

The Availability of Copper in Soils Historically Amended with Sewage Sludge, Manure, and Compost

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Metals in soils amended with sewage sludge are typically less available compared with those in soils spiked with soluble metal salts. However, it is unclear if this difference remains in the long term. A survey of copper (Cu) availability was made in soils amended with sewage sludge, manure, and compost, collectively named organic amendments. Paired sets of amended and control soils were collected from 22 field trials where the organic amendments had aged up to 112 yr. Amended soils had higher total Cu concentrations (range, 2–220 mg Cu kg⁻¹; median, 15 mg Cu kg⁻¹) and organic C (range, 1–16 g kg⁻¹; median, 4 g kg⁻¹) than control soils. All samples were freshly spiked with CuCl₂, and the toxicity of added Cu to barley was compared between amended and control soils. The toxicity of added Cu was significantly lower in amended soils than in control soil in 15 sets by, on average, a factor of 1.4, suggesting that aged amendments do not largely increase Cu binding sites. The fraction of added Cu that is isotopic exchangeable Cu (labile Cu) was compared between control soils freshly spiked with CuCl₂ and amended soils with both soils at identical total Cu concentrations. Copper derived from amendments was significantly less labile (on average 5.9-fold) than freshly added Cu in 18 soils. This study shows that Cu availability after long-term applications of organic amendments is lower than that of freshly added Cu²⁺ salts, mainly because of its lower availability in the original matrix and ageing reactions than because of increased metal binding sites in soil.

THE INPUT OF COPPER (Cu) to agricultural land mainly occurs via the application of manure, sewage sludge, and compost, collectively referred to here as “organic amendments.” Soil biota, such as plants and invertebrates, are most critically affected by elevated soil Cu concentrations (Basta et al., 2005). Soil limits for Cu have recently been proposed after testing of Cu toxicity to different soil biota in different soils and at different equilibration times after soil contamination (Smolders et al., 2009). It was shown that Cu toxicity is related to the number of Cu binding sites in soil and, to account for ageing effects, to the reversibly sorbed Cu in soil (“labile Cu”). The derivation of Cu limits in soil was largely based on soils amended with soluble Cu²⁺ salts. In soils where Cu is added via organic amendments, the situation is different, and two mechanisms can be distinguished by which Cu effects in soil differ from those of Cu²⁺ salts.

First, the organic matter in the amendments has Cu binding properties, and the same is true for Fe/Al oxyhydroxides in sewage sludge (also termed biosolids). As a result, soils amended with organic amendments may have larger Cu binding capacity than unamended soils. Second, the “labile Cu,” or, more generally, Cu speciation in these organic amendments added to soil, is not equal to that in soils to which Cu²⁺ salts were added (Heemsbergen et al., 2010; Oliver et al., 2004). For example, Cu extractability reduces during the sewage sludge processing phase (Alvarez et al., 2002), and recent evidence showed that a fraction of Cu in fresh biosolids and in pig slurry is present as Cu(I) (Donner et al., 2011; Legros et al., 2010). Both mechanisms (i.e., increased binding sites and lower labile fraction) may partly offset the risk of increasing total Cu concentrations compared with Cu²⁺ salts because of differences in bioavailability. Similarly, an assessment of both mechanisms may allow correcting soil limits calibrated to Cu²⁺ salts to those

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Abbreviations: APF, amendment protection factor; eCEC, effective cation exchange capacity.

applicable to organic amendments. The protection mechanisms in sewage sludge-amended soils have been formulated as the sludge protection hypothesis for Cu and other metals (Basta et al., 2005). This hypothesis has been controversial because long-term mineralization of organic matter from sewage sludge can increase the metal:organic C ratio and increase risk (McBride, 1995). Experimental evidence, however, does not suggest a large release of Cu on mineralization of sludge-derived organic matter (Oliver et al., 2005). It is unclear to what extent this applies to Cu added with manure and compost where the matrix is predominantly organic. In general, the issue in risk assessment of Cu added via organic amendments is to identify if such protection mechanisms are sustained in the long term.

The identification of lower metal availability in soils historically amended with sewage sludge, manure, and compost is best addressed in field trials that include parallel reference treatments with metal salts added at equivalent total concentrations, equilibrated for the same ageing time, and maintained at the same pH as the treatments with organic amendments. Field trials with this design have shown protective effects of sewage sludge on metal bioavailability up to 15 yr after application for Cd and Zn in most, but not all, cases (Basta et al., 2005; Brown et al., 1998; Chaudri et al., 2007; Heemsbergen et al., 2010; McLaughlin et al., 2006). For Cu, experimental evidence about potential sludge protection is weak or nonexistent when Cu is added as manure or compost. This is partly related to diagnostic problems. Biological uptake of Cu (i.e., plant shoot Cu or earthworm Cu concentrations) is strongly regulated (Chaignon et al., 2003; Peijnenburg et al., 1999) and does not sensitively reveal soil Cu bioavailability at incipient toxicity. For example, Cu concentrations in corn grown in sludge-treated soils are unaffected by soil Cu loadings (Chang et al., 1992).

Alternatively, direct toxicity based approaches to test the effects of Cu on plant growth in soils with organic amendments are confounded by the mixed metal contamination and, more generally, by the beneficial effects of the organic amendments on soil fertility or on soil physical processes that counteract toxicity at moderate doses (Korcak and Fanning, 1985). This means that neither Cu uptake nor Cu toxicity can be used unequivocally in amended soils, and, therefore, soil chemical analysis is the main avenue left to determine potential Cu toxicity. Only one recent multisite study has compared Cu extractability between aged biosolids and Cu²⁺ salt-amended soils. However, solubility (soil solution Cu- or CaCl₂-extractable Cu) was greater in biosolids-amended soils than in metal salt-amended soils, likely due to enhanced dissolved organic matter in the former treatments and due to limited equilibration (3 yr) in the field (Heemsbergen et al., 2010). The isotopic dilution assay (*E* value) with radioactive ⁶⁴Cu or stable ⁶⁵Cu on amended soils offers an additional approach; it measures the isotopic exchangeable and potentially available Cu in soil. In fact, *E* values measure the same fraction of soil Cu that is accessible to plants (Oliver et al., 2006), and the *E* values can, therefore, be used as a proxy for the accessible soil Cu.

The objective of this study was to evaluate the long-term protective effect of organic amendments on Cu availability across a wide range of types of organic amendments, ageing

times, and locations (soil type and climate). The availability of Cu in these soils was measured with a biological assay (toxicity) and with soil chemical analysis. These assays specifically identify the two different mechanisms referred to above (i.e., additional metal sorption sites due to the addition of soil amendment and the change in Cu availability in the soils amended with organic amendments compared with those amended with soluble Cu²⁺ salts). The former mechanism was tested with a biological assessment (i.e., a comparison of Cu²⁺ toxicity to barley plants between amended and control soil in which both soils were spiked with soluble Cu²⁺ salts). Any residual component of the organic amendments in soil may bind Cu²⁺ and reduce toxicity compared with the control soil. Such a design has been used for identifying sludge protection for Cd (Mahler et al., 1987; Kukier et al., 2010). The latter mechanism is tested with a chemical assay and used the isotope dilution principles to identify the relative availability of the Cu that was added with the organic amendments compared with freshly added Cu²⁺ salt. This relative measure is affected by the availability of Cu in the original amendments and by the transformation reactions that take place during in-field ageing. Both assays yield toxicity or availability factors relative to freshly added Cu²⁺ salts and offer the potential for correcting soil limits that are developed using soluble Cu²⁺ salts.

Materials and Methods

Soils

Soils were sampled at 22 sites where field trials were established (Table 1). At each site, a control and one or two treatments with different doses of amendments were sampled, yielding 52 soil samples in total. Soil samples were pooled from replicate plots (the number varied depending on location, from zero replicate treatment plots in Chinese trials to four replicate plots in Swedish trials) in the top 20 cm of soil (0–10 cm for Australian and Thai soils). All samples were air-dried at 25°C (Australian and Thai soils were oven-dried at 40°C) and sieved to <2 mm before analyses. At sites 11 and 12, additional samples were collected from metal salt trials that had been set up at the same time as the sewage sludge trials. Samples were taken from the metal salt plots that had total Cu concentrations closest to those in the treatments that had received sewage sludge. This allowed for separation of ageing effects from the effects due to the different source of the Cu added (salt vs. organic amendments). These additional samples were only used for the chemical assays and not for the bioassay.

Soil pH was measured in 0.01 mol L⁻¹ CaCl₂ (1:5 soil:solution ratio). Total carbon (C) and nitrogen content was measured by ignition with a Variomax CN analyzer (Pro-Tech). The carbonate-C was determined from the pressure increase after the addition of HCl to the soil in closed containers, with FeSO₄ used as a reducing agent. Organic C was calculated as the difference between total and inorganic C content. Total Cu concentrations were determined by a boiling aqua regia extraction and analysis of the extracts with inductively coupled plasma-optical emission spectroscopy (PerkinElmer Optima 3300 DV). A European soil standard was included as reference material (BCR142: light sandy loam, Community Bureau of Reference) with

certified aqua regia soluble Cu of 70 mg Cu kg⁻¹; measured values were (mean ± SD) 73 ± 3 mg Cu kg⁻¹. The silver-thiourea method (Chhabra et al., 1975) was used to measure the effective cation exchange capacity (eCEC) and exchangeable cations at the pH of the soil. Silver and exchangeable cations were determined in the extract by inductively coupled plasma–optical emission spectroscopy. Soil water contents at saturation and at –10 kPa were determined by the sand-box method using 100 cm³ soil cores (P1.80–1, Eijkelkamp Agrisearch Equipment).

Plant Test and Derivation of Amendment Protection Factor

A bioassay was performed to test for differences in sensitivity to added Cu between amended and corresponding control soils. Before soil spiking, soil pH differences between amended and control soils were corrected by liming. The historical use of organic amendments had increased or decreased soil pH by less than 1 pH unit in most soils, except for one large increase of 3 pH units in the sewage ash–amended soil (site 14). The soil sample with the lowest pH of each site was limed with Ca(OH)₂ if the pH difference between treated and control soils was larger than 0.3 pH units. In total, 17 soils (of 14 sites) were limed. Finely ground Ca(OH)₂ was added as a suspension in

water corresponding to 50 mL kg⁻¹ soil. Soils were left to equilibrate for 2 wk, after which they were dried at 25°C and sieved to <2 mm. All soils were spiked with 10 rates of soluble Cu²⁺ (control + nine Cu doses: 25, 40, 63, 100, 158, 251, 400, 630, and 1000 mg Cu kg⁻¹) with the appropriate amount of CuCl₂ solution (50 mg mL⁻¹) diluted with water to a volume corresponding to 5 mL 100 g⁻¹ soil. Soil and the CuCl₂ solution were mixed and allowed to equilibrate overnight. After equilibration, the spiked soils were transferred to perforated pots and leached with artificial rain water consisting of 5 × 10⁻⁴ mol L⁻¹ CaCl₂, 5 × 10⁻⁴ mol L⁻¹ Ca(NO₃)₂, 5 × 10⁻⁴ mol L⁻¹ MgCl₂, 10⁻⁴ mol L⁻¹ Na₂SO₄, and 10⁻⁴ mol L⁻¹ KCl to remove artifacts in the toxicity experiment induced by spiking soils with soluble metal salts (Stevens et al., 2003). Soils were first saturated from beneath, and, after saturation, soils were drained while adding artificial rain water on top to allow a minimum of 2 pore volumes of leachate. The leached soils were then air-dried at 30°C and sieved (2 mm). Soil pH and total Cu concentration after liming, spiking, and leaching were measured as described above. The pH differences between amended and corresponding control soils were within 0.4 pH units (mean 0.1), except for two cases (sites 5 and 14) that were therefore not considered further (i.e., soils of only 20 sites were tested). Spiking the soils with increasing Cu doses decreased the pH by, on average, 0.6 units at the highest Cu dose. However, pH shifts greater

Table 1. Description of soils sampled in 2005 from field trials with different application rates of organic amendments.

Site	Location†	Amendment‡	Application rates§	Period	pH¶	%OC#	Total Cu# mg kg ⁻¹
1	Madrid (SP)	SS 1	50 and 100 Mg ha ⁻¹ yr ⁻¹	1983–1991	7.8	0.5–2.1	13–232
2	Madrid (SP)	SS 2	50 and 100 Mg ha ⁻¹ yr ⁻¹	1983–1991	7.8	0.5–1.8	13–109
3	Igelösa (SW)	SS	4 and 12 Mg ha ⁻¹ 4 yr ⁻¹	1981–2005	6.7	1.7–2.0	13–28
4	Petersborg (SW)	SS	12 Mg ha ⁻¹ 4 yr ⁻¹	1981–2005	6.6	0.9–1.0	11–20
5	Leuven (BE)	SS 2	25 and 50 Mg ha ⁻¹ yr ⁻¹	2002–2005	6.8	0.8–0.9	14–22
6	Leuven (BE)	SS 3	25 and 50 Mg ha ⁻¹ yr ⁻¹	2002–2005	6.8	0.8–1.1	14–20
7	Leuven (BE)	SS 4	25 and 50 Mg ha ⁻¹ yr ⁻¹	2002–2005	6.8	0.8–1.4	14–30
8	Boutersem (BE)	Co	45 Mg ha ⁻¹ yr ⁻¹	1997–2005	5.7	0.8–1.5	8–11
9	Askov (DK)	M	1 and 1.5 times “normal” doses††	1894–2005	5.6	1.1–1.6	6–12
10	Hohenheim (DE)	SS	15 and 30 Mg ha ⁻¹ yr ⁻¹	1972–1989	6.5	1.1–2.1	26–61
11	Kingaroy (AU)	SS	112 Mg ha ⁻¹	2002	5.2	1.8–2.1	69–84
12	Spalding (AU)	SS	132 Mg ha ⁻¹	2003	5.9	2.2–2.8	23–70
13	Tallimba (AU)	M	n.d.	n.d.	5.8	1.8–2.1	8–10
14	Glenfield (AU)	SA	60 Mg ha ⁻¹	1993	4.2	2.4–2.5	7–32
15	Glenfield (AU)	SS	600 Mg ha ⁻¹	1983–1987	5.3	3.4–5.0	26–139
16	Jilin (CN)	M	225 Mg ha ⁻¹	1990–2004	5.8	1.3–1.9	25–36
17	Yunnan (CN)	M	135–270 Mg ha ⁻¹	1999–2004	6.7	1.5–1.9	80–177
18	Hunan (CN)	M	430–660 Mg ha ⁻¹	1990–2004	5.1	0.8–1.5	29–65
19	Zhejiang (CN)	M	320 Mg ha ⁻¹	1990–2004	5.7	1.6–1.9	28–42
20	Xinyang (CN)	M	450–675 Mg ha ⁻¹	1990–2004	7.7	0.7–1.5	17–29
21	Chiang Mai (TH)	SS	55 Mg ha ⁻¹	2005	6.6	0.6–0.8	10–47
22	Phra Puttbatat (TH)	SS	55 Mg ha ⁻¹	2005	5.2	0.9–1.0	17–31

† AU, Australia; BE, Belgium; CN, China; DE, Germany; DK, Denmark; SP, Spain; SW, Sweden; TH, Thailand.

‡ Co, compost (urban waste); M, manure; SA, sewage ash; SS, sewage sludge. Numbers are added when different organic amendments were added at the same site.

§ Rates separated with a dash show range of rates over the different years; rates separated by “and” represent different treatments. n.d., not determined.

¶ Soil pH (CaCl₂, 0.01 mol L⁻¹) is that of the unamended soil.

Organic carbon content (%OC) and soil total Cu concentrations are given as a range representing the unamended (control) and the highest application rate plots, respectively.

†† Normal doses have been varied over years (Munkholm et al., 2002).

than 0.3 units were observed only in the three highest Cu doses (i.e., >400 mg Cu kg⁻¹ added). Before commencing the root elongation test, all soils were equilibrated for 1 wk at a water content corresponding to 80% of that at -10 kPa.

The barley root elongation test was based on ISO 11269-1 (ISO, 1993). Summer barley seeds (*Hordeum vulgare* cv. Mauritia) were pregerminated on moist tissue paper for 24 h at 20°C in the absence of light. Pregerminated barley seeds with a radicle shorter than 2 mm in length were planted in each of two replicate pots of each soil treatment. Due to a limited amount of available soil, parallel-sided pots with 10 cm soil depth as described in ISO 11269-1 (1993) were reduced to 2.75- or 4-cm-diameter pots, and the number of seeds was reduced to three or four per pot, respectively. All soils from the same site were tested in the same sized pot. To reduce evaporation, the soil and seeds were covered by a thin layer of polyethylene beads. The pots were placed in a growth cabinet (Weiss, 18' SP/+5 Ju-Pa) with a 16 h/8 h day/night cycle. Air temperatures in the cabinet were 20°C (day) and 16°C (night), with relative humidity fixed at 70% throughout. Light intensity at canopy height was 650 μmol photons m⁻² s⁻¹. Water loss from the pots was restored daily with deionized water. After 4 d, the intact roots were sampled by washing soil from roots, and the length of the longest root on each plant was recorded.

The dose-response curves for the root elongation assay were fitted by a log-logistic model allowing description of hormesis effects (Schabenberger et al., 1999) or by a log-logistic dose-response model (Doelman and Haanstra, 1989) if hormesis was insignificant. The models were fitted to the data with the Marquardt method (SAS 8.02, SAS Institute Inc.). The "dose" in this model was the added Cu concentration (background corrected measured concentration), with the dose in the control soil attributed to a very small value (0.1 mg kg⁻¹). The ED_x (i.e., effective dose where there was x% effect) values, expressed as added Cu concentrations, were predicted from these curves. The amendment protection factor (APF) was defined as

$$\text{APF} = \frac{\text{ED}_{x,\text{AM}}}{\text{ED}_{x,\text{C}}} \quad [1]$$

where ED_{x,AM} and ED_{x,C} are the added Cu toxicity threshold at 10 or 50% (= x) inhibition in amended (AM) and control (C) soils, respectively. The ED₁₀-based value is most relevant for risk assessment. However, ED₁₀ values are (statistically) less precise than ED₅₀ values; therefore, both values are used and compared here. An APF >1 indicates a protective effect of organic amendments on Cu toxicity. The APF was considered significantly different from 1 if the 95% confidence intervals of the ED_x values did not overlap between amended and control soils. Comparing toxicity of the added Cu (expressed as ED_x) between control and amended soils, however, assumes that the bioavailability and toxicity of the background Cu in these soils was the same or that differences were minor compared with the toxic Cu dose. This assumption holds for the amended soils with a moderate increase in Cu concentrations (<30 mg Cu kg⁻¹) but may be problematic for amended soils with a large increase in Cu concentration (e.g., the Madrid SS 1 and SS 2 series). Therefore, added Cu toxicity thresholds (ED_x) of the amended soils were calculated as the sum of the ED_x based on the added Cu salt and the difference in soil

labile Cu (*E* value, see next section) between the amended and control soil. Conceptually, this means that the organic amendments have already "added" some Cu and that the labile Cu (ΔE value) represents this dose, which is, by definition, equally available as the freshly added CuCl₂ salt. This correction of the threshold in the amended soil was very small: Most ED₅₀ values were >200 mg Cu kg⁻¹, and the largest ΔE value in the amended soil was 30 mg Cu kg⁻¹.

Isotopic Exchangeable Copper and the Derivation of the Relative Copper Availability Factor

A chemical assay was performed to test for the differences in Cu availability of "added Cu" (relative to the unspiked control) between the amended soil and the corresponding control soil that was spiked with soluble CuCl₂. This assay was the isotopic exchangeable Cu (*E* value, in mg Cu kg⁻¹). The *E* values were measured on all control samples, on the samples from plots with organic amendments at the highest application rate, and on all control samples with soluble Cu²⁺ salts added to match total Cu of the corresponding amended soils. In addition, a series of pH amendments was made on control soil and on Cu²⁺ salt-amended soils to produce a range of pH values that bracketed the pH of the corresponding treatment with organic amendments because soil pH can affect Cu *E* values. The *E* values were measured in triplicate (amended soils) or duplicate (control soils and salt-spiked soils at different pH values). The *E* value determination followed the methodology of Nolan et al. (2004), but an additional resin-purification step, as suggested by Lombi et al. (2003), was used to minimize colloidal interference, which was marked for the tropical soils. In summary, 20 mL of deionized water was added to 2 g of soil, and for soils requiring pH adjustment an appropriate amount of diluted HCl or Ca(OH)₂ was added. Two drops of toluene (to inhibit microbial activity) were added to all tubes containing soil. The soil suspensions were equilibrated for 48 h on an end-over-end shaker. For the Cu²⁺ salt-amended control samples, an aliquot of Cu(NO₃)₂ was added to raise total Cu concentrations to those of the corresponding amended samples, and the samples were equilibrated for a further 48 h. The samples were then spiked with radioactive ⁶⁴Cu or, in the case of the Thai samples, enriched ⁶⁵Cu and equilibrated for 24 h (1% of total Cu as suggested by Nolan et al. [2004]). The samples were then filtered (<0.2 μm), and an aliquot of filtrate was equilibrated in an end-over-end shaker with 50 to 100 mg of calcium-form Chelex resin for 24 h. The resin was allowed to settle, and the supernatant was decanted. Elution of the metal from the resin was then performed through the addition of 0.5 mol L⁻¹ HNO₃ with end-over-end shaking for 1 h. Copper isotopic ratios were determined by inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7500c, Agilent Technologies) in the samples spiked with ⁶⁵Cu. Solution Cu concentrations in the samples spiked with radioisotope were measured using graphite furnace-atomic absorption spectrometry (AAAnalyst 600, PerkinElmer), and ⁶⁴Cu activities were determined by γ counting (1480 Wizard, Wallac). The relative available fraction (*f*_{av}) of Cu was defined as:

$$f_{\text{av}} = \frac{E_{\text{AM}} - E_{\text{C}}}{E_{\text{FS}} - E_{\text{C}}} \quad [2]$$

with E representing the isotopic exchangeable Cu in amended soil (AM), control soil (C), or soil freshly spiked (FS) with soluble Cu^{2+} salt. The f_{av} was calculated with the E values of control and amended treatments at the same soil pH values. This entire procedure was applied to the soils of all sites except for site 6, where relative Cu enrichment was too little to obtain meaningful data. The SE of f_{av} was calculated from the SEM of each E value among replicates assuming independent measurements. One-sided t tests (at $p \leq 0.05$) were used to test if f_{av} was significantly different from 1.0, with a conservative (low) value of $n = 2$ to identify significance.

Results

Soils

The 22 sites covered a range of cumulative application rates of 16 to 900 Mg ha^{-1} and a range of maximal ages of amendments in soil of 1 to 112 yr and represented the following types of mixed organic amendments applied to land: manure (farmyard manure, pig slurry), sewage sludge (anaerobically digested, lime stabilized, raw sludge), sewage ash, and compost (Table 1). These amendments increased soil organic C content by 1 to 16 g kg^{-1} , with a median increase of 4 g kg^{-1} . The increases of Cu concentrations in amended soils were detectable in all soils and ranged from 2 to 220 mg Cu kg^{-1} . The median increases in trace metal enrichment were (in mg kg^{-1}) 15 (Cu), 30 (Zn), 1.5 (Ni), <5 (Pb), and <0.2 (Cd). The largest increases were found at sites 1 and 2 (Madrid) and at site 16 (Glenfield). These metal additions did not reach toxic values for the bioassay used here as suggested from literature data in freshly spiked soils (details not given), although borderline toxicity was suggested for the soils of site 2 (see below).

Plant Test and the Amendment Protection Factor

The root length in the soils that were not spiked with Cu salts ranged from 3.6 to 12.2 cm, with most values being about 10 cm. Poor root growth (<6 cm) was evident in the soils of sites 18 and 21, but this was likely unrelated to metal concentrations, which were similar in amended soils and control samples; these data were considered too unreliable for inclusion and were omitted from further analysis. Therefore, there were 18 paired sets of soils from which the amendment protection factor was derived. Generally, there were no large differences (<20%) in root length between amended and control soils. In the Yunnan and Phra Puttabat soils, the control response in the amended soils was about 30% smaller than in the control soils, whereas in the Madrid SS 2 the opposite was true.

The Cu EC_{10} values (= ED_{10} + background Cu) ranged from 70 to 600 mg Cu kg^{-1} , and EC_{50} values ranged from 180 to 1400 mg Cu kg^{-1} . The plant toxicity of added Cu^{2+} salt is partially related to the acidification induced by the metal salt application. However, the focus here is not on the absolute value of toxicity but on the ratio of toxicity values of amended to corresponding control soils, expressed as the amendment protection factor. This factor should be only weakly affected by the CuCl_2 -induced acidification because the pH decrease was similar in the soils with or without organic amendments. For example, soil pH decreased from 6.2 to 5.4 by the CuCl_2 application in the control soil of Petersborg, whereas the Cu^{2+}

salt decreased pH from 6.2 to 5.2 in the sludge-amended treatment. The differences in pH between the corresponding soils at the ED_{50} (i.e., the soils for which the APF was derived) range between +0.4 and -0.4 pH units (mean, 0.0). The pH of the soils with organic amendments can be higher or lower than that of control at EC_{50} . The average of the absolute values of the pH difference was 0.2 pH units. The differences in pH at EC_{50} were similar to differences in pH between corresponding control soils, indicating similar acidification by the addition of CuCl_2 in control and amended soils.

For most series, the ED_{50} was marginally, but significantly, larger in the amended soil compared with the control soils (e.g., Fig. 1), resulting in an amendment protection factor >1 (Table 2). All amendment protection factors were relatively small, and the average value was only about 1.4, meaning that aged organic amendments in soil do not protect against toxicity of added soluble Cu salt.

There was no significant relationship ($p > 0.05$) between the APFs and the factor increase in soil organic C content resulting from the application of organic amendments (Fig. 2). At seven sites, two rates of organic amendments were applied to soils. In three of these sites, the APFs significantly increased with the rate, whereas it significantly decreased at one site (site 1, Madrid SS1). In soils of that latter site, Cu toxicity was significantly greater (ED_{50} of added soluble Cu^{2+} salt was lower) in the 100 $\text{Mg ha}^{-1} \text{ yr}^{-1}$ dose compared with the 50 $\text{Mg ha}^{-1} \text{ yr}^{-1}$ dose (data not shown). The APF in both sets of soils from Madrid (SS1 and SS2; open symbols in Fig. 2) were relatively low despite their large increase in soil organic C due to sewage sludge applications. This may be explained by the toxicity of other metals because the SS1 sewage sludge from Madrid resulted in high metal loadings to soil, suggesting that the sewage sludge-derived metals (including Cu) contributed to the toxicity. That site contained the highest total metal concentrations of this collection: (in mg kg^{-1}) 230 (Cu), 315 (Zn), 100 (Pb), 12 (Cd), and 16 (Ni). The Cu doses due to applications of organic amendments were taken into account in the calculation of APF; however, the effects of other phytotoxic metals (Zn and Ni) were not considered. Therefore, APFs as

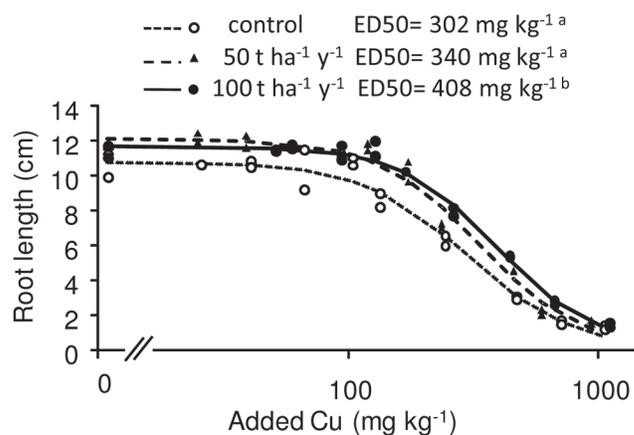


Fig. 1. Response of barley root growth to freshly added soluble Cu^{2+} salt in soils of site 2 at Madrid (Table 1) that was historically amended with sewage sludge at two rates (in $\text{Mg ha}^{-1} \text{ yr}^{-1}$). The Cu ED_{50} doses followed by the same lowercase letter are not significantly different at $p \leq 0.05$. The soil organic carbon (%OC) contents of the soils were 0.5 (control), 1.6 (lowest dose), and 1.8 (highest dose).

Table 2. The amendment protection factors (Eq. 1) and relative Cu availability factors (Eq. 2) in the soils of the different field trial sites.

	Min.	Max.	Mean	$n > 1^{*\dagger}$	$n < 1^*$	$n, n.s.^*$
Amendment protection factors‡						
At ED ₁₀	0.81	3.23	1.63	10	0	8
At ED ₅₀	0.49	2.11	1.37	15	1	2
Relative Cu availability factors§	<0.00	0.42	0.17	18	0	3

† The number of factors (n) that are significantly different from unity ($*p \leq 0.05$) or not (n.s.).

‡ The amendment protection factor (APF) is based on a bioassay and indicates the factor by which added soluble Cu²⁺ salt was less toxic in the amended soil (at the highest rate) compared with the corresponding control soil. An APF > 1 indicates a protective effect of the amendments.

§ The relative Cu availability factor (f_{av}) is based on isotopic exchangeable Cu and is the relative availability of Cu added with the amendment (at the highest rate) compared with freshly added soluble Cu²⁺ salt in the corresponding control soil. A $f_{av} < 1$ indicates that Cu from aged organic soil amendments was less available than soluble Cu²⁺ salt.

defined here might be considered as a lower estimate of the capacity of the binding sites from the amendments to protect against metals present in the amended soils.

Isotopic Exchangeable Copper and the Relative Copper Availability Factor

The Cu *E* values in amended soils were higher than those of their respective control soils at 18 of the 21 sites. The higher Cu *E* values are obviously related to higher total soil Cu concentrations in these amended soils. The relative Cu availability between treatments can be expressed as the Cu *E* values relative to total soil Cu (i.e., % *E* values). The % *E* values in amended soils were significantly lower than for soils freshly spiked with soluble Cu²⁺ salt in all but one case (Fig. 3). These % *E* values of amended soils were the same ($n = 17$) or lower ($n = 3$) than corresponding control soils, whereas the reverse was true in only one case. This means that Cu in organic amendments has a similar or lower availability than background Cu (mainly geogenic).

The *E* value of Cu in soils freshly spiked with soluble Cu²⁺ salt was always higher than that of the unspiked soils and higher than the corresponding amended soils at equal total soil Cu concentrations (Fig. 4). The *E* value measurements of these three treatments allow derivation of a relative Cu availability factor f_{av} (Eq. 2 and Fig. 4). In three cases (sites), there was no detectable increase of the Cu *E* value due to organic

amendments, which therefore yielded a negative value for the f_{av} factor. In addition, the increment in soil total Cu due to organic amendments was often relatively small, and the standard error of the f_{av} became as large as the factor itself in six cases, including the four cases of negative f_{av} values. All but three f_{av} values were significantly below 1 ($p \leq 0.05$), with an average value of only 0.17 (Table 2). This means that, on average, Cu availability in the amended soils was 17% relative to soluble Cu²⁺ salt that had equilibrated for 48 h in soil. The f_{av} values were not significantly affected by the type of amendments (i.e., manure versus sewage sludge versus compost). Similarly, the average age of the amendments in soil (Fig. 5), soil pH, soil total Cu concentration, and soil organic C had no significant effect on the f_{av} values (data not shown).

At two of the National Biosolid Research Program field sites in Australia (McLaughlin et al., 2007), the experimental design included field-aged soluble Cu²⁺ salt treatments in addition to the sewage sludge treatments. Because in these cases the equilibration time in the field was similar, it is possible to separate ageing processes from the other factors that may have caused differences in Cu availability between the field treatments and the laboratory-spiked samples used here to derive f_{av} . The results obtained for these two sites are reported in Fig. 6. The data presented include all the data obtained with the pH adjustments. The results for the Kingaroy site are quite variable, and no significant difference between the treatments

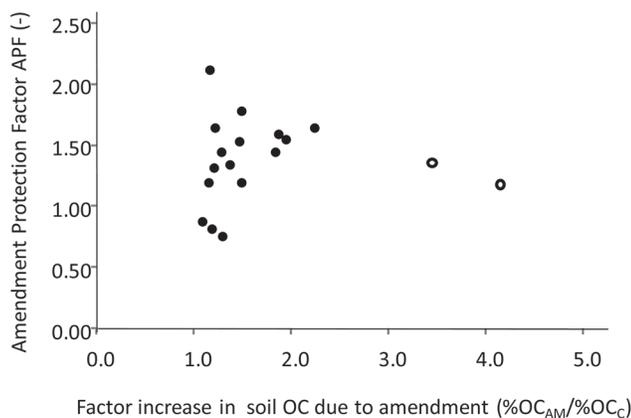


Fig. 2. The amendment-protection factors (APFs) based on Cu ED₅₀ for barley root elongation in relation to the change in soil organic carbon (OC) content in amended (AM) versus control (C) soils. The two open symbols are those of Madrid soils where soil metal contamination due to sludge application may have contributed to metal toxicity and, hence, to a relative low APF value.

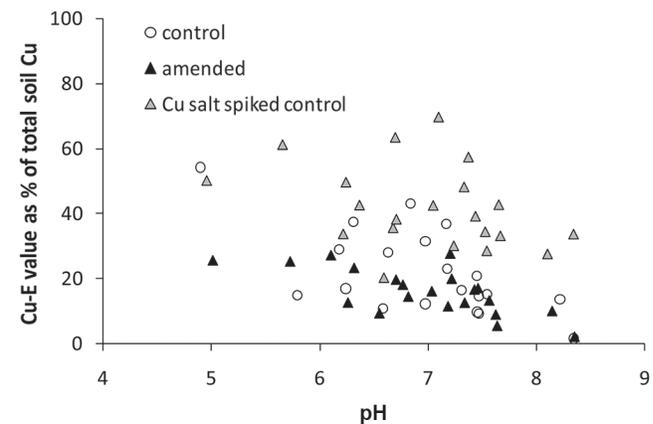


Fig. 3. The isotopic exchangeable Cu (*E* value) as percent of total Cu concentrations. Data at about the same pH reflect the three treatments for each of the 21 sites (i.e., control soil, Cu²⁺ salt-spiked soil, and amended soil). Copper in soils treated with organic amendments was less available (lower % *E*) than freshly spiked Cu in all but one case and had a lower availability than background Cu in three cases (see text).

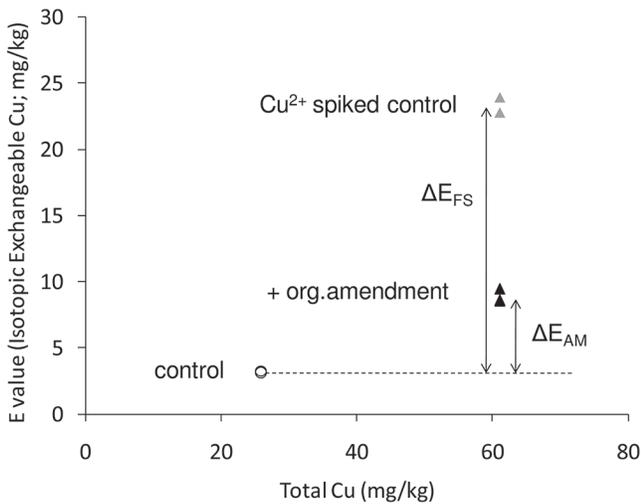


Fig. 4. The E value of Cu in soils of site 10, Hohenheim. The control soil was freshly amended with Cu^{2+} salt (FS) in the laboratory up to the same total Cu concentration as the sewage sludge-amended soil, and its E value was compared with that in the amended soil (AM; here $30 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ between 1972 and 1989). The relative Cu availability factor (f_{av} ; Eq. [2]) was calculated from the ratio of E -value increments and was $f_{av} = 0.28 \pm 0.04$ (mean \pm SEM; $n = 2$).

could be detected. The difference in total Cu concentrations between the control soil and the other treatments was only 15 mg kg^{-1} . This increase is too small to detect significant differences in labile Cu between spiked and corresponding amended soils. Also, this soil (an Oxisol) was the most analytically challenging due to the large amount of Fe-containing colloids. The results obtained for the Spalding site were more conclusive and showed a statistical difference ($P < 0.001$) between the laboratory and field salt-treated samples. The lability of Cu in the soil amended in the field with soluble Cu^{2+} salt was about half of that measured in soil that was freshly spiked in the laboratory, illustrating ageing effects on Cu availability. There was also a small but significant difference between the Cu lability in the metal salt treatment in the field and the amended soil. This indicates that, in addition to ageing, other mechanisms were responsible for the low lability of Cu in the amended soils at this site.

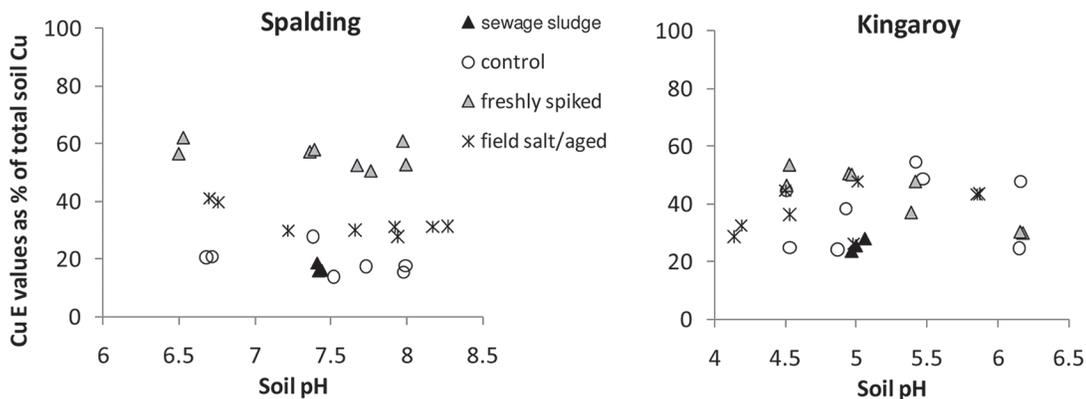


Fig. 6. Copper lability (E values as % of total Cu) for the Spalding and Kingaroy soils where metal salts spiked plots were installed next to sewage sludge treatments plots at equal doses. Field metal salts were aged in the field for the same time as the sewage sludge. Freshly (48 h) spiked soils refer to laboratory spiked soils.

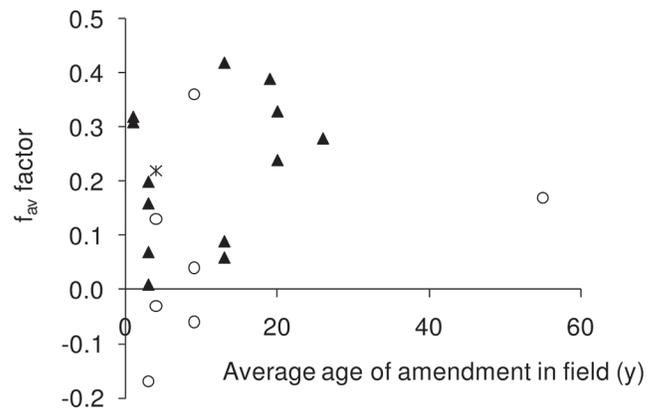


Fig. 5. Relationship between the relative Cu availability factor (f_{av}) in amended soils and the age of the amendments in soil. 6 = sewage sludge; C = manure; x = compost.

Discussion

The APFs, operationally defined here by a relative toxicity assessment, were relatively low and averaged only 1.4 at ED_{50} or slightly higher (1.6) at the environmentally more relevant ED_{10} concentration range. A previous study showed that soil eCEC explained the toxicity of Cu to barley root elongation (Rooney et al., 2006). The factor increase in eCEC in these soils through organic amendments was only about 1.1 (maximum 1.4), and the empirical $\text{ED}_{50}/\text{eCEC}$ equation developed by (Rooney et al., 2006) predicts that ED_{50} values should have increased due to increased eCEC by, on average, a factor of 1.07, which is even lower than the average value of 1.4 for the APFs observed here.

The APFs defined here only quantify how amended soils may bind added Cu^{2+} better and mitigate its toxicity. The relative Cu availability factor (f_{av}) assesses the second mechanism of protection of organic amendments (i.e., the relative availability of the Cu in organic amendments relative to freshly added soluble Cu^{2+} salt, equilibrated for 48 h). These factors were, on average, 0.17, meaning that Cu from aged organic amendments was about 5.9-fold ($1.0/0.17$) less available than the freshly added Cu^{2+} . This lower bioavailability of Cu from organic amendments was confirmed for almost all sites (Table 2). This availability is probably most influenced by the lability

of Cu in the original matrix and not by the long-term reactions in the field. A previously published survey of Australian biosolids showed that *E* values of Cu in biosolids ranged from 7 to 38% (mean, 23%) of total Cu concentrations (Oliver et al., 2004). The average value of 23% can be similarly interpreted as above (i.e., Cu derived from organic amendments is about 4.3-fold [$1/0.23$] less available than freshly added Cu^{2+} salt). In contrast, ageing experiments in a wide range of soils amended with soluble Cu^{2+} salts showed that ageing does not generally reduce Cu lability to this extent except in soils with pH at and above 7.5 (Ma et al., 2006a, 2006b). The %E of Cu^{2+} decreased in these experiments from 100% to 25 to 90% (average, 57%) after 2.3 yr (Buekers et al., 2007) or between 20 and 80% (average, 56%) at pH 7 after 1 yr (Ma et al., 2006b). This means that ageing reactions reduce soluble Cu^{2+} availability less than 2-fold on average, which is clearly below the averages of relative availability of Cu in biosolids in comparison to Cu^{2+} salts (4.3-fold) (Oliver et al., 2004) or of Cu derived from different organic amendments (5.9-fold; this study). The Cu^{2+} salt ageing data in soils at pH 7.5 yield similar %E values (Ma et al., 2006b) as the Cu derived from organic amendments in soils across a wide range of pH values. This suggests that the typical high pH in the sewage sludge or manure (pH >7.0) may induce a Cu fixation reaction that is not reverted when incorporated in more acid soils as in the collection here. The analysis of the Spalding site (Fig. 6) confirms the lower availability of sewage sludge Cu compared with soluble Cu^{2+} salts when both were aged for the same time and at the same site. A biosolid–soil laboratory incubation study that extended for 7 yr in different soils showed that *E* values increased by maximally 5% of total added Cu on ageing. In contrast, during the same period, 28 to 53% of the organic C was lost (i.e., the organic matter mineralization did not release a large fraction of occluded Cu) (Oliver et al., 2005).

Overall, it appears that the so-called sludge-protection mechanism of Cu availability is valid for all organic amendments (sewage sludge, manure, and compost) but that it is related to the speciation of Cu in the original amendment and not to the creation of additional metal binding capacity in the soil. Recently, a similar study was performed to detect the sludge protection mechanisms for Cd (Kukier et al., 2010). Paired sets of long-term sewage sludge–amended and control soils were spiked with Cd^{2+} salt enriched in ^{111}Cd . Cadmium uptake (lettuce) was compared between amended and unamended soils. In that study, the sludge protection mechanism was also confirmed, although the mechanisms were different compared with the situation for Cu: The uptake versus soil Cd slopes in soils freshly amended with Cd^{2+} salt were largest for control soils and decreased in amended soils. This decrease was related to additional metal binding in the amended soils. In contrast, sludge-derived Cd was generally isotopically labile (>60%; most values, 80–95%).

Our study may assist the risk assessment of Cu in organic amendments. Long-term application of organic amendments increases the potential of soils to immobilize added Cu; however, the factor change noted here (1.4 on average) is marginal, and it is questionable if such a factor is of any

relevance in risk assessment, also considering the variability among the different sites (Fig. 2; Table 2). In contrast, the metals added via organic amendments are much less available than soluble metal salts, and this makes a clear difference for a risk assessment scheme that is based on toxicity studies with aged or freshly added metal salts (Smolders et al., 2009). For example, toxicity tests in soils spiked with freshly added Cu may suggest a safe threshold expressed as freshly added Cu (in kg Cu ha^{-1} or mg Cu kg^{-1} soil). Such a value may then be multiplied with the factor 5.9 ($1/f_{av}$), denoting the differences in availability of added Cu between soils with aged organic amendments and that with freshly added soil. In addition, this outcome may be multiplied with the factor 1.4 to account for the additional alleviation of Cu toxicity due to additional properties. A risk assessment scheme may include safety factors; however, such a discussion is beyond the scope of this work. Significant release or mobilization of Cu in amended soils (i.e., following a time-bomb hypothesis) does not occur within the time frames of the field trials that have been sampled, and this confirms results from previous studies (Oliver et al., 2005).

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References

- Alvarez, E.A., M.C. Mochon, J.C.J. Sanchez, and M.T. Rodriguez. 2002. Heavy metal extractable forms in sludge from wastewater treatment plants. *Chemosphere* 47:765–775. doi:10.1016/S0045-6535(02)00021-8
- Basta, N.T., J.A. Ryan, and R.L. Chaney. 2005. Trace element chemistry in residual-treated soil: Key concepts and metal bioavailability. *J. Environ. Qual.* 34:49–63.
- Brown, S.L., R.L. Chaney, J.S. Angle, and J.A. Ryan. 1998. The phytoavailability of cadmium to lettuce in long-term biosolids-amended soils. *J. Environ. Qual.* 27:1071–1078. doi:10.2134/jeq1998.00472425002700050012x
- Buekers, J., L. Van Laer, F. Amery, S. Van Buggenhout, A. Maes, and E. Smolders. 2007. Role of soil constituents in fixation of soluble Zn, Cu, Ni, and Cd added to soils. *Eur. J. Soil Sci.* 58:1514–1524. doi:10.1111/j.1365-2389.2007.00958.x
- Chaignon, V., I. Sanchez-Neira, P. Herrmann, B. Jaillard, and P. Hinsinger. 2003. Copper bioavailability and extractability as related to chemical properties of contaminated soils from a vine-growing area. *Environ. Pollut.* 123:229–238. doi:10.1016/S0269-7491(02)00374-3
- Chang, A.C., T.C. Granato, and A.L. Page. 1992. A methodology for establishing phytotoxicity criteria for chromium, copper, nickel, and zinc in agricultural land application of municipal sewage sludges. *J. Environ. Qual.* 21:521–536. doi:10.2134/jeq1992.00472425002100040001x
- Chaudri, A., S. McGrath, P. Gibbs, B. Chambers, C. Carlton-Smith, A. Godley, J. Bacon, C. Campbell, and M. Aitken. 2007. Cadmium availability to wheat grain in soils treated with sewage sludge or metal salts. *Chemosphere* 66:1415–1423. doi:10.1016/j.chemosphere.2006.09.068
- Chhabra, R.J., J. Pleysier, and A.C. Cremers. 1975. The measurement of the cation exchange capacity and exchangeable cations in soils: A new method. In: *International Clay Conference*. Applied Publishing, Mexico City, Mexico. p. 439–449.
- Doelman, P., and L. Haanstra. 1989. Short- and long-term effects of heavy metals on phosphatase activity in soils: An ecological dose response model approach. *Biol. Fertil. Soils* 8:235–241. doi:10.1007/BF00266485
- Donner, E., D. Howard, M. de Jonge, D. Paterson, M.H. Cheah, R. Naidu, and E. Lombi. 2011. X-ray absorption and micro X-ray fluorescence spectroscopy investigation of copper and zinc speciation in biosolids. *Environ. Sci. Technol.* 45:7249–7257.

- Heemsbergen, D.A., M.J. McLaughlin, M. Whatmuff, M.S. Warne, K. Broos, M. Bell, D. Nash, G. Barry, D. Pritchard, and N. Penney. 2010. Bioavailability of zinc and copper in biosolids compared to their soluble salts. *Environ. Pollut.* 158:1907–1915. doi:10.1016/j.envpol.2009.10.037
- International Organisation for Standardisation (ISO). 1993. ISO 11269-1. Soil quality: Determination of the effects of pollutants on soil flora. Part 1: Method for the measurement of inhibition of root growth. International Organization for Standardization, Geneva, Switzerland.
- Korcak, R.F., and D.S. Fanning. 1985. Availability of applied heavy metals as a function of type of soil material and metal source. *Soil Sci.* 140:23–34. doi:10.1097/00010694-198507000-00004
- Kukier, U., R.L. Chaney, J.A. Ryan, W.L. Daniels, R.H. Dowdy, and T.C. Granato. 2010. Phytoavailability of cadmium in long-term biosolids-amended soils. *J. Environ. Qual.* 39:519–530. doi:10.2134/jeq2007.0671
- Legros, S., P. Chaurand, J. Rose, A. Masion, V. Briois, J.H. Ferrasse, H. Saint Macary, J.Y. Bottero, and E. Doelsch. 2010. Investigation of copper speciation in pig slurry by a multitechnique approach. *Environ. Sci. Technol.* 44:6926–6932. doi:10.1021/es101651w
- Lombi, E., R.E. Hamon, S.P. McGrath, and M.J. McLaughlin. 2003. Lability of Cd, Cu, and Zn in polluted soils treated with lime, beringite, and red mud and identification of a non-labile colloidal fraction of metals using isotopic techniques. *Environ. Sci. Technol.* 37:979–984. doi:10.1021/es026083w
- Ma, Y.B., E. Lombi, A.L. Nolan, and M.J. McLaughlin. 2006a. Short-term natural attenuation of copper in soils: Effects of time, temperature, and soil characteristics. *Environ. Toxicol. Chem.* 25:652–658. doi:10.1897/04-601R.1
- Ma, Y.B., E. Lombi, I.W. Oliver, A.L. Nolan, and M.J. McLaughlin. 2006b. Long-term aging of copper added to soils. *Environ. Sci. Technol.* 40:6310–6317. doi:10.1021/es060306r
- Mahler, R.J., J.A. Ryan, and T. Reed. 1987. Cadmium sulfate application to sludge-amended soils: I. Effect on yield and cadmium availability to plants. *Sci. Total Environ.* 67:117–131. doi:10.1016/0048-9697(87)90205-1
- McBride, M.B. 1995. Toxic metal accumulation from agricultural use of sludge: Are USEPA regulations protective? *J. Environ. Qual.* 24:5–18. doi:10.2134/jeq1995.00472425002400010002x
- McLaughlin, M.J., M. Whatmuff, M. Warne, D. Heemsbergen, G. Barry, M. Bell, D. Nash, and D. Pritchard. 2006. A field investigation of solubility and food chain accumulation of biosolid-cadmium across diverse soil types. *Environ. Chem.* 3:428–432. doi:10.1071/EN06061
- McLaughlin, M.J., M.S.J. Warne, D.P. Stevens, M.S. Whatmuff, D. Heemsbergen, K. Broos, G. Barry, M.J. Bell, D. Nash, D. Pritchard, and N. Penney. 2007. Australia's National Biosolid Research Program: How it came about, and what has it discovered? *Water Practice Technol.* 2:art88.
- Munkholm, L.J., P. Schjonning, K. Deboz, H.E. Jensen, and B.T. Christensen. 2002. Aggregate strength and mechanical behaviour of a sandy loam soil under long-term fertilization treatments. *Eur. J. Soil Sci.* 53:129–137. doi:10.1046/j.1365-2389.2002.00424.x
- Nolan, A.L., Y.B. Ma, E. Lombi, and M.J. McLaughlin. 2004. Measurement of labile Cu in soil using stable isotope dilution and isotope ratio analysis by ICP-MS. *Anal. Bioanal. Chem.* 380:789–797. doi:10.1007/s00216-004-2816-6
- Oliver, I.W., G. Merrington, and M.J. McLaughlin. 2004. Australian biosolids: Characterization and determination of available copper. *Environ. Chem.* 1:116–124. doi:10.1071/EN04056
- Oliver, I.W., A. Hass, G. Merrington, P. Fine, and M.J. McLaughlin. 2005. Copper availability in seven Israeli soils incubated with and without biosolids. *J. Environ. Qual.* 34:508–513. doi:10.2134/jeq2005.0508
- Oliver, I.W., Y.B. Ma, E. Lombi, A.L. Nolan, and M.J. McLaughlin. 2006. Stable isotope techniques for assessing labile Cu in soils: Development of an L-value procedure, its application, and reconciliation with E values. *Environ. Sci. Technol.* 40:3342–3348. doi:10.1021/es051845j
- Peijnenburg, W., R. Baerselman, A.C. de Groot, T. Jager, L. Posthuma, and R.P.M. Van Veen. 1999. Relating environmental availability to bioavailability: Soil-type-dependent metal accumulation in the oligochaete *Eisenia andrei*. *Ecotoxicol. Environ. Saf.* 44:294–310. doi:10.1006/eesa.1999.1838
- Rooney, C.P., F.J. Zhao, and S.P. McGrath. 2006. Soil factors controlling the expression of copper toxicity to plants in a wide range of European soils. *Environ. Toxicol. Chem.* 25:726–732. doi:10.1897/04-602R.1
- Schabenberger, O., B.E. Tharp, J.J. Kells, and D. Penner. 1999. Statistical tests for hormesis and effective dosages in herbicide dose response. *Agron. J.* 91:713–721. doi:10.2134/agronj1999.914713x
- Smolders, E., K. Oorts, P. van Sprang, I. Schoeters, C.R. Janssen, S.P. McGrath, and M.J. McLaughlin. 2009. Toxicity of trace metals in soil as affected by soil type and aging after contamination: Using calibrated bioavailability models to set ecological soil standards. *Environ. Toxicol. Chem.* 28:1633–1642. doi:10.1897/08-592.1
- Stevens, D.P., M.J. McLaughlin, and T. Heinrich. 2003. Determining toxicity of lead and zinc runoff in soils: Salinity effects on metal partitioning and on phytotoxicity. *Environ. Toxicol. Chem.* 22:3017–3024. doi:10.1897/02-290