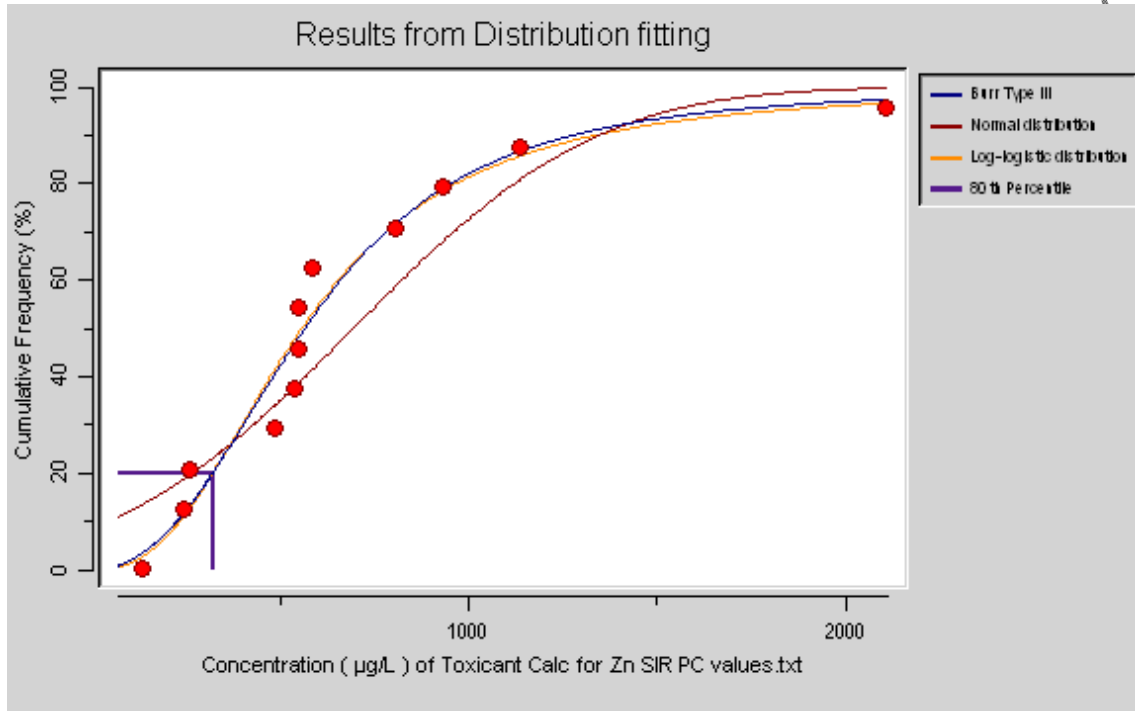


Figure 2. The distribution of the concentrations of added copper (mg/kg) that inhibited microbial substrate induced respiration (SIR) by 20% (EC20) for all sites from the National Biosolids Research Program. Three different distributions were fitted to the data, with the Burr type III distribution fitting the best to the data. The purple line indicates the maximum concentration of added copper that should protect 80% of sites from experiencing a 20% reduction in SIR.



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Figure 3. The distribution of the concentrations of added zinc (mg/kg) that inhibited microbial substrate induced respiration (SIR) by 10% (EC10) for all sites from the National Biosolids Research Program. Three different distributions were fitted to the data, with the Burr type III distribution fitting the best to the data. The purple line indicates the maximum concentration of added Zn that should protect 80% of sites from experiencing a 20% reduction in SIR.



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