# PREDICTING METAL UPTAKE AND RISK TO THE HUMAN FOOD CHAIN FROM LEAF VEGETABLES GROWN ON SOILS AMENDED BY LONG-TERM APPLICATION OF SEWAGE SLUDGE

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**Abstract.** The success of risk assessment of metal contaminated soils depends on how precisely one can predict the bio-availability of metals in soil and transfer to the human food chain. In the present investigation, we tested several formulations of the 'free-ion activity model (FIAM)' to predict uptake of Cd, Zn and Cu by perpetual spinach (*Beta vulgaris*, Cicla) grown on a range of soils amended with sewage sludge. The model was parameterised using data measured on samples of pore water extracted by centrifugation and with porous *Rhizon* samplers installed within the rhizosphere of the growing plants. Free ion activities ($M^{2+}$) were estimated following speciation of solution data using version 6 of the 'Windermere Humic Aqueous Model (WHAM-VI). For all three metals, the best formulation of the FIAM appeared to require only one hypothetical root sorption site without competition from protons. Values of ($M^{2+}$) could also be predicted satisfactorily from a pH-dependent Freundlich relation. Thus, from a combined FIAM–Freundlich relation and population dietary information, it was possible to estimate risk (hazard quotients) to consumers from very simple soil measurements: extractable metal content (0.05 M EDTA (Zn and Cu) or 1 M $CaCl_2$ (Cd)), soil humus content and pH. The role of increased soil organic matter content and soil pH, in reducing risk to consumers, is illustrated for Cd in a hypothetical soil at the current UK statutory Cd limit for sludge application to agricultural land.

**Keywords:** biotic ligand model, free ion activity model, metal solubility, metal uptake, risk assessment, soil solution, speciation, spinach

## 1. Introduction

There is increasing concern regarding the exceedance of statutory and advisory food standards for trace metals throughout the world. On the other hand, peri-urban agriculture is assuming greater importance, particularly in developing countries like India, due to increasing population pressure. In many cases, metal-enriched waste products have been added to such marginal agricultural land. This is a matter of concern because of the persistence of these metals in soils, uptake by crops, and possible accumulative effects in animals and human beings (Rattan *et al.*, 2002).

Assessing risk from such metal-contaminated soils depends on how precisely one can predict the solubility of metals in soil and subsequent transfer of these metals from soil to plants. Over the years various approaches have been employed

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to estimate the ‘bio-availability’ of metals in soil. Most studies focus on trying to quantify the solid-phase reservoir of available metal – the ‘capacity’ factor. For example, current legislation in most countries still utilizes total soil metal concentrations as a simple index of hazard in contaminated soils. However, total metal load takes no account of the soil characteristics that modify the bio-availability of metal pollutants in contaminated soils. The total metal content of soil will usually include fractions that are not immediately available to plants, micro-organisms and soil fauna (Zhang *et al.*, 2001) although many studies have demonstrated good correlations between total metal content and uptake (Sauerbeck, 1991; Hooda *et al.*, 1997). The toxic effects of heavy metals have also been related to some operationally defined extractable fractions (Sauve *et al.*, 1998; McLaughlin *et al.*, 2000). The European Commission have adopted a standardized extraction with 0.05 M EDTA to represent the ‘available fraction’ of heavy metals in soil and sediment (Quevauviller, 1998). The radio-labile pool of metal (Young *et al.*, 2000) may be a better representation of a chemically responsive metal reservoir in soils, but isotopic dilution procedures are unlikely to be adopted on a routine basis.

Attempts have also been made to characterize the bio-availability of metals in soil using an ‘intensity factor’ i.e. metal concentrations or activity in soil solution (Sauve *et al.*, 1998; Chaudri *et al.*, 1999; McGrath *et al.*, 1999; Vulkan *et al.*, 2000). There is considerable experimental evidence to suggest that responses of plants and soil organisms to metal toxicity are explained by the variation in free metal ion activity in soil pore water (Spark, 1995; Sauve *et al.*, 1998; Hough *et al.*, 2003, 2004). In such studies, extraction of a representative soil aqueous phase to which, in theory, plant roots or microorganisms are continually exposed, is of the utmost importance. Among recent techniques, centrifugation and *Rhizon* samplers are used to extract soil pore water (Di Bonito *et al.*, 2002). The latter approach is particularly interesting in that it can involve *in-situ* extraction of soil pore water from the rhizosphere of growing plants. However, there is not much information on the relative effectiveness of these techniques. Estimation of free metal ion activity in the soil solution from chemical analysis of extracted samples can now be achieved with reasonable precision using speciation models which incorporate humic and fulvic binding of metals. The ‘Windermere Humic Aqueous Model’ (WHAM-VI) is one of the most comprehensive examples (Tipping, 1998).

Extraction of soil pore water and subsequent chemical analysis and speciation may be difficult to adopt on a routine basis to assess ecotoxicity of metal in contaminated soils. In addition, solution phase information is almost universally absent from existing large soil geochemical surveys. To be generally useful, estimates of metal solubility must be based on accessible soil characteristics. Thus attempts have been made to predict metal solubility from more ‘widely-accessible’ soil characteristics (Jopony and Young, 1994; Tye *et al.*, 2003).

Several researchers have validated uptake models based on ion transport from soil to roots (mass flow and diffusion) and the kinetics of ion absorption by plants (Jungk and Classen, 1997; Singh and Pandeya, 1998). However, application of models which rely on knowledge of root morphological characteristics and ion diffusion over the course of a growing season may be difficult to apply meaningfully in field situations. Thus, it may be worthwhile assessing the validity of simpler approaches like the free ion activity model (FIAM) to predict metal concentration in plants at the point of harvest (Hough *et al.*, 2004).

In this paper, an attempt was made to integrate these considerations within a simple model framework in order to predict toxic hazard of metal from soil geochemical data. The specific objectives of this investigation were (i) to predict the metal solubility (cadmium, zinc and copper) in metal-contaminated soils from accessible soil characteristics, (ii) to predict the uptake of metal by a leaf vegetable (perpetual spinach) from the soil solution phase using several forms of the FIAM and (iii) to predict the risk to human consumers from consideration of transfer of metals to the human food chain under prescribed soil conditions. A simple pot trial using a leafy vegetable grown in soils subject to long-term sewage sludge amendment is used to illustrate the approach.

## 2. Materials and Methods

### 2.1. SOILS: LOCATION AND COLLECTION

Seventeen topsoil samples (0–15 cm) were collected from different fields of a sewage disposal farm in the East Midlands of England which has been used for sewage sludge disposal for over 100 years. The farm has ‘dedicated site’ status under the UK Sludge Regulations (European Council Directive, 1986) and is subject to a geochemical survey every 4 or 5 years. Fields used for soil collection were selected to include a variation in soil metal contents which approximated the range found on the farm as a whole. All soil samples (moist) were passed through a coarse sieve (4 mm) for use in a pot experiment. A sub-sample of one kg of each soil was stored for chemical analysis after air drying, grinding and sieving to $<2$ mm.

### 2.2. POT EXPERIMENT

Four replicate 4 L capacity plastic pots were filled with soil from each collection site and arranged in a randomised block design. About 10–12 seeds of leaf beet (perpetual spinach, *Beta vulgaris*, Cicla) were sown in each pot and, after 7 days of germination, a uniform plant population of six plants in each pot was maintained. Pots were irrigated daily to return the soil moisture close to field capacity (FC). Four weeks after germination, the above-ground parts of plants from one replication were harvested and dried at 60 °C for 48 h. Harvested plant samples were ground using a stainless steel mill. Similarly after six weeks of germination, plants from the remaining three replicates were also harvested and processed. Ground plant samples were digested with *Primar* high purity concentrated $HNO_3$ and analysed for Cd, Zn and Cu using flame atomic absorption spectrophotometry (FAAS). A standard reference material (tomato leaves, National Institute of Standard and Technology, USA, SRM 1573a) was used to validate the digestion and analysis by AAS.

### 2.3. SOIL PORE WATER EXTRACTION, ANALYSIS AND SPECIATION

Soil pore water was obtained by centrifugation and using *in-situ Rhizon* samplers. Porous *Rhizon* soil moisture samplers (Rhizosphere Research Products, Wageningen, Holland) were inserted at an angle of 45° through the rhizosphere of growing plants three weeks after the germination of the spinach. In the sixth week following germination, pore solution was extracted through the *Rhizon* samplers by applying negative pressure (0.07 MPa); the soils were returned to field capacity (FC) and left overnight prior to solution extraction. Part of the extracted soil solution was acidified to give 0.1 M $HNO_3$ and used for assay of trace metals and cations; the remaining soil solution was used for analysis of anions and dissolved organic carbon (DOC).

Soil solution samples were also recovered by centrifugation following harvest at 4 weeks. Pots were returned to FC after harvest and left overnight. Soil was removed from pots and sieved to remove roots. About 200 g of moist soil was centrifuged at 5000 rpm for 90 min in partitioned tubes with an outer collection vessel separated from the soil by a 20 $\mu$m stainless steel mesh filter (Di Bonito *et al.*, 2002).

Concentrations of Zn, Cu, Ca, Mg, Na and K in soil pore water were determined using flame-AAS (FAAS). A quartz atom concentrator tube was used in the case of Zn and Cu to increase the sensitivity of the method; calibration was with graded concentrations of standard Zn and Cu solution up to 1 mg $L^{-1}$. Soil solution was analysed for Cd using graphite furnace AAS (GFAAS); calibration was undertaken using graded concentrations of standard Cd solution up to 5 $\mu$g $L^{-1}$. Solution concentrations of $SO_4$, Cl, $NO_3$ and phosphate were measured by *Dionex* ion chromatography. Dissolved organic and inorganic carbon (DOC and DIC) were measured using an automated *LABTOC* aqueous carbon analyser.

The distribution of Cd, Zn and Cu into different species in soil pore water was determined using the Windermere Humic Aqueous Model (WHAM-VI Tipping, 1994; Tipping, 1998). Total concentrations of Cd, Zn, Cu, Ca, Mg, K, Na, $SO_4$, Cl, $NO_3$, phosphate, $CO_3$, DOC, pH and estimated ($Fe^{3+}$) activity were used as inputs to the model. The total concentration of carbonate was estimated from DIC assays. The value of ($Fe^{3+}$) was computed from the solubility product ($K_{so}$) of $Fe(OH)_3$ and its standard reaction enthalpy:

$$\log_{10} K_{so} = \log_{10} K^{\circ}_{so} + 0.219 \Delta H^{\circ} \left( \frac{1}{298} - \frac{1}{T} \right),$$

$$\log_{10}(Fe^{3+}) = \log_{10} K_{so} - 3\text{pH};$$

at 298 K, $\log_{10} K^{\circ}_{so} = 2.7$ and the standard reaction enthalpy $\Delta H^{\circ} = -25000$ cal $mol^{-1}$ (S. Lofts, personal communication). The temperature was set at 293 K for the speciation modelling. It was assumed that 50% DOC was fulvic acid containing 50% C as suggested by E. Tipping (personal communication).

### 2.4. SOIL ANALYSIS

Soil samples were digested using *aqua regia* (75% conc. HCl + 25% $HNO_3$) and analysed for Cd, Zn and Cu using FAAS. Soils were extracted with 1 M $CaCl_2$ for Cd, which can be used as an alternative measure of the 'labile' pool of soil Cd (Young *et al.*, 2000) and 0.05 M ethylenediaminetetraacetic acid (EDTA) for Zn and Cu (Quevauviller, 1998). After harvest, soil was removed from each pot and pH was measured (approximate soil: water ratio = 1:2.5) with a glass electrode. Loss on ignition of soil was determined at 550 °C. Available phosphate in soil was extracted with 0.5 M $NaHCO_3$ (pH 8.5) and measured colorimetrically (Rowell, 1994).

### 2.5. PREDICTION OF METAL SOLUBILITY AND PLANT METAL UPTAKE

Metal solubility was predicted by using a simple pH-dependent Freundlich equation (Jopony and Young, 1994). This model predicts the free ion activity of trace metals in soil solution as function of labile soil metal and pH with the simplifying assumption that all labile metal is adsorbed on humus (Tye *et al.*, 2003).

$$(\mathrm{M}^{2+}) = \frac{M_\mathrm{L}}{k_\mathrm{M}(\mathrm{H}^+)^{-n_\mathrm{M}}} \tag{1}$$

where $(\mathrm{M}^{2+})$ is the free metal ion activity in soil solution, $M_\mathrm{L}$ is the labile pool of soil metal assumed to be exclusively adsorbed on humus (mol $\mathrm{kg}^{-1}$ carbon); $k_\mathrm{M}$ and $n_\mathrm{M}$ are empirical constants which express the pH-dependence of the metal distribution coefficient. In the present study, 1 M $CaCl_2$-extractable Cd, and 0.05 M EDTA–extractable Zn and Cu were used as estimates of the labile metal pools.

The form of FIAM called the biotic ligand model (BLM) was used to predict the metal concentration in plants as a function of free metal ion in soil pore water. This approach is based on sorption of metal from soil solution to hypothetical plant root sorption sites considering the competition between cations and protons for sorption sites (Parker and Peddler, 1997; Brown and Markich, 2000). Ignoring competition between different metals, including alkali-earth cations, a summation of binding sites can be written as:

$$\mathrm{R_T} = \mathrm{R} + \mathrm{RM} + \mathrm{RH} \tag{2}$$

where $\mathrm{R_T}$, R, RM and RH refer to total, free, metal-bound and protonated sites respectively. The absorption reaction for metal ions and protons may be described by Equations (3) and (4).

$$M^{2+} + R \Leftrightarrow RM; \quad K_M = \frac{RM}{(M^{2+})R} \tag{3}$$

$$H^{+} + R \Leftrightarrow RH; \quad K_H = \frac{RH}{(H^{+})R} \tag{4}$$

The simple reaction constants ($K_M$ and $K_H$) in Equations (3) and (4) ignore possible effects on membrane characteristics, such as electrostatic potential and permeability, resulting from cation sorption or changes in pH. Combining Equations (2)–(4), and assuming that metal uptake to spinach shoots [$M_{spinach}$] is proportional to RM:

$$M_{spinach} = \frac{K_t R_T K_M (M^{2+})}{1 + K_M(M^{2+}) + K_H(H^{+})}, \tag{5}$$

where $K_t$ is a proportionality constant which expresses the assumption that metal concentrations in plant shoots reflect the concentration of metal ions adsorbed on root sites integrated over the growing time of the plant. Equation (5) can be adapted to include consideration of competition between several metals and/or alkali-earth cations for several hypothetical site types. Equation (5) was parameterized, by non-linear error minimization, using the 'Solver' facility in Microsoft 'Excel'; the error sum of squares was calculated for numerical, rather than logarithmic, plant metal content data.

A transfer factor (TF) was calculated as the ratio of metal concentrations in plants ($M_{spinach}$) to metal ion activity in soil pore water ($M^{2+}$) as follows:

$$TF = \log\left(\frac{M_{spinach}}{M^{2+}}\right) \tag{6}$$

### 2.6. RISK ASSESSMENT

The risk to human health, through consumption of green vegetables, was expressed as a 'hazard quotient' ($HQ_{gv}$) using the following relationship (Pierzynski *et al.*, 2000):

$$HQ_{gv} = \frac{ADD}{RfD} \tag{7}$$

where $HQ_{gv}$ is the hazard quotient arising from consumption of green vegetables (in this case perpetual spinach), ADD is the average daily dose per kg body weight (mg kg$^{-1}$ d$^{-1}$) and RfD, the 'reference dose', is the ADD at which no adverse effects are evident. The values of RfD used for Cd and Zn were 0.001 and 0.3 mg kg$^{-1}$ d$^{-1}$, respectively (IRIS, 2003). For Cu, there is a value for 'provisional maximum tolerable daily intake' (PMTDI) of 0.5 mg kg$^{-1}$ d$^{-1}$ (WHO, 1982) which may be used in place of RfD (Alam *et al.*, 2003). Daily intake of green vegetable was assumed to be 0.2 kg d$^{-1}$ which is the recommended amount from a nutritional point of view (Hassan and Ahmed, 2000). A factor of 0.085 was used to convert the fresh to dry weight of perpetual spinach (Baes *et al.*, 1984). Average body weight for an adult was assumed to be 70 kg (U.S. EPA, 1991). Thus the HQ for an adult was calculated as:

$$\mathrm{HQ} = \frac{M_{\mathrm{spinach}} \times 0.2 \times 0.085}{\mathrm{RfD} \times 70} \tag{8}$$

## 3. Results and Discussion

### 3.1. SOIL CHARACTERISTICS

The characteristics of the 17 test soils are shown in Table I. The data reflect a varied history of sewage sludge application, with elevated humus, metal and phosphate

TABLE I

Characteristics of experimental soils

| Soil no. | pH | Loss on ignition[a] (%) | Total metal[b] (mg kg$^{-1}$) Cd | Zn | Cu | Extractable metal[c] (mg/kg) Cd | Zn | Cu | Available *P* (mg/kg)[d] |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 6.82 | 16.0 | 7.53 | 621 | 249 | 3.25 | 220 | 129 | 284 |
| 2 | 6.86 | 23.2 | 15.5 | 1232 | 540 | 5.30 | 570 | 277 | 467 |
| 3 | 7.06 | 9.59 | 8.25 | 372 | 149 | 3.13 | 151 | 77.1 | 221 |
| 4 | 6.60 | 11.8 | 9.35 | 471 | 208 | 4.23 | 216 | 109 | 213 |
| 5 | 6.76 | 12.1 | 9.55 | 502 | 208 | 3.65 | 223 | 111 | 278 |
| 6 | 6.69 | 16.9 | 5.86 | 443 | 177 | 2.04 | 182 | 105 | 252 |
| 7 | 6.78 | 16.3 | 20.6 | 1211 | 471 | 6.99 | 617 | 209 | 387 |
| 8 | 7.24 | 7.11 | 2.21 | 214 | 78 | 0.84 | 96.7 | 42.5 | 249 |
| 9 | 6.62 | 16.8 | 17.1 | 1184 | 462 | 6.31 | 458 | 220 | 411 |
| 10 | 6.66 | 24.8 | 34.8 | 2174 | 897 | 12.1 | 961 | 365 | 443 |
| 11 | 6.46 | 29.4 | 55.0 | 2165 | 930 | 17.6 | 1100 | 419 | 533 |
| 12 | 6.71 | 22.50 | 31.2 | 1768 | 759 | 8.21 | 828 | 342 | 450 |
| 13 | 6.57 | 25.2 | 17.6 | 1798 | 734 | 6.08 | 796 | 313 | 516 |
| 14 | 6.64 | 7.72 | 1.31 | 202 | 74.5 | 0.36 | 87.1 | 33.5 | 233 |
| 15 | 6.71 | 7.80 | 1.18 | 186 | 66.0 | 0.53 | 69.2 | 32.3 | 200 |
| 16 | 6.22 | 29.7 | 39.9 | 1955 | 966 | 17.9 | 1148 | 435 | 501 |
| 17 | 6.82 | 13.8 | 8.20 | 833 | 321 | 3.39 | 501 | 176 | 393 |

[a] Values of loss on ignition were used as estimates of total soil organic matter.

[b] Aqua-regia digestion.

[c] Cd was extracted with 1 M $CaCl_2$, Zn and Cu were extracted with 0.05 M EDTA.

[d] Available *P* was extracted with 0.5 M $NaHCO_3$.

contents and a pH broadly in line with the target for the site (>6.5). For comparison, UK legislation permits sludge application to normal agricultural land (non-dedicated sites) provided the Cd, Zn and Cu concentrations are less than 3,300 and 135 mg kg$^{-1}$ respectively in the pH range 6.0–7.0.

### 3.2. SPECIATION OF TRACE METALS IN SOIL SOLUTION

The analysis of the soil solution for trace metals is shown in Table II. In all cases, metal concentrations extracted with centrifugation were reasonably well correlated with values obtained from *Rhizon* samplers: Cd ($r^2 = 0.88$), Zn ($r^2 = 0.58$) and Cu ($r^2 = 0.77$). On average, more Cd was extracted with *Rhizon* samplers compared to centrifugation whereas Zn and Cu concentrations were similar with both methods. However, statistical comparison between the two methods was not possible because centrifugation yielded so little solution that the samples had to be pooled for chemical analysis.

Speciation of the soil solution extracted with *Rhizon* samplers using WHAM-VI revealed that 42–80% (mean 61%) of Cd and 60–91% (mean 79%) of Zn was associated with DOC. Similar results were found for samples isolated by centrifugation.

TABLE II
Trace metal concentrations in soil pore water

| | Pore water extracted by centrifugation | | | Pore water extracted with *Rhizon* samplers | | |
|---|---|---|---|---|---|---|
| Soil | Cd ($\mu$g L$^{-1}$) | Zn (mg L$^{-1}$) | Cu (mg L$^{-1}$) | Cd ($\mu$g L$^{-1}$) | Zn (mg L$^{-1}$) | Cu (mg L$^{-1}$) |
| 1 | 1.26 | 0.20 | 0.12 | 2.74 | 0.09 | 0.07 |
| 2 | 1.11 | 0.22 | 0.13 | 3.86 | 0.15 | 0.16 |
| 3 | 1.36 | 0.13 | 0.09 | 3.54 | 0.07 | 0.10 |
| 4 | 2.11 | 0.19 | 0.15 | 4.59 | 0.10 | 0.05 |
| 5 | 1.89 | 0.19 | 0.16 | 3.36 | 0.09 | 0.14 |
| 6 | 0.85 | 0.19 | 0.12 | 3.25 | 0.12 | 0.10 |
| 7 | 4.38 | 0.26 | 0.25 | 6.60 | 0.20 | 0.27 |
| 8 | 0.73 | 0.14 | 0.05 | 1.69 | 0.05 | 0.03 |
| 9 | 2.87 | 0.24 | 0.22 | 4.87 | 0.21 | 0.21 |
| 10 | 4.11 | 0.27 | 0.34 | 8.44 | 0.31 | 0.23 |
| 11 | 3.63 | 0.30 | 0.32 | 6.74 | 0.24 | 0.30 |
| 12 | 1.90 | 0.19 | 0.26 | 4.40 | 0.23 | 0.32 |
| 13 | 1.37 | 0.20 | 0.21 | 3.66 | 0.22 | 0.33 |
| 14 | 0.54 | 0.09 | 0.03 | 0.48 | 0.01 | 0.03 |
| 15 | 0.77 | 0.17 | 0.04 | 1.03 | 0.02 | 0.04 |
| 16 | 8.70 | 0.26 | 0.32 | 11.1 | 0.49 | 0.33 |
| 17 | 1.33 | 0.18 | 0.19 | 3.40 | 0.15 | 0.18 |

More than 99.5% of Cu in the soil pore water was associated with organic species for both the methods of extraction. As a proportion of the total metal concentration in solution, free metal ion activities ranged from 9.24 to 30.6% (mean = 19.4%) for $Cd^{2+}$ and 2.47–20.5% (mean = 9.53%) for $Zn^{2+}$. Again similar values were obtained for samples isolated by centrifugation. The average activity of free $Cu^{2+}$ was only 0.01% of Cu concentration in solution for both methods.

## 3.3. METAL SOLUBILITY

Figure 1 shows the fit of the simple adsorption model for predicting metal activity in solution from accessible soil characteristics, for all three metals. Equation (1) explained 84 and 95% of the variation in solution activity of ($Cd^{2+}$) extracted by Rhizon samplers and centrifugation, respectively. In the case of ($Zn^{2+}$) and ($Cu^{2+}$), Equation (1) accounted for 59 and 58% of the variation observed, respectively, for both Rhizon samplers and centrifugation. The greater success of the model for prediction of ($Cd^{2+}$) may be partly because EDTA is less effective at discriminating between the chemically responsive and fixed pools of soil Zn and Cu compared to the extraction of labile Cd with $CaCl_2$. However, this is difficult to assess on such a small data set.

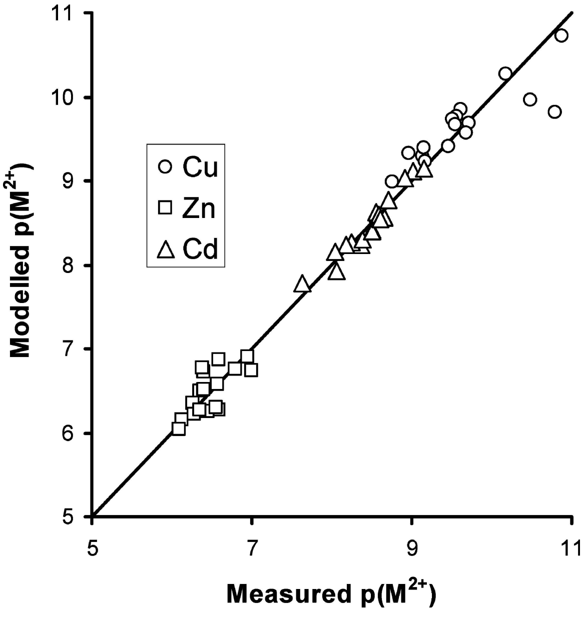

*Figure 1.* Measured and modelled values of $p(M^{2+})$ for Cu (circle), Zn (triangle) and Cd (square). Modelled values were calculated by independently parameterising Equation (1) for each metal data set (Table I); ‘measured’ values were derived from analysis of the soil pore water and speciation with WHAM VI.

Tye *et al*. (2003) found a good fit to data from a larger number of diverse soils using an equation similar to the one employed here, expressing metal solubility only as a function of labile metal, %C and pH. However, one feature of soils subject to long-term application of sewage sludge is their exceptionally large phosphate content. It is well recognised that the bioavailability of metals in contaminated soils is reduced by application of phosphate (Rattan *et al.*, 2002). Hence, stepwise regression was undertaken to evaluate the effect of available P on metal ion activity in soil solution (data not shown). It was found that available P failed to improve the predictability of metal ion activity although a negative partial regression coefficient of P indicated the general depressing effect of P on Cd solubility. The lack of improvement was probably due to a strong correlation between available P and organic carbon ($r^2 = 0.62$).

### 3.4. PLANT METAL CONCENTRATION

Concentrations of Cd, Zn and Cu in the above-ground portion of spinach (Table III) showed considerable variation between soils: 2.01–34.7, 127–1340 and 5.85–34.3 mg kg$^{-1}$, respectively. The mean Cd, Zn and Cu concentrations were

TABLE III

Mean metal concentrations in spinach leaves

| | Plant metal content on dry matter basis (mg kg$^{-1}$) | | |
|---|---|---|---|
| Soil | Cd | Zn | Cu |
| 1 | 13.1 | 771 | 27.9 |
| 2 | 15.7 | 863 | 20.7 |
| 3 | 10.7 | 362 | 19.2 |
| 4 | 17.7 | 965 | 26.0 |
| 5 | 11.2 | 728 | 20.6 |
| 6 | 19.0 | 985 | 22.5 |
| 7 | 15.1 | 689 | 20.9 |
| 8 | 2.34 | 127 | 5.85 |
| 9 | 22.3 | 957 | 26.1 |
| 10 | 20.4 | 970 | 20.2 |
| 11 | 16.5 | 945 | 23.9 |
| 12 | 14.3 | 821 | 24.7 |
| 13 | 14.2 | 936 | 29.4 |
| 14 | 2.01 | 266 | 6.18 |
| 15 | 2.42 | 188 | 12.5 |
| 16 | 34.7 | 1340 | 34.3 |
| 17 | 8.90 | 709 | 25.1 |

14.2, 742 and 21.5 mg kg$^{-1}$. These values are considerably larger than normally found in plants grown on agricultural soils and yet no foliar symptoms of metal toxicity (e.g. chlorosis) were observed in the test plants.

Total soil Cd content accounted for 41% of the variation in plant Cd concentrations; the corresponding values for Zn and Cu were 48 and 36%, respectively. Hooda *et al.* (1997) also found a significant correlation between metal concentration in plants and total metal contents of sludge amended soils. However, Cd extractable with 1 M $CaCl_2$ explained 56% of the variation in plant Cd concentrations. By contrast, EDTA-extractable Zn and Cu were only as good as total metal content in predicting the metal concentrations in plants. The activity of ($Cd^{2+}$) in solution alone explained 79% (*Rhizon* extraction) or 70% (centrifugation) of the variation in plant-Cd; the corresponding values for Zn were 53 and 73%. By contrast, free ($Cu^{2+}$) ion activity produced no improvement over extractable Cu in predicting Cu uptake. Zhang *et al.* (2001) also reported nonlinearity between free $Cu^{2+}$ activity in solution and plant-Cu.

Total metal concentration in soil pore water and free metal activity were almost equally effective in predicting the plant metal concentrations for all three elements across the methods of extraction. However, the two variables were strongly correlated: with correlation coefficients of 0.94, 0.95 and 0.77 for Cd, Zn and Cu, respectively when solution was extracted with *Rhizon* samplers. This was expected for the narrow pH range found in such carefully managed soils. Moreover, there is some evidence to show that absorption of metal by plants is not solely dependent on free metal ion activity in soil solution (Smolders and McLaughlin, 1996; Collins *et al.*, 2001). A large concentration of complexed metal in the rhizosphere is also likely to increase uptake by shortening the diffusion path to the root surface. Unfortunately, our data set is not adequate to resolve whether the principle determinant of metal uptake rate is free metal ion activity or total metal concentration in solution. The question is probably not even meaningful if the rate of depletion of the soil solution around the root surface significantly exceeds the ability of the soil to replenish metal concentration in solution by desorption and diffusion.

Table IV shows values of $R^2$ for various implementations of the free ion activity model (FIAM/BLM) for Cd, Zn and Cu. For the data set used, the model was not improved by assuming two hypothetical root sorption sites or by including proton competition (with metal ions) for sites. This was probably due to the narrow range of soil pH found in the test soils. A plot of solution-to-plant transfer factor ($M_{\mathrm{spinach}}/(M^{2+})$) against pH showed only a weak tendency to increase with pH. Using values of ($M^{2+}$) estimated from extractable metal, pH and LOI (Equation 1) resulted in a substantial reduction in fit to the FIAM equation. This highlights the limitation of such simple solubility ‘models’ in providing reliable estimates of ($M^{2+}$) but also underlines the strength of the relationship between *measured* values of free ion activity (chemical analysis and modelled speciation) and plant uptake.

The metal concentrations in plants ($M_{\mathrm{spinach}}$) were plotted against the free ($M^{2+}$) activity in solution derived from centrifugation (Figures 2–4). For all three metals,

TABLE IV

Values of $R^2$ for the prediction of metal concentrations in spinach leaves using the FIAM (Equation 5) implemented with either one or two root sorption sites, with or without proton competition, and using either measured or modelled (Equation 1) values of ($M^{2+}$)

| Root adsorption sites | $H^+$ competition | ($M^{2+}$)[a] | Rhizon (R) or centrifugation (C) | Correlation coefficients Cd | Zn | Cu |
|---|---|---|---|---|---|---|
| 1 | No | Measured | R | 0.83 | 0.83 | 0.72 |
| 2 | No | Measured | R | 0.83 | 0.83 | 0.72 |
| 1 | Yes | Measured | R | 0.83 | 0.83 | 0.72 |
| 1 | No | Modelled | R | 0.68 | 0.64 | 0.42 |
| 1[b] | No | Measured | C | 0.78 | 0.82 | 0.80 |
| 2 | No | Measured | C | 0.78 | 0.82 | 0.80 |
| 1 | Yes | Measured | C | 0.78 | 0.82 | 0.80 |
| 1 | No | Modelled | C | 0.69 | 0.56 | 0.50 |

[a] Measured values of ($M^{2+}$) were computed from analysis of soil pore water speciated using WHAM-VI; modelled values are from a parameterized version of Equation (1) using values of extractable metal, pH and organic carbon.

[b] This is the model presented in Figures 2–4; values of the resolved parameters (from Equation 5) for this form of the model are given in the figure descriptions for each metal.

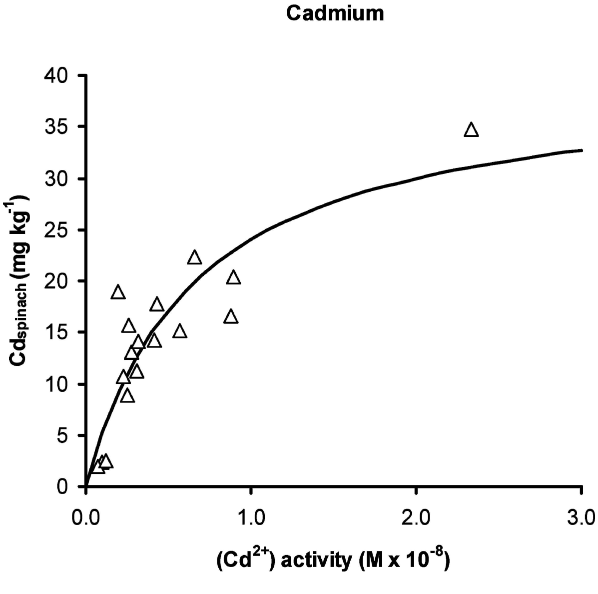

*Figure 2.* Uptake of Cd by spinach as a function of $Cd^{2+}$ activity in the soil pore water, calculated using the WHAM-VI speciation model. The fit of the FIAM is shown as a solid line; the model was implemented with a single root sorption site without proton competition. From Equation (5), $\log_{10}(K_t R_T) = 1.6$ and $\log_{10} K_M = 8.18$.

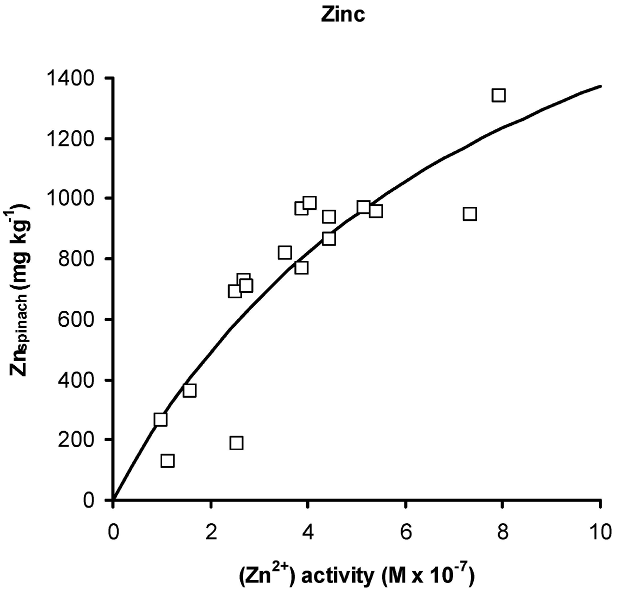

*Figure 3*. Uptake of Zn by spinach as a function of $Zn^{2+}$ activity in the soil pore water, calculated using the WHAM-VI speciation model. The fit of the FIAM is shown as a solid line; the model was implemented with a single root sorption site without proton competition. From Equation (5), $\log_{10}(K_t R_T) = 3.39$ and $\log_{10}(K_M) = 6.09$.

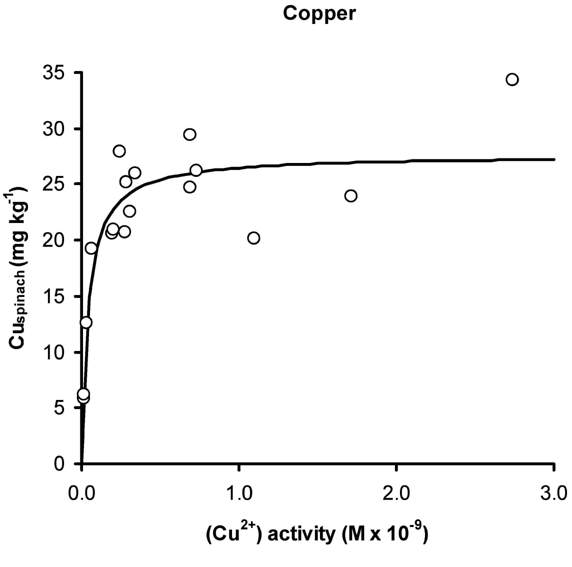

*Figure 4*. Uptake of Cu by spinach as a function of $Cu^{2+}$ activity in the soil pore water, calculated using the WHAM-VI speciation model. The fit of the FIAM is shown as a solid line; the model was implemented with a single root sorption site without proton competition. From Equation (5), $\log_{10}(K_t R_T) = 1.4$ and $\log_{10}(K_M) = 10.4$.

but especially for Cu, the need for a description which includes a limitation to uptake is apparent; there is an approach to an asymptote as ($M^{2+}$) increases. It is clearly inappropriate to assume a constant solution-to-plant transfer factor for trace metals in such soils.

### 3.5. RISK ASSESSMENT

Hazard quotients ($HQ_{gv}$), with respect to human health, associated with the consumption of green vegetables grown on the test soils are presented in Table V. The values of $HQ_{gv}$ varied from 0.49 to 8.43, 0.10 to 1.08 and 0.003 to 0.017 for Cd, Zn and Cu, respectively; thus only Cd represented a significant risk. For Cd, values of $HQ_{gv}$ were more than 1 for 14 out of 17 of the test soils. The implications of such values are aggravated by the fact that green vegetables constitute only a small portion of the human diet (e.g. 16% Bangladesh, Alam *et al.*, 2003) – although they may be a substantial source of Cd. In the case of Cu, values of $HQ_{gv}$ were considerably less than 1. Figure 5 shows values of $HQ_{gv}$ calculated for the test soils in three different ways: values of ($M^{2+}$) and $M_{spinach}$ were either, respectively, (i)

TABLE V

Single metal hazard quotients ($HQ_{gv}$) for human consumption of perpetual spinach grown on the test soils

| | Hazard quotient ($HQ_{gv}$) | | |
|---|---|---|---|
| Soil | Cd | Zn | Cu |
| 1 | 3.18 | 0.62 | 0.014 |
| 2 | 3.81 | 0.70 | 0.010 |
| 3 | 2.59 | 0.29 | 0.009 |
| 4 | 4.31 | 0.78 | 0.013 |
| 5 | 2.73 | 0.59 | 0.010 |
| 6 | 4.61 | 0.80 | 0.011 |
| 7 | 3.67 | 0.56 | 0.010 |
| 8 | 0.57 | 0.10 | 0.003 |
| 9 | 5.41 | 0.77 | 0.013 |
| 10 | 4.95 | 0.78 | 0.010 |
| 11 | 4.02 | 0.77 | 0.012 |
| 12 | 3.48 | 0.66 | 0.012 |
| 13 | 3.44 | 0.76 | 0.014 |
| 14 | 0.49 | 0.21 | 0.003 |
| 15 | 0.59 | 0.15 | 0.006 |
| 16 | 8.43 | 1.08 | 0.017 |
| 17 | 2.16 | 0.57 | 0.012 |

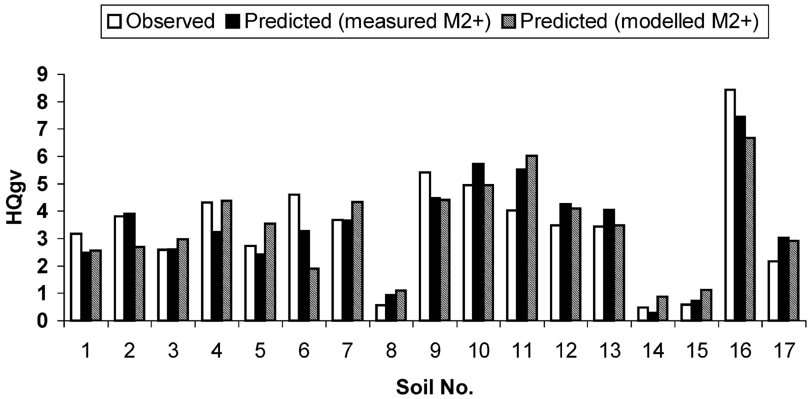

*Figure 5.* Comparison of observed and predicted hazard quotients for Cd in spinach; prediction was from the free ion activity model using values of ($Cd^{2+}$) either from speciation of the extracted soil pore water or using the generic solubility model (Equation 1).

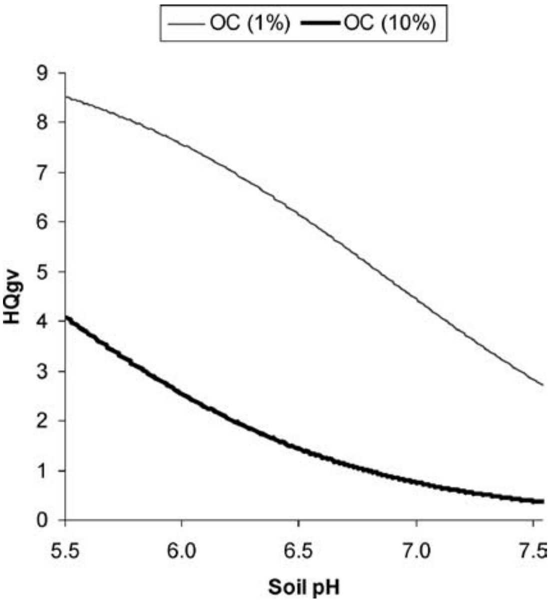

*Figure 6.* Relationship between soil pH and $HQ_{gv}$ for Cd in spinach leaves in soil having 1.11 mg $CaCl_2$-extractable Cd $kg^{-1}$ (corresponding to a total soil Cd content of 3.0 mg $kg^{-1}$) at 1% (thin line) and 10% soil organic carbon (OC) (thick line).

both measured, (ii) measured and modelled using the FIAM or (iii) both modelled using Equation (1) (predicting metal solubility) and the FIAM. There was reasonable agreement in all three cases which may partly justify the use of simple soil variables to estimate risk from food consumption.

Although in this data set there was apparently no advantage in considering proton competition for root adsorption sites, soil pH still plays the major role in determining ($M^{2+}$) in soil pore water (and hence plant uptake). The variation in estimated $HQ_{gv}$ with soil pH, at constant concentration of $CaCl_2$ extractable Cd and soil organic carbon is shown in Figure 6. Here values of $HQ_{gv}$ were predicted by integrating the solubility (Equation 1) and FIAMs – procedure (iii) in the preceding paragraph. The level of $CaCl_2$-extractable Cd used here was 1.11 mg $kg^{-1}$ which corresponds to the average extractability of Cd by $CaCl_2$ for these soils at the statutory limit of total soil Cd (3 mg $kg^{-1}$). In this illustration, the levels of organic matter used were 1 and 10%. Thus Figure 6 shows how $HQ_{gv}$ varies with reasonable ranges of soil pH and organic carbon content with total soil Cd set at the current statutory limit for sludge application. Such relationships may be of assistance in the management of metal-contaminated soils; the benefits of a large humus content and high pH value are clear.

## 4. Conclusions

Both methods of extraction of soil solution (*Rhizon* samplers and centrifugation) were effective in extracting soil pore water for metal speciation. *Rhizon* samplers may be preferred over centrifugation in view of their simplicity and the *in situ* nature of their placement. The simple solubility model used to predict ($M^{2+}$) in the soil pore water, as a function of extractable metal, pH and total soil organic carbon, was reasonably effective. However, this approach produced greater errors when predicting plant uptake compared to values of ($M^{2+}$) derived from speciation of *measured* pore water concentrations. Compared to Zn and Cu, predicted values of ($Cd^{2+}$) in solution were closer to observed values. Metal uptake data were reasonably well described by a very simple form of the FIAM. Thus predictions of hazard quotients were possible from accessible soil characteristics (pH, total metal content and %C) by integrating the solubility model and FIAM. The approach described in this study to assess the toxic level of metal may be useful in routine risk assessment of contaminated soils and in assessing management options for specific agricultural and horticultural sites.

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