Copper Availability in Seven Israeli Soils Incubated with and without Biosolids
I. W. Oliver,* A. Hass, G. Merrington, P. Fine, and M. J. McLaughlin

ABSTRACT

Land application is becoming a preferred option for disposal of sewage sludge (biosolids) from wastewater treatment plants. However, it creates potential risks due to the heavy metal contents of these materials, with copper (Cu) being of chief concern. The long-term fate of biosolid metals applied to agricultural soils is not well understood, particularly in the soils of the Middle East. This investigation was conducted to determine whether the availability of Cu changes with time in biosolid-amended and nonamended soils from Israel. Seven soils, typifying the span of properties and formation environments encountered in Israel, were incubated with and without biosolids for 7 yr, and changes in organic carbon (OC) content and labile Cu concentration were determined. Isotopic exchange techniques, using $^{64}$Cu, and ion activity measurements, using a Cu$^{2+}$ ion selective electrode, revealed that the available Cu concentration remained relatively low and stable over the 7-yr incubation. This was despite substantial reductions in OC. This study shows that, with regard to Cu, application of such biosolids to these soils at rates of up to 250 Mg ha$^{-1}$ does not pose a threat to the environment in the short to medium term.

THE SAFE AND EFFICIENT disposal of sewage by-products is a challenge facing all countries, with land application becoming increasingly popular because of its potential benefits to soil fertility and structural stability (Hall and Coker, 1981; Oberle and Keeney, 1994; Peverley and Gates, 1994; Joshua et al., 1998; Johansson et al., 1999; Mosquera-Losada et al., 2001). However, such benefits need to be balanced with the risks of accumulation of metals and other potentially toxic elements in soils that can pose a risk to environmental health (MacLean et al., 1987; McGrath, 1987; King and Hajjar, 1990; Chaudri et al., 2000; Bhogal et al., 2003). Copper (Cu) is one of the metals of chief concern in this regard, as it has been shown to be particularly toxic to microbes, with many species of fungi and N-fixing bacteria being especially sensitive (McGrath et al., 1988; Chaudri et al., 1992; Dahlin et al., 1997). Copper is also one of the potentially toxic metals that is present in relatively high concentrations in many biosolids (commonly 500–3000 mg kg$^{-1}$, dry weight). This, combined with the knowledge that Cu associates strongly with organic matter that can degrade over time, suggests that it is vital to determine the likely long-term impact of Cu and other sewage biosolids on soil and ecosystem health so that applications of the material can be kept at safe levels. Some long-term studies have been conducted in Europe and the United States (Chang et al., 1987; McGrath, 1987; Dowdy et al., 1991; Jarausch-Wehrheim et al., 1996; Aitken and Cummins, 1997; Brown et al., 1998), but the conclusions drawn from these investigations are often conflicting. Further, any conclusions drawn from these temperate regions may not be applicable to the situation in Mediterranean climates. Much less is known about the likely fate of biosolid metals applied in the semiarid to subhumid soils of the Middle East. This study investigated the availability of Cu in seven soils from Israel incubated with and without biosolids for 7 yr to determine some of the potential long-term effects of biosolid land application on the chemistry and availability of Cu in soil. The implications of the changes in Cu availability are discussed in regard to the long-term disposal of sludges to land in Israel.

MATERIALS AND METHODS

Soils and Biosolids

Seven soils were selected for the study that encompassed a range of climatic conditions (semiarid to subhumid) and parent materials in Israel: (i) a dune sand; (ii) a loamy Calcic Haploxeralf (Nahal-Oz, from loessial desert dust origin); (iii) a fine loamy, calcareous, Typic Xerochrept (Mitzpe Masuah, on many species of fungi and N-fixing bacteria being old basaltic surfaces). Soils were collected from the A or Ap horizon except in the case of the Netanya soil, for which the Bt horizon was sampled. Soils were air-dried and passed through a 2-mm sieve. Biosolids were obtained from the drying beds at the municipal sewage treatment plant in Haifa, Israel. The biosolids had been anaerobically digested before drying to a water content of 100 g kg$^{-1}$. Biosolids were crushed to pass a 1-mm sieve and stored in sealed containers at 4°C until use. Total contents of N and P in the biosolids were 15.4 and 15.0 g kg$^{-1}$ respectively, and the C to N ratio was 14. Selected physicochemical properties of the soils and biosolids are shown in Table 1.

Total Cu concentrations were determined using a modification of USEPA Method 3051A (Link et al., 1998). Five-hundred-milligram samples (crushed to pass a 0.25-mm sieve) were microwave-digested in 12 mL of aqua regia (9 mL concentrated HNO$$_3$$ + 3 mL concentrated HCl) plus 1 mL perchloric acid. The digestion was performed as follows: 10 min at 580 W then ventilation of the microwave chamber for 10 min.
followed by another 10-min operation at 580 W and ventilation for 10 min. The tubes were allowed to cool to room temperature, and the contents quantitatively transferred to 50-mL volumetric flasks containing yttrium at 1 mg L\(^{-1}\) as an internal standard. Solutions were then analyzed by inductively coupled plasma–atomic emission spectroscopy (ICP–AES) (Spectroflame Modula E; Spectro GMBH, Kleve, Germany), and total Cu concentrations determined (Table 1).

Table 1. Selected properties of soils and biosolids before incubation.

<table>
<thead>
<tr>
<th>Soils and biosolids</th>
<th>Clay</th>
<th>Silt</th>
<th>Sand</th>
<th>Carbonate</th>
<th>OC(^{†})</th>
<th>Cu</th>
<th>pH(^{‡})</th>
<th>CEC(^{§})</th>
<th>Dominant clay¶</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dune sand</td>
<td>3</td>
<td>12</td>
<td>985</td>
<td>18</td>
<td>&lt;1</td>
<td>2 ± 0.1</td>
<td>7.80</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Nahal-Oz</td>
<td>181</td>
<td>302</td>
<td>517</td>
<td>129</td>
<td>4</td>
<td>21 ± 0.3</td>
<td>7.54</td>
<td>30</td>
<td>M, I</td>
</tr>
<tr>
<td>Mitzpe Masuah</td>
<td>196</td>
<td>590</td>
<td>214</td>
<td>572</td>
<td>29</td>
<td>29 ± 1.5</td>
<td>7.39</td>
<td>26</td>
<td>M, I</td>
</tr>
<tr>
<td>Netanya 6</td>
<td>352</td>
<td>145</td>
<td>503</td>
<td>0</td>
<td>3</td>
<td>18 ± 0.3</td>
<td>6.29</td>
<td>22</td>
<td>K, M</td>
</tr>
<tr>
<td>Terra Rossa</td>
<td>350</td>
<td>550</td>
<td>100</td>
<td>56</td>
<td>15</td>
<td>19 ± 1.2</td>
<td>7.37</td>
<td>45</td>
<td>K, M</td>
</tr>
<tr>
<td>Golan 37</td>
<td>296</td>
<td>536</td>
<td>168</td>
<td>0</td>
<td>12</td>
<td>36 ± 0.4</td>
<td>6.15</td>
<td>21</td>
<td>H, A</td>
</tr>
<tr>
<td>Golan 4</td>
<td>744</td>
<td>106</td>
<td>150</td>
<td>8</td>
<td>7</td>
<td>37 ± 1.1</td>
<td>7.25</td>
<td>72</td>
<td>M</td>
</tr>
<tr>
<td>Biosolids</td>
<td>70</td>
<td>210</td>
<td></td>
<td></td>
<td></td>
<td>633 ± 108</td>
<td>6.46</td>
<td>38</td>
<td></td>
</tr>
</tbody>
</table>

‡ Organic carbon.

§ Cation exchange capacity determined by the NH\(_4\)-N acetate method (Thomas, 1982).

¶ H, halloysite; I, illite; K, kaolinite; M, montmorillonite.

Incubation Procedure

One-kilogram oven-dry equivalent masses of soils and mixtures of soil and biosolids (9:1 soil to biosolid ratio) were placed in 2-L pots. The soil–biosolid mixtures equate to a biosolid application rate of 250 Mg ha\(^{-1}\), considering the upper 20-cm plow layer. This rate is approximately 10 to 15 times the common seasonal application in Israel (15–25 Mg ha\(^{-1}\)), and was deliberately chosen as an extreme value to make a measurable impact on the metal content of the amended soils. The selected rate also exceeds the current Israeli guidelines for annual maximum recommended load of Cu to soils (namely, 158 kg ha\(^{-1}\) compared with the guideline of 9 kg ha\(^{-1}\)). All samples were wetted to 60% of the water holding capacity, as determined at 33 kPa, and thoroughly mixed. They were then incubated for 7 yr in a temperature controlled room at 30°C. Moisture loss during the incubation was minimized by placing the pots in closed 50-L containers with free water at the base. The containers were also aerated with moistened air.

The moisture contents of the soils and soil–biosolids mixtures of soil and biosolids (9:1 soil to biosolid ratio) were incubated. The CuE was determined following a slightly modified version of the method of McLaren and Crawford (1974). Triplicate samples (3 g) were equilibrated with 30 mL 0.01 M CaCl\(_2\) (containing three drops of toluene to inhibit microbial activity) for 40 h on end-over-end shakers, after which the radioisotope \(^{64}\)Cu was added (in 0.1 mL solution containing approximately 2 MBq activity). After a further 24-h equilibration period, samples were centrifuged for 10 min at 1200 \(\times\) g, the supernatant solutions filtered through 0.45-μm filters, and then measured for radioactivity (gamma counter) and concentrations of total solution Cu (graphite furnace atomic absorption spectrometer [GFAAS], Aanalyst 600; PerkinElmer, Wellesley, MA). Isotopically exchangeable Cu (the E-value, or CuE) was calculated as follows:

\[
\text{CuE} = \frac{[\text{Cu(sol)} \times \text{TA}]}{(\text{CR} \times \text{SM})}
\]

where CuE is isotopically exchangeable Cu (mg kg\(^{-1}\)); Cu(sol) is concentration of Cu in 0.01 M CaCl\(_2\) solution (mg L\(^{-1}\)); TA is total activity of \(^{64}\)Cu added (Bq); CR is count rate in solution (Bq L\(^{-1}\)); and SM is sample mass (kg).

As a further measure of available Cu, cupric ion activity (pCu\(^{2+}\)) was measured in solution extracts using a cupric ion selective electrode (Orion 9429), a silver–silver chloride double junction reference electrode (Orion 900200), and a mV meter (Orion 720). Although it may not be the only form assimilated, the ionic species Cu\(^{2+}\) is widely believed to be the form of Cu most readily taken up by organisms (Brams and Fiskell, 1971; Graham, 1981; Kochian, 1991), thus Cu bioavailability and/or toxicity may be directly related to the concentration (or activity) of Cu\(^{2+}\) in solution (Kim et al., 1999; Sauve et al., 1996). Measurement of Cu\(^{2+}\) activity in soil solution extracts can therefore determine the immediately available Cu in the sample (i.e., the intensity, \(I\)), and thus may give an indication of the average conditions of Cu availability to which organisms present in the sample would be subjected. Duplicate 3-g samples were equilibrated with 50 mL 0.01 M CaCl\(_2\) solution for 24 h by end-over-end shaking. Samples were then centrifuged for 10 min at 1200 \(\times\) g, the supernatant decanted, and the pCu\(^{2+}\) (negative log molar Cu\(^{2+}\) activity)
Fig. 1. Calibration curve constructed to determine cupric ion activity (pCu²⁺) from mV readings. The slope deviates slightly from the ideal Nernstian response of −29.58 mV.

Statistical Analysis

Statistical analyses (ANOVA) were performed using Genstat 5 for Windows (Release 4.1) (Laws Agricultural Trust, 1998) and Microsoft Excel (Microsoft Corporation, 2000).

RESULTS AND DISCUSSION

Changes in Soil Organic Carbon and pH

The unamended Golan, Mitzpe Masuah, and Terra Rossa soils all showed significant (p ≤ 0.05) reductions in OC by the end of the 7-yr incubation (Fig. 2), while the other nonamended soils showed no change. In terms of the percentage of initial OC lost, the reductions amounted to 55, 35, 39, and 50% for Golan 4, Golan 37, Mitzpe Masuah, and Terra Rossa soils, respectively. All of the biosolid-amended soils lost significant amounts (p ≤ 0.05) of OC during the incubation (Fig. 2), with the percentage of initial OC lost ranging between 28% for Nahal-Oz to 53% for Golan 4. In the case of Golan 4, Mitzpe Masuah, and Terra Rossa biosolid-amended soils, the OC content had decreased back to that of the original unamended soils by the end of the 7-yr incubation (Table 2), while for the remaining amended soils the OC levels were still elevated relative to their unamended states. This compares to the study by Brown et al. (1998), where approximately 80% of the OC added in biosolid amendments to a fine sandy loam soil in Maryland, USA (pH 5.5–6.5, and application rates 100–224 Mg ha⁻¹) had been mineralized after 14 yr. By contrast, the study by Hyun et al. (1998) found added biosolid OC had only declined by 30 to 40% after 10 yr in a sandy loam soil in California, USA (application rates 135–1080 Mg ha⁻¹).

By the end of the incubation the pH had increased marginally in Nahal-Oz, dune sand, Golan 4, and Golan 37 soils, whereas the Netanya 6 soil recorded a very large increase of 1.5 units (Fig. 3). This soil may have a low buffering capacity due to its lack of carbonates and low OC (Table 1), making it susceptible to pH change. The Mitzpe Masuah and Terra Rossa soils showed no change in pH. For the soil–biosolid mixtures all pH values were essentially the same after 7 yr as at time 0 (Fig. 3). Increases in soil pH can occur as the result of organic matter decomposition, because mineralization and ammonification processes release OH⁻ ions and consume H⁺ ions (Ritchie and Dolling, 1985).

Isotopically Exchangeable Copper

For five out of the seven unamended soils, CuE was not significantly different (p ≤ 0.05) between original samples and those incubated for 7 yr (Fig. 4). For the two soils showing statistical differences, Terra Rosa and Golan 4, the increases were less than 1 mg kg⁻¹, so the increases were less than 1 mg kg⁻¹, so

Table 2. Organic C content of original unamended soils and amended soils after incubation.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Unamended soil OC†</th>
<th>Amended soil OC post-incubation</th>
<th>LSD (0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dune sand</td>
<td>0.4</td>
<td>15.9</td>
<td>5.4</td>
</tr>
<tr>
<td>Nahal-Oz</td>
<td>4.3</td>
<td>14.3</td>
<td>0.7</td>
</tr>
<tr>
<td>Mitzpe Masuah</td>
<td>28.8</td>
<td>24.8</td>
<td>NS‡</td>
</tr>
<tr>
<td>Netanya 6</td>
<td>2.7</td>
<td>14.5</td>
<td>1.3</td>
</tr>
<tr>
<td>Terra Rossa</td>
<td>14.5</td>
<td>16.5</td>
<td>1.6</td>
</tr>
<tr>
<td>Golan 37</td>
<td>11.7</td>
<td>15.5</td>
<td>1.1</td>
</tr>
<tr>
<td>Golan 4</td>
<td>7.4</td>
<td>9.1</td>
<td>NS</td>
</tr>
</tbody>
</table>

† Organic carbon.
‡ Not significant.
furnace atomic absorption spectrometry (0.001 mg L\(^{-1}\)), so CuE values calculated for them can only be viewed as estimates. The CuE values for the unamended soils (0.15–2 mg kg\(^{-1}\), representing 0.8–7% of the total soil Cu) were similarly low to those measured by McLaren and Crawford (1974) in 24 English soils. They found a CuE range of 0.19 to 12 mg kg\(^{-1}\), representing from 2 to 19% of the total soil Cu, with half of the soils having less than 10% of their total Cu isotopically exchangeable. Thus, a large percentage of soil Cu is held in forms not readily exchangeable, or in equilibrium, with the soil solution (i.e., non-labile Cu).

In this study, the CuE of the original, nonincubated biosolid-amended soils ranged from 1.4 mg kg\(^{-1}\) for Netanya 6 to 10.9 mg kg\(^{-1}\) for Mitzpe Masuah (Fig. 4). When put in terms of the percentage of total Cu that was isotopically exchangeable, the spread was between 2 and 12%. The low percentages indicate that the great majority of Cu added to the soils by biosolid application was in non-labile forms. In contrast to the unamended soils, the majority of the biosolid-amended soils did show significant (\(p < 0.05\)) changes over the incubation period (Fig. 4). The amended Golan soils, Mitzpe Masuah, Terra Rossa, and Netanya 6 all showed increases in CuE with time. Because pH remained stable during the incubation period, the increases in CuE observed were due to chemical redistributions, most likely due to losses of organic matter through mineralization (which were considerable in terms of percentages of initial OC; Fig. 2). However, the increases in CuE were only between 2 and 5 mg kg\(^{-1}\) (and were less than 5% of total soil Cu in all cases), thus their likely environmental impact would be small. A similar lack of environmentally relevant change in metal availability was noted for Cd by Brown et al. (1998), who observed no difference in Cd uptake by lettuce (\(Lactuca sativa\) L.) in the 14 yr following biosolid application to a fine sandy loam. Similarly, Hyun et al. (1998) had still failed to find any changes in Cd availability 10 yr after biosolid application, using both plant uptake and chemical measures. One may expect Cu to show a greater response than Cd in terms of possible increases in the labile fraction over time due to Cu having a much stronger affiliation with organic matter (McBride, 1981), particularly in cases such as the present study where appreciable amounts of organic carbon were mineralized over the incubation period (Fig. 2). However, this did not prove to be the case, as environmentally significant changes in labile Cu were not observed. This suggests that any Cu previously bound to the mineralized organic fraction of the biosolids was able to be retained by other biosolid components, most likely the mineral fraction but also possibly the resistant, non-readily decomposable organic materials present. If this was indeed the case, then such a result provides supporting evidence for the protection theory, which hypothesizes that mineral components may compensate for any loss of metal retention capacity caused by mineralization of organic components (Chaney and Ryan, 1993).

Changes in Copper Ion Activities

As would be expected given the minimal changes in pH observed during the incubation, cupric ion activities
(pCu$^{2+}$) in CaCl$_2$ extract solutions were generally consistent for pre- and post-incubation samples (Fig. 5), with the exceptions of Nahal-Oz and Netanya 6 for the unamended soils (no significant differences were observed for any of the amended soils). The differences between pre- and post-incubation pCu$^{2+}$ were significant for the unamended Netanya 6 soil ($p \leq 0.05$), with values of 10.4 and 10.7, respectively, yet the change is unlikely to be of any real consequence for the environment. The change observed for the Nahal-Oz soil was greater (0.8 units), but may still be of little environmental relevance because the pCu$^{2+}$ values were already above those found to cause toxicity to plants (pCu$^{2+} < 8$; McBride, 2001). Therefore the pCu$^{2+}$ measurements support the findings from the CuE determinations, in that even when biosolids were applied at rates vastly exceeding the normal application rates, and in excess of guideline regulations, adverse environmental consequences were not observed in terms of Cu toxicity. Thus it can be seen that land application of biosolids in Israel, following current practices, will not lead to adverse outcomes in terms of soil Cu toxicity. In fact, it could be argued that the regulatory guidelines may be overprotective. However, supporting or refuting such an argument is not the focus or goal of this paper.

**CONCLUSIONS**

Unamended soils incubated for 7 yr lost up to 55% of their initial OC content, while soils amended with biosolids lost between 28 and 53%. Soil pH remained relatively constant in most treatments but increased by 1.5 units in one soil. Despite the losses of OC, isotopically exchangeable Cu did not change by more than 5 mg kg$^{-1}$ in any of the unamended or amended soils (representing less than 5% of total soil Cu). There was also no environmentally significant change in pCu$^{2+}$ in 0.01 M CaCl$_2$ extracts over time, thus from an environmental impact viewpoint the availability of Cu remained low and constant. We conclude that in these samples, which are typical Mediterranean semi-arid to subhumid soil types, Cu was either not associated with the readily degradable OC or, more likely, was readily retained by other biosolid and/or soil components once released. Therefore OC degradation, which was extensive in many soils, did not lead to significant increases in Cu availability, and thus these data do not support the “time-bomb” hypothesis, which theorizes that metals will be released once organic matter is reduced below a critical level (McBride, 1995). Therefore, with respect to Cu, applications of such biosolids to these soils at rates of up to 250 Mg ha$^{-1}$ are not likely to pose any environmental risks in the short to medium term, and thus the current common practice of applying 10 to 15 Mg ha$^{-1}$ certainly does not pose a risk. Conclusions on the longer-term fate of biosolid Cu (i.e., $>10$ yr) cannot be directly drawn from these results, but, given the chemical distribution into more stable forms observed here, it seems unlikely that biosolid Cu will pose a long-term threat in these soils.

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**REFERENCES**


