Environ. Sci. Technol. **2006**, *40,* 3342−3348

Stable Isotope Techniques for Assessing Labile Cu in Soils: Development of an *L*-Value Procedure, Its Application, and Reconciliation with *E* Values

IAN W. OLIVER,*,† YIBING MA,†,‡ ENZO LOMBI,† ANNETTE L. NOLAN,† AND MIKE J. MCLAUGHLIN†,§

CSIRO Land and Water, Centre for Environmental Contaminant Research, PMB 2, Glen Osmond, South Australia 5064, Australia, Institute of Soil and Fertilisers and Ministry of Agriculture Key Laboratory of Plant Nutrition and Nutrient Cycling, Chinese Academy of Agricultural Sciences, Beijing 100081, China, and Department of Soil and Land Systems, School of Earth and Environmental Sciences, The University of Adelaide, PMB1, Glen Osmond, South Australia 5064, Australia

Isotopic techniques have become a valuable tool for assessing the lability or potential availability of elements in soil. Until now, work on soil Cu has been limited to *E*-value methods where soil solution extracts are obtained by physical means due to the very short (12.4 h) half-life of the radio isotope ^{64}Cu. However, a stable isotope method has recently been developed for determining soil Cu *E* values that utilizes enhancement of the ^{65}Cu isotope in soil and measurement of the subsequent ratio with ^{63}Cu. We have developed an *L*-value technique for soil Cu, where plants are used to sample the soil solution and therefore give a direct measure of the plant available Cu. The *L*-value technique developed was then compared, and found to be equivalent, with *E* values using equilibration periods up to and including the growth period of plants in the *L*-value method.

Introduction

Isotopic exchange techniques quantify the amount of a given element that is capable of freely exchanging, over a certain period of time, between the soil solid and solution phases and thus quantify the labile pool or that fraction potentially available to organisms (*1*−*4*). These techniques are based on the principle that when a small amount of an isotope is introduced to a soil, its distribution will reflect that of the corresponding labile element pool in the soil. The distribution of the isotope between the solution and solid phases and the labile pool can be determined by sampling and analyzing the solution phase. In this case the labile pool is traditionally called an *E* value (*E* for exchangeable). Alternatively, plants grown in the labeled soil can be used to sample the isotopically exchangeable pool. In this case the labile pool is called an *L* value (*5*). Theoretically, *E*- and *L*-value methods should measure the same pool and therefore produce similar results. That is, the *E* and *L* values generated should be equivalent for a given element in a given soil. This is unless the biological component (i.e. the plant) modifies the soil rhizosphere in a way that mobilizes nonisotopically exchangeable metals (*6*).

Good agreement between *E* and *L* values has been found for Ni across a range of soil types (*7*) and for Zn in neutral to acidic soils (*2*, *8*). However, in the case of alkaline soils Zn *E* values have often been found to be significantly higher than *L* values, possibly due to irreversible fixation of the added tracer and/or increased isotopic exchange in batch techniques compared to pot trials (*2*, 8). Colloidal interferences may also be a source of inconsistency between *E* and *L* values (*9*). In some instances, the presence of soil colloids (containing nonexchangeable metal) in solution may cause differences to be observed, as *E*-value measurements for Zn and other elements can be affected by these (*10*) while *L* values are not. Conflicting results have been recorded for Cd, with one study showing *E* value − *L* value equivalence (*11*), while another reported significant differences (with *L* values being greater) (*12*). No such work has been done on soil Cu, due to the very short half-life of the most accessible Cu radioisotope (^{64}Cu, $t_{1/2}$ = 12.4 h). This short half-life makes the *L*-value procedure a practical impossibility, thus the *E*-value technique has been relied upon in isotopic investigations of soil Cu availability/lability (*10*, *13*−*15*). However, Nolan et al. (*16*) recently developed a procedure for determining Cu *E* values using the stable isotope ratio ^{65}Cu/^{63}Cu, which had excellent agreement with values determined using the ^{64}Cu radioisotope. With the development of this technique, the next logical step would be to adapt it to an *L*-value procedure involving plants. If such an *L*-value procedure could be developed, then direct comparisons between *E* and *L* values could be made for soil Cu, allowing their equivalence to be tested without radiological risks to the user. Furthermore, once developed, such a procedure could be generalized and applied to the examination of any element having at least two stable isotopes.

To make a fair comparison between *E* and *L* values the effect of soil-isotope equilibration time needs to be considered. Typically, *E*-value procedures involve less than 1 week equilibration, whereas *L*-value procedures involve several weeks to months, due to the growth period of plants required. The equilibration time allowed can affect the degree to which the added tracer exchanges with metal in the soil solids (with more tracer diffusing into solids as time passes), which in turn affects the *E* and *L* values calculated (*17*, *18*). It is therefore important to compare *E* and *L* values with equivalent equilibration times. Some researchers (i.e. refs *7* and *8*) have measured isotope exchange kinetics over periods of 100 minutes and used the data to extrapolate *E* values to equilibration times equivalent to those used in *L*-value procedures. However, isotope exchange kinetics has not been tested for Cu, and, furthermore, direct measurement of *E* and *L* values with equivalent equilibration times will avoid any criticisms associated with such extrapolations. Therefore, in this study, we conducted preliminary trials to develop an *L*-value procedure for soil Cu based on the stable Cu isotope *E*-value technique of Nolan et al. (*16*). We then conducted a more in-depth study that compared *E* values determined in parallel samples equilibrated for various times up to and

* Corresponding author phone: +44 (0) 1355223332; fax: +44 (0) 1355229898; e-mail: I.Oliver@suerc.gla.ac.uk. Current address: Scottish Universities Environmental Research Centre (SUERC), Rankine Avenue, Scottish Enterprise Technology Park, East Kilbride G75 0QF, Scotland, U.K.

† CSIRO Land and Water, Centre for Environmental Contaminant Research.

‡ Chinese Academy of Agricultural Sciences.

§ The University of Adelaide.

10.1021/es051845j CCC: $33.50
Published on Web 04/06/2006

TABLE 1. Selected Characteristics of Soils Used in the Preliminary Tomato *L*-Value Study

soil	pH ($CaCl_2$)	clay (%)	total C (%)	total Cu (mg/kg)	^{65}Cu spike (mg/kg)
Tavistock + lime (U.K.)	5.50	11	4.7	1058	15.5
Kapunda + Bolivar (Aus)	6.41	11	8.0	432	5.5
Kapunda + Murray Bridge (Aus)	6.39	21	5.8	602	12.3
vineyard (Italy)	7.14	4.6	2.7	389	9.0

including the growth period used in the *L*-value procedure. This allowed examination of the equivalence of *E* and *L* values for soil Cu.

Methods

Preliminary Investigation 1 − L Values Determined with Tomato Plants. Soils. As an initial test of the stable isotope *L*-value technique, the isotopically labile Cu, or *L* value, was determined using tomato (*Solanum lycopersicum*) as the test plant in four soils. The soils used in this initial investigation were sourced from Australia, the U.K., and Italy (Table 1). The soil from Tavistock, a region within Devonshire County in England, U.K., had a relatively high background metal concentration due to contamination from historic mining activities (*19*). The soil used in this preliminary investigation had been limed (0.3% by mass) to raise the pH to 5.5. The two soils from Kapunda, an agricultural region in South Australia, Australia, approximately 80 km north of the state's capital city, Adelaide, were taken from adjacent fields that had been amended 9 years previously with sewage biosolids from the treatment plants at Bolivar and Murray Bridge, respectively (*16*). Bolivar is the principal sewage treatment plant for Adelaide, while Murray Bridge treatment plant services the small rural center of Murray Bridge (~77 km east of Adelaide). The vineyard soil originates from the Trentino Alto Adige Region in northern Italy, from a site where Cu salts have been applied as a fungicide for over 80 years (*16*).

Copper Spiking Solution. Enriched ^{65}Cu (99.5% atom abundance) was obtained as Cu metal (Trace Sciences International, Ontario, Canada) and dissolved in 50% concentrated HNO_3 (70%, Mallinckrodt, Phillipsburg, NJ) to give a 10 000 mg/L stock solution. From this, a working stock solution of 100 mg of ^{65}Cu/L was produced (with a measured ^{65}Cu abundance ratio of 94.43%, ±0.012%).

***L*-Value Determination.** Triplicate 100 g air-dry samples of each soil were spiked with the 100 mg ^{65}Cu/L stock. The amount of ^{65}Cu added to each soil was based on approximately 5% of the isotopically exchangeable Cu (Cu *E* value) determined previously in our laboratory for each soil using the radioisotope ^{64}Cu (data not published). The volume of deionized water required to raise the water content of each soil to the equivalent of that at 70% maximum water holding capacity (determined by the funnel method (*20*)) was added immediately after the ^{65}Cu spike and was thoroughly mixed through using spatulas. Three control pots were also established for each soil, in which no ^{65}Cu was added, but all other aspects were identical. The samples were then covered and allowed to equilibrate for 5 days at 20 °C, after which five tomato seeds were sown in each pot. All pots were transferred to an incubation room where they were kept for 33 days under day/night conditions of 14 h at 22 °C and 10 h at 16 °C. At the end of the growth period the above ground plant tissues were harvested and dried at 60 °C. Yields (oven dry) were recorded on a per pot basis. The plant tissues were digested in boiling nitric acid (Mallinckrodt) and analyzed for total element concentrations using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES, Spectroflame Modula, Spectro). The ratio of ^{63}Cu:^{65}Cu was determined in the digest solutions using Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Agilent 7500c, Agilent Technologies), with instrument settings as described by Nolan et al. (*16*). Where necessary, samples were diluted to fall within the range 50−200 μg Cu/L immediately prior to ICP-MS analysis. The isotopically labile Cu, or *L* value, was then calculated according to eq 1

$$L\text{ value} = R \times \frac{\mathrm{AW(Cu_{nat})}}{\mathrm{AW(^{65}Cu)}} \times \frac{\mathrm{IR_{sp} - IR_{meas}}}{\mathrm{IR_{meas} - IR_{nat}}} \times (\mathrm{IR_{nat}} + 1) \quad (1)$$

where *L* value = plant determined isotopically exchangeable Cu (mg/kg), *R* = total ^{65}Cu* added to soil during spiking (mg/kg soil), AW(Cu $_{nat}$) = atomic weight of natural Cu, AW(^{65}Cu) = atomic weight of ^{65}Cu isotope, IR_{nat} = natural abundance ratio of ^{63}Cu/^{65}Cu in plant (from control pots), IR_{sp} = abundance ratio of ^{63}Cu*/^{65}Cu* in spiking solution (determined as 0.0057 ± 0.0001), IR_{meas} = measured abundance ratio of (^{63}Cu+^{63}Cu*)/(^{65}Cu+^{65}Cu*) in plant tissue, and an asterisk (*) indicates Cu sourced from the added ^{65}Cu spike.

Derivation of the *L*-Value Equation. The empirical form of the *L*-value equation can be stated as follows (eq 2)

$$L = R \times \frac{C}{C^*} \quad (2)$$

where *C* denotes Cu (Cu^{63}+Cu^{65}) assimilated into plant tissues from soil (mg/kg), *C** denotes spiked Cu^{65*} assimilated by the plant (mg/kg), and other terms are as above (eq 1).

Therefore, three Cu isotope ratios are required to determine Cu *L* values in soils using stable isotope techniques: (a) the native abundance ratio of ^{63}Cu/^{65}Cu (IR_{nat}), (b) the ratio of ^{63}Cu*/^{65}Cu* in the spiking solution (IR_{sp} = 0.5/99.5), and (c) the measured Cu isotope ratio (^{63}Cu+^{63}Cu*)/(^{65}Cu+^{65}Cu*) in plant tissue grown in spiked soils (IR_{meas}). Although most stable isotope ratios are considered invariable in nature, there have been reports of natural Cu isotope variation (*21*), hence the native abundance ratio (IR_{nat}) was measured in control samples that were not spiked with ^{65}Cu (in all cases, IR_{nat} fell within 4% of the 2.244 IUPAC representative value). The ratio of the concentration of natural Cu (mg/ kg) to the concentration of spiked ^{65}Cu* (mg/ kg) assimilated into plant tissues from soils is defined as

$$\frac{C_{sol}}{C^*_{sol}} = \frac{\mathrm{AW(Cu_{nat})}}{\mathrm{AW(^{65}Cu)}} \times \frac{^{63}\mathrm{Cu} + {}^{65}\mathrm{Cu}}{^{65}\mathrm{Cu^*}} = \frac{\mathrm{AW(Cu_{nat})}}{\mathrm{AW(^{65}Cu)}} \times \frac{\mathrm{IR_{nat}}\,{}^{65}\mathrm{Cu} + {}^{65}\mathrm{Cu}}{^{65}\mathrm{Cu^*}} = \frac{\mathrm{AW(Cu_{nat})}}{\mathrm{AW(^{65}Cu)}} \times \frac{^{65}\mathrm{Cu}}{^{65}\mathrm{Cu^*}} \times (\mathrm{IR_{nat}} + 1) \quad (3)$$

where ^{63}Cu and ^{65}Cu are the isotope atomic abundances, and other terms are as above (eq 1).

If the relationship IR_{meas} = (^{63}Cu+^{63}Cu*)/(^{65}Cu+^{65}Cu*) is divided by ^{65}Cu*, then

$$\frac{^{65}\mathrm{Cu}}{^{65}\mathrm{Cu^*}} \times \mathrm{IR_{nat}} + \mathrm{IR_{sp}} = \frac{^{65}\mathrm{Cu}}{^{65}\mathrm{Cu^*}} \times \mathrm{IR_{meas}} + \mathrm{IR_{meas}} \quad (4)$$

Equation 4 can be rearranged as

$$\frac{^{65}Cu}{^{65}Cu^*} = \frac{IR_{sp} - IR_{meas}}{IR_{meas} - IR_{nat}} \quad (5)$$

Combining eqs 2, 3, and 5 yields the equation for calculating L values from measured ratios IR_{nat} and IR_{meas} (eq 1, above).

Preliminary Investigation 2 − Confirming the Effect of Equilibration Time on Isotopic Exchangeability Measurements Made Using Stable Cu Isotopes. The effect of varying soil-spike equilibration time prior to isotopic analysis has been recognized as a source of discrepancies in the results of isotope dilution experiments, particularly when comparing E and L values (*7*, *8*, *22*). We conducted this second preliminary investigation to confirm whether such effects of time influenced measurements made using stable Cu isotopes. Following the stable Cu isotope E-value method of Nolan et al. (2004), E-value measurements were made on the soil from Tavistock (Table 1) after soil-spike equilibration periods ranging from 1 h to 1 week. Measurements were also made on Tavistock soil samples that had not been previously limed (pH 4.9). Calculations of E values were made according to eq 6. The results (as fully described in the Results section) confirmed the potential effects of equilibration time variation on isotopic exchangeability measurements made using stable Cu isotopes and thus confirmed the need for further investigations incorporating equilibration time differences if L values determined using stable isotope techniques are to be reconciled with E values.

Principal Investigation − Reconciling *E* and *L* Values Determined with Stable Cu Isotopes. Soils. Six soils were selected from an ongoing Cu aging study involving 19 European soils (Table 2). Soils that had been treated with Cu salts ($CuCl_2$) and aged for 3 months in the field (in pots) were investigated. Prior to field placement, Cu was added to the soils at concentrations that had been previously shown to cause a 10% reduction in root elongation of barley (*Hordeum vulgare*) seedlings (i.e. the EC10 concentration). After retrieval from the field, samples were allowed to air-dry in glasshouses, sieved to 2 mm, and stored dry until used.

***L*-Value Determination with Ryegrass.** For each soil, a 170 g air-dry sample was spiked with the 100 mg ^{65}Cu/L stock (Table 2). As in the preliminary study above, the amount of ^{65}Cu added to each soil was based on approximately 5% of the isotopically exchangeable Cu (Cu E value) determined previously for each soil using the radioisotope ^{64}Cu (*23*). The volume of deionized water required to raise the water content of each soil to the equivalent of that at field capacity (100 cm water suction or pF 2) was added immediately after the ^{65}Cu spike and was thoroughly mixed through using spatulas. Three subsamples from each prepared soil, equivalent to 50 g of oven-dry material, were placed into separate pots. A spoonful of ryegrass seeds (*Lolium multiflorum*, approximately 0.5 g) was then scattered across the surface, which was subsequently covered with a thin layer of small plastic beads to reduce evaporation. Two control pots were also established for each soil, in which no ^{65}Cu was added, but all other aspects were identical. All pots were then transferred to a growth room with day/night conditions of 14 h at 22 °C and 10 h at 16 °C. The plants were harvested 28 days after sowing by cutting at 0.5 cm above the surface of the plastic beads. Plant materials were dried in an oven (70 °C for 48 h), and the dry mass per pot was recorded. Plant materials were digested and analyzed for total element concentrations and ^{63}Cu:^{65}Cu ratios as outlined above. The L values were calculated according to eq 1, with correction for contributions from seed Cu (see below).

Determining Contributions from Seed Cu. The use of ryegrass in the L-value determination presented an obstacle due to the possible contribution of seed Cu to the Cu present in shoots at the end of the growth period. This was not a concern in the case of tomatoes, because their small seed size and minimal Cu concentrations meant seed Cu translocation to shoots was a negligible percentage of total Cu uptake (<5%). However, the ryegrass seeds used had a Cu concentration approximating 5 mg/kg, and 0.5 g of seeds was added to each pot. Therefore, seed Cu could have influenced the outcome as any significant contributions from the seeds would affect not only shoot Cu concentrations but also the Cu isotope ratios measured (i.e. the results would reflect an isotope ratio of mixed seed and soil Cu origin, rather than giving a true indication of the Cu isotope ratios as extracted from the soil by the plants).

The distribution of seed Cu was measured using a 10 day germination test. Ryegrass seeds were germinated in a Petri dish containing a filter paper wetted with MilliQ water. After 10 days, the shoots of seedlings, roots, and seed shells were separated by cutting, with the components then being air-dried, weighed, and digested in concentrated nitric acid. The concentration of Cu in the digest solutions was measured using ICP-OES. From these values the percentage of seed Cu transferred to shoots was determined to be 32.1%. The seed Cu concentration (4.34 mg/kg), together with this derived 32.1% value for seed-to-shoot Cu transfer, was used to correct L-value calculations by subtracting seed contributed Cu from plant shoot Cu concentrations.

***E*-Value Determination.** Two g samples, 10 for each soil, were placed in 50 mL centrifuge tubes. An amount of ^{65}Cu equivalent to that applied in the L-value procedure (mg/kg basis) was added to each tube along with enough deionized water to raise the moisture content to field capacity. In addition, two control tubes (no ^{65}Cu addition but otherwise identical) were established for each soil. The tubes were then sealed and placed inside the growth room at the same time as the L-value samples (above). Every 2 days the tubes were briefly opened to allow gas exchange and then resealed. In duplicate, samples were taken after 0, 2, 6, 13, and 27 days exposure, and the isotopically exchangeable Cu was determined using the stable isotope measurement technique developed by Nolan et al. (*16*). This involved adding 20 mL of deionized water to each tube, along with two drops of toluene to inhibit microbial activity, and placing on an *end-over-end* shaker for 24 h. Tubes were then centrifuged at 1200*g* for 30 min, and the supernatant solutions were filtered through 0.2 μm syringe filters. The filtered solutions were

TABLE 2. Selected Properties and Dosing Rates of Soils Investigated in the Study

no.	soil	pH ($CaCl_2$)	sand (%)	silt (%)	clay (%)	total C (%)	background Cu (mg/kg)	added Cu (EC10, mg/kg)	^{65}Cu spike (mg/kg)
2	Nottingham (U.K.)	3.4	64	23	13	5.2	17	67	5.9
9	Kövlinge II (Sweden)	5.1	77	14	9	2.3	8	126	5.9
10	Montpellier (France)	5.2	87	4	9	0.8	5	126	5.9
14	Woburn (U.K.)	6.4	60	19	21	4.4	22	217	11.8
15	Ter Munck (Belgium)	6.8	14	71	15	1.0	15	178	5.9
17	Rots (France)	7.4	19	54	27	3.0	14	626	17.6

TABLE 3. Tomato *L* Values for Soils Examined in the Preliminary Study

	L value (mg/kg)	SE[a]	SE[a] as % *L* value	*L* value as % total Cu	SE[a]
Tavistosk + 0.3% lime	481	45.9	9.6	45.4	4.3
Kapunda + Bolivar	374	24.2	6.5	86.5	5.6
Kapunda + Murray Bridge	333	26.5	8.0	55.3	4.4
vineyard	333	20.3	6.1	85.6	5.2

[a] SE = standard error.

TABLE 4. Plant Yield (g/pot)

soil	2	9	10	14	15	17
EC10-treated	0.241	0.125	0.169	0.408	0.223	0.274
^{65}Cu-spiked	0.302	0.122	0.208[a]	0.368	0.262	0.358
L.S.D.$_{(0.05)}$	*0.124*	*0.041*	*0.034*	*0.064*	*0.083*	*0.091*
soil mean yield[b]	0.278ae	0.123b	0.192c	0.384d	0.246a	0.3242e
across soil *L.S.D.*$_{(0.05)}$	*0.048*					

[a] Yield difference between EC10-treated and ^{65}Cu-spiked treatments for soil 10 were statistically significant but were within 1.2 × *L.S.D.*$_{(0.05)}$. [b] Yield mean for EC10 and ^{65}Cu treatments combined (soils followed by different letters recorded significantly different yields at the 0.05 level).

acidified with ultrapure HNO_3 (Mallinckrodt) and kept at 4 °C until analysis. Total solution Cu concentrations and ^{63}Cu:^{65}Cu isotope ratios were determined as per *L*-value samples. Isotopically exchangeable Cu (*E* value) was calculated using eq 6 (*16*)

$$E\text{ value} = R \times \frac{\mathrm{AW}(^{63}\mathrm{Cu} + {}^{65}\mathrm{Cu})}{\mathrm{AW}(^{65}\mathrm{Cu})} \times \frac{\mathrm{IR_{sp}} - \mathrm{IR_{meas}}}{\mathrm{IR_{meas}} - \mathrm{IR_{nat}}} \times (\mathrm{IR_{nat}} + 1) \quad (6)$$

where *E* value = isotopically exchangeable Cu (mg/kg), *R* = total concentration of ^{65}Cu* in the spike (mg/kg), AW = atomic weight of Cu isotope, IR_{nat} = natural abundance ratio of ^{63}Cu/^{65}Cu in solution, IR_{sp} = abundance ratio of ^{63}Cu*/^{65}Cu* in spiking solution, and IR_{meas} = measured abundance ratio of (^{63}Cu+^{63}Cu*)/(^{65}Cu+^{65}Cu*) in solution after equilibration.

Statistical analyses (ANOVA, employing least significant differences) of yield, plant Cu concentration, and *L*-value and *E*-value data were performed using Genstat 6 for Windows (*24*).

Results and Discussion

Preliminary Investigation 1 − *L* Values Determined with Tomato Plants. The *L* values determined for the four soils investigated ranged from 333 to 481 mg/kg (Table 3). The values were highly reproducible, in all cases returning standard errors among replicates of less than 10%. In terms of percentage of total soil Cu, the *L* values ranged from 45 to 87%. Among the four soils, differences in the percentage of total Cu being plant available likely stemmed from their different sources of Cu contamination (i.e. mining waste vs sewage biosolids vs Cu fungicides), as each source would contribute Cu in a different form, with differing degrees of solubility and degradability. For example, the soil collected from the Tavistock mining area showed the lowest lability of Cu, despite the low pH of the soil, probably due to the presence of primary minerals (mainly chalcopyrite) where Cu is occluded and not readily exchangeable with soil solution. This result is in agreement with the low lability of Cu in this soil determined by *E* values using the radioisotope ^{64}Cu (*25*). Also, the Kapunda soil amended with biosolids from Murray Bridge had a much higher clay content than that amended with biosolids from Bolivar, indicating that the matrices of the two biosolids were quite different, resulting in differences in Cu availability. Differences in physical and chemical characteristics between the soils would have also contributed to the different percentage availabilities observed.

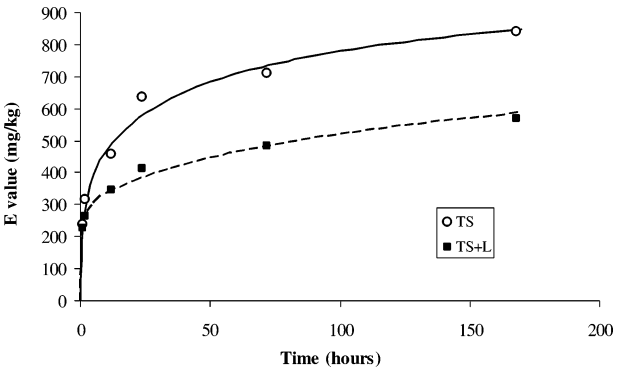

FIGURE 1. Effect of soil-spike equilibration time on *E* values measured using the stable Cu isotope technique. TS − Tavistock soil; TS+L − Tavistock soil treated with lime.

Preliminary Investigation 2 − Effect of Equilibration Time on Stable Cu Isotope *E* Values. Results of the second preliminary investigation confirmed that variation in soil-spike equilibration time prior to *E*-value measurement affected the outcome, with greater equilibration time leading to higher *E* values (Figure 1). Thus *E*-value determinations made using the stable Cu isotope technique are affected by time of equilibration in a similar way to radioisotopic investigations (i.e. refs *7*, *17*, *18*, and *22*).

Principal Investigation. Results from the principal investigation showed that while ryegrass yields varied between soils, there was practically no difference between yields in EC10-treated controls and the ^{65}Cu-spiked treatments within soils (Table 4). The exception was soil 10, which did show a yield difference between EC10-treated controls and the ^{65}Cu-spiked treatments; however, the difference was marginal (Table 4). Plant Cu concentrations also varied across soils but generally did not vary between EC10-treated controls and the ^{65}Cu spiked treatments within a given soil (Table 5).

***E* Values.** Soils 2, 10, and 15 all showed significant increases in *E* values with increasing equilibration time relative to the 1-day equilibration (Figure 2). Soils 9 and 17 did not show significant increases, but this was due to deviation of a single replicate at 1-day equilibration for soil 9 and 14 days for soil 17 (Figure 2). If these seemingly aberrant values are omitted from the data set, soils 9 and 17 also show increasing *E* values with equilibration time.

***L* Values.** *L* values ranged from 53 mg/kg for soil 2 to 440 mg/kg for soil 17. The values showed high precision among replicates, with standard errors being less than 5 mg/kg for

TABLE 5. Plant Cu Concentrations (mg/kg)

soil	2	9	10	14	15	17
EC10-treated	12.34	21.57	42.00	11.83	18.94	16.80
^{65}Cu-spiked	13.98[a]	23.07	45.02	16.04[a]	18.48	16.48
L.S.D.$_{(0.05)}$	*1.59*	*2.86*	*5.76*	*3.72*	*3.23*	*4.78*
soil mean plant [Cu][b]	13.33a	22.47c	43.81d	14.36a	18.66b	16.61b
across soil *L.S.D.*$_{(0.05)}$	*2.24*					

[a] Plant [Cu] differences between EC10-treated and ^{65}Cu-spiked treatments were statistically significant but were within 1.1 × *L.S.D.* for soil 2 and 1.2 × *L.S.D.* for soil 14. [b] Plant [Cu] mean for EC10 and ^{65}Cu treatments combined (soils followed by different letters recorded significantly different plant [Cu]).

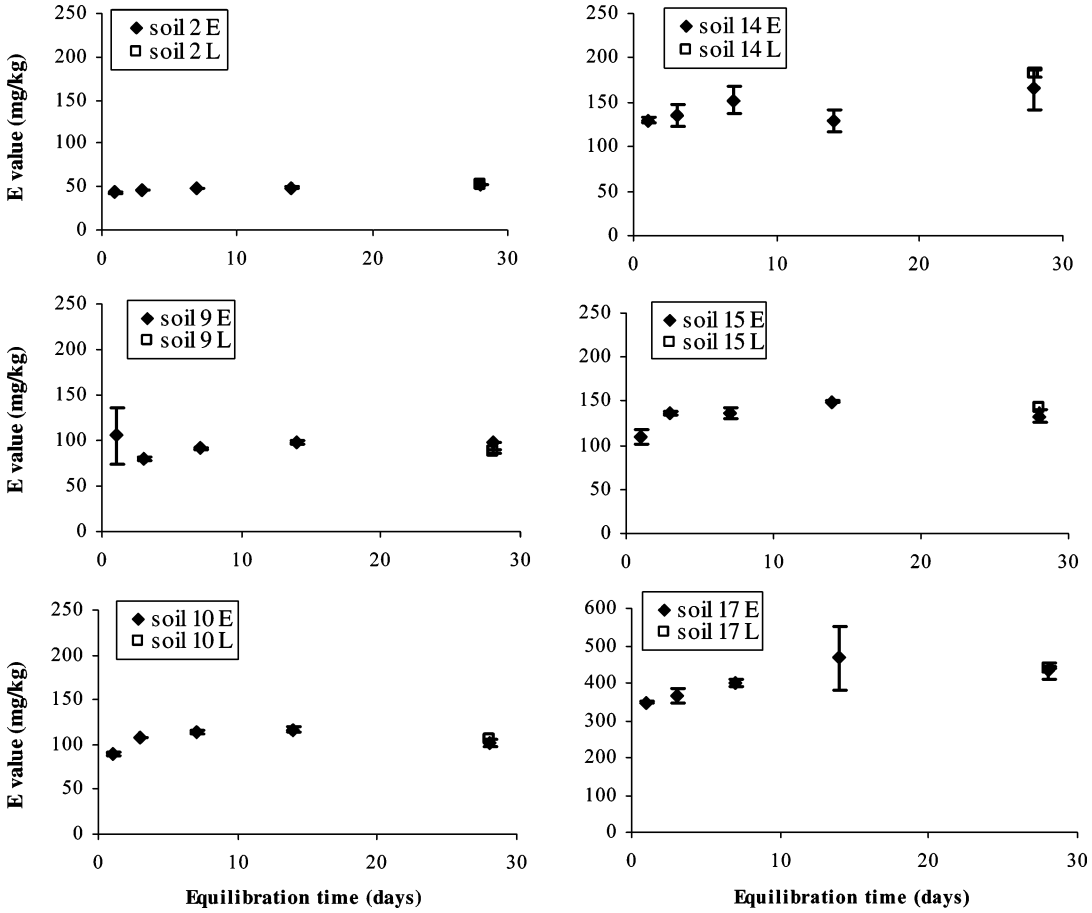

FIGURE 2. Cu *E* values (mg/kg) showing the effect of equilibration time (1−28 days) and rye grass Cu *L* values determined for each soil. Note change of scale for soil 17. Error bars indicate standard errors, where they extend beyond symbol margins.

TABLE 6. *E* and *L* Values in Absolute Terms and as Percentages of Total Cu and EC10 Cu Addition[a]

			E value as %				*L* value as %	
soil	*E* value (mg/kg)	SE[b]	total Cu	EC10 addition	*L* value (mg/kg)	SE[b]	total Cu	EC10 addition
2	52	0.06	58	77	53	0.84	63	79
9	99	0.17	70	78	88	2.50	66	70
10	101	3.40	74	80	106	1.91	81	84
14	165	22.94	66	76	183	4.32	76	84
15	133	7.16	67	75	142	1.12	74	80
17	433	21.46	66	69	441	2.02	69	70

[a] Percentage EC10 Cu addition = [*E* or *L* value (mg/kg)/Cu added (EC10) prior to incubation (mg/kg)] × 100. [b] SE = standard error.

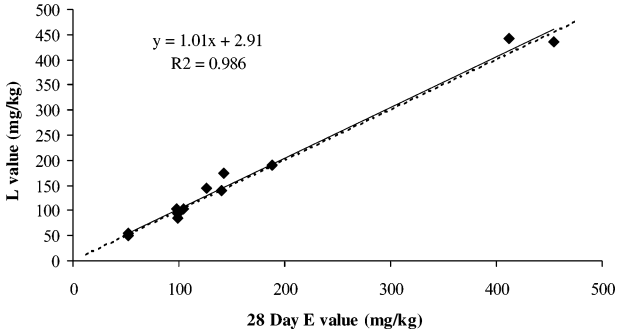

FIGURE 3. *L* values (mg/kg) plotted against 28 day *E* values (mg/kg). The regression (solid) and 1:1 (dashed) lines are indicated.

all soils (with most having SE less than 2 mg/kg, Table 6). The *L* values and the 28 day *E* values were highly correlated, with a linear regression returning an R^2 of 0.99 (Figure 3). More importantly, complete convergence between *E* and *L* values was observed for each soil (Figure 2 and Table 6), indicating that both *E*- and *L*-value techniques measure the same pool of soil Cu. Direct comparison of the stable Cu

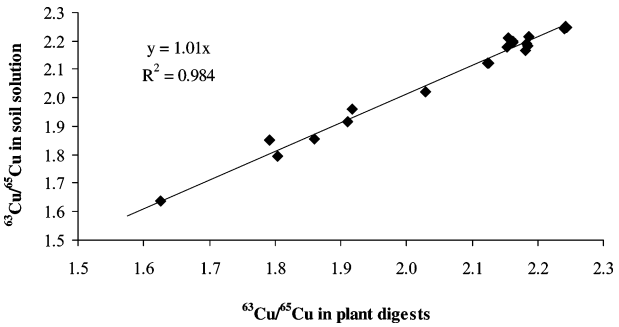

FIGURE 4. Comparison of stable Cu isotope ratios in plant digests with soil extract solutions (data from rye grass and tomato experiments combined).

isotope ratio in plant tissue digests with that in soil extract solutions also confirmed this (Figure 4). Similarly, in terms of the percentage of the EC10 Cu addition made to the soils prior to incubation, across all six soils 70−85% of the added Cu remained isotopically exchangeable after 3 months incubation in soil (Table 6). The lowest lability of Cu, in terms of percentage of added Cu, was observed in soil 17, which had the highest pH and was the only soil tested with appreciable carbonate content (14.9%).

The results obtained here are in accordance with those reported from studies on Ni (*7*), P (*22*), and Zn (*8*), which claimed to show *L*- and *E*-value measurements to be equivalent for the respective elements. However, the R^2 for the *E* value − *L* value regression from the P study by Frossard and co-workers was 0.90, which is lower than the value observed in the present study ($R^2 = 0.99$). When presented as percentages of *L* values, the *E* values from the Zn study by Sinaj et al. (*8*) ranged from 90 to 134% (or up to 267% if the alkaline soils are included), thus showing greater variation than our results. It is likely that our results showed greater and more consistent agreement between *E* and *L* values because our investigation directly measured *E* values with soil-spike equilibration times that were identical to those in the *L*-value procedure, whereas the cited studies involved extrapolation of short-term *E*-value measurements out to equivalent times used for the respective *L*-value measurements.

However, while being in general agreement with some published outcomes, the current results are in contrast to those described by Smolders et al. (*12*), who observed higher *L* values relative to *E* values for Cd and Zn in a range of Belgian soils (using wheat, *Triticum aestivum*, as the test plant). They hypothesized that rhizosphere dynamics were potential causes for the differences observed, concluding that metal radioisotope specific activity may have differed slightly between soil solution in the rhizosphere and solution in the bulk soils they examined. Such a situation could arise if the release of plant exudates promoted metal solubilization from nonlabile pools through organic chelation or by altering rhizosphere pH, as demonstrated by Collins et al. (*26*, *27*). We can conclude from the current study that such rhizospere interactions did not significantly modify the lability of Cu in the test soils. The nature and range of exudates released by plants can be species specific as well as dependent on environmental factors, thus further work is required to determine whether the *E* value−*L* value convergence obtained here with stable Cu isotopes is consistent across a wider range of soils and plant species.

In summary, a procedure for determining Cu *L* values using enriched stable isotope ratios has been successfully developed and applied in tests using tomatoes and rye grass. Values determined by the procedure were not statistically different than *E* values measured in soils that had equivalent soil-isotope equilibration times (regression $R^2 = 0.99$). Complete convergence of *E* and *L* values was observed. This method could be generalized to examine the lability of any element having two or more stable isotopes.

Acknowledgments

We thank the International Copper Association for providing support for this research. The authors also thank Prof. Erik Smolders (KUL, Belgium) for soil collection, Dr. Steve McGrath (Rothamsted Station, U.K.) for root elongation inhibition data, and Mrs. Sharyn Zrna (CSIRO Land and Water) for technical assistance.

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Received for review September 19, 2005. Revised manuscript received January 19, 2006. Accepted February 13, 2006.

ES051845J