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Hazard and risk assessment of human exposure to toxic metals using *in vitro* digestion assay

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ABSTRACT

Clean-up targets for toxic metals require that the site be "fit for purpose". This means that targets are set with respect to defined receptors that reflect intended land-use. In this study, the likely threat of human exposure to toxic metals has been evaluated by simulating the human digestion process *in vitro*. The effects of key attributes (i.e. sample fraction size, pH, K_d and total metal concentrations) on the bioavailability of Cu and Ni were also investigated. Total metal concentration was the key explanatory factor for Cu and Ni bioavailability. A comparative ranking of metal concentrations in the context of tolerable daily intakes for Cu and Ni confirmed that the pH has the greatest impact on metals bioavailability. Rapid screening of key attributes and total toxic metal doses can reveal the relative hazard imposed on human, and this approach should be considered when defining threshold values for human protection.

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Oral bioavailability; human exposure; *in vitro* digestion assay; hazard and risk assessment; copper and nickel; TDI

1. Introduction

In determining the suitability of contaminated sites for future use, it is important to ensure that they are "fit for purpose". This means that a quantitative assessment of the hazard and risk of metal pollutants is placed in the context of the future use and receptors for that site. The potential effects of metal contaminants on human health and biota have been widely discussed through the interpretation of empirical data.[1–3] Copper (Cu) and nickel (Ni), considered in this study, are essential elements with nutritional and biochemical functions.[3,4] In excess, however, they cause adverse effects.[5] Many countries have defined guidance values based on the total metal content of soil that are regarded as protective for identified receptors. However, when considering metal impact on numerous receptors through several exposure pathways, a measure of the total metal content may be inadequate.[6,7] Selective extractions that simulate receptor's exposure to soil metal provide a more realistic approach and consequently a better estimate of human exposure to hazard.[7] Environmental hazard is often described in terms of metal mobility and bioavailability in soils. The mobility of metals is used to explain metal partitioning between soil solids and solution.[8] The terms oral bioavailability, on the other hand, and human ingestion have been used synonymously for human exposure to metals. The human bioavailable concentration is the fraction of an ingested substance that is soluble in the gastrointestinal environment and available for absorption into the blood.[7] To quantify this, *in vitro* digestion assays have been widely established.[7,8] Such assays empirically quantify the bioavailable fraction of metal contaminants by simulating the oral exposure pathway for children (<6 years old) in relation to their physiology. Those children are prone to soil ingestion while playing outdoors. Children ingest soil both deliberately (by ingesting soil) and involuntarily (by putting dirty hands and objects in their mouths).[9,10] Models for the protection of human health ensure that the total assimilated dose of a given metal does not exceed the tolerable daily intake (TDI). The TDI is an estimation of the mass of the chemical that can be ingested daily throughout a human lifetime without adverse health effects.[4,5] The TDI values are derived from various mammalian assays where a soluble salt of a metal is administered to a test animal.[1,5] Consequently, reported TDI values differ and do not reflect the hazard posed by an involuntary intake of metals from a matrix such as soil or from dietary assimilation.[1]

Soil physio-chemical properties such as, texture, pH or organic matters content vary. Such properties affect partitioning and biological availability of metals but their inclusion in bioavailability studies is limited.[11–16] Furthermore, the bioavailability is acknowledged to vary with the particle size fraction of the sample presented to the assay.[11,12] A variety of particle size fractions,

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ranging from <45 µm to <2000 µm have been studied. [17] The size fraction adopted for use in the *in vitro* digestion assay is generally <250 µm, because this represents the fraction most likely to adhere to children's fingers and thus ingested.[18] Previous studies have considered the influence of particle size fraction (<50, <125, <2000 µm, and 2 mm) on bioavailability but the results have been inconclusive because of limited consideration of the intrinsic properties of the soil under investigation. [19–23]

The objectives of this study were to (1) understand the likely threat of human exposure to toxic metals by simulating the human digestion process *in vitro*; (2) to evaluate the effects of particle size fractionation on the bioavailability of Cu and Ni; (3) to quantitatively assess the significance of intrinsic soil properties in determining Cu and Ni bioavailability in selected laboratory conditioned and field contaminated soils; (4) and to relate measured bioavailable concentrations to the tolerable daily intake (TDI) of Cu and Ni.

2. Materials and methods

2.1. Samples preparation

Four coarse-textured soils (Brechin, Culbin, Insch and St. Fergus) were sequentially conditioned with amendments of Cu and Ni. A description of the soil collection, preparation, metal amendments and conditioning were detailed by Maderova et al. [13]. In brief, the soils were initially incubated for 16 weeks after amendment with a range of Cu and Ni sulphate amendments to provide total metal concentrations of 50, 250, 1000 and 2500 mg/kg for Cu, and 25, 250, 1000 and 2500 mg/kg for Ni. Soils historically contaminated with Cu (Cu Amended Sludge Site [CASS]) and Ni and Cu (Smelter Site [SS]) were also used.

Soil samples were sieved to derive two particle size fractions: a coarse (<2 mm) and a fine (<180 µm) fraction. Soil pH was measured in reverse osmosis (RO).H_2O using standard techniques at a soil to solution ratio of 1:2.5 and pH meter (HANNA Instruments H19812). Partitioning coefficients (K_d) were calculated as described by Sauvé et al. [24] using soil solution extracted using Rhizon samplers.[13] The total metal concentrations of the two soil particle size fractions were determined after microwave-assisted total metal digestion as described by US EPA 3051 Method.[25] Samples were analysed for Cu and Ni using flame atomic absorption spectrophotometry (FAAS-Perkin Elmer Analyst 100 Spectrometer) at λ 324.8 and 233 nm for Cu and Ni respectively. A certified reference material CRM GBW 07406-soil was used.

2.2. The in vitro digestion assay

The bioavailable fractions of Cu and Ni were determined using the *in vitro* gastrointestinal digestion described by Oomen et al. [21]. In brief, 6 ml of synthetic saliva (pH 6.5 ± 0.2) was added to 0.6 g of soil (dry weight basis) and mixed using an end-over-end shaker for 5 min at 55 rpm. Synthetic gastric juice (10.5 ml, pH 1.1 ± 0.1) was added and the mixture mixed for a further 2 h. Twenty-four ml of synthetic duodenal juice (pH 7.8 ± 0.2) and 6 ml of chicken bile (pH 8.0 ± 0.2) were added and the mixture rotated using an end-over-end shaker for another 2 h. The mixing process was carried out at 37 ± 2 °C. All reagents were pre-incubated at 37 ± 2 °C. The digested samples were centrifuged for 10 min at 1730 G and 37 ± 2 °C. The *chyme* (the supernatant after centrifugation) was transferred into fresh sterile 50 ml centrifuge tube, and 0.2 ml concentrated HNO_3 was added. Samples were chemically analysed for Cu and Ni using FAAS (previous section). The pH values of the synthetic gastrointestinal juices were adjusted to 6.5 using concentrated HCl and NaOH, and were measured using a portable pH meter (HANNA Instruments H19812). Chemicals and reagents were purchased from Sigma (St. Louis, MO, USA), BDH Chemicals Ltd. (Poole, UK), and Fisher Scientific UK Ltd. (Loughborough, Leicestershire). The assay was conducted using three independent replicates and appropriate reagent and sample controls were adopted.

2.3. Data analysis

Normality testing and equal variances were carried out. Correlation tests (Pearson product-moment or Spearman rank-order) and regression were used to relate measured bioavailability to intrinsic soil properties. Significance testing was performed with ANOVA. Significance testing and least significant differences (LSD) were calculated at $p \leq 0.05$ value. All statistical analysis was performed using Minitab 15 for Windows.

3. Results and discussion

3.1. Influence of total metal concentration on bioavailability

Total Cu and Ni concentrations had a significant influence ($p < 0.05$) on the measured bioavailable fractions of Cu and Ni for the amended soils (Tables 3 and 4) and the CASS soils (Table 5). There was a significant positive correlation between the total Cu concentrations (mg/kg) and Cu bioavailability (mg/kg) for soils amended with Cu, ($r = 0.99$, $r = 0.99$, $r_s = 0.87$, and $r_s = 0.97$, $p < 0.01$), and CASS soils ($r = 0.821$, $p < 0.01$). Similarly, Ni bioavailability correlated with total Ni concentrations ($r_s = 0.96$, 0.97, 0.92 and $r = 0.99$, $p < 0.05$) for Brechin, Culbin, Insch and St. Fergus soils (Table 4). These findings were evident both for soils sieved to <2 mm and <180 µm particle size fractions (Tables 3–5). Cu and Ni gastrointestinal bioavailability also increased with increasing total metal concentrations. These findings were in agreement with results reported by other authors.[26,27]

There were no correlations between the bioavailability of Cu and Ni and the total Cu and Ni concentrations of SS soils ($p = 0.914$ and 0.602 for Cu, 0.122 and 0.783 for Ni; Table 6). These soils had particularly elevated concentrations of both Ni and Cu and as such the sportive capacity of the matrix was very different from the other soils used in this study; both those amended and the CASS soils (Table 5).

3.2. Influence of particle size fraction on bioavailability

Soils with the highest bioavailable concentration of Cu followed the order: St. Fergus > Brechin > Insch > Cul bin for soils sieved to <180 µm (Table 3). Nickel bioavailability varied with total Ni concentrations among the four soils amended with Ni regardless of the particle size fraction (Table 4). The maximum bioavailability concentration of Ni for soils sieved to <180 µm had the same order as Cu bioavailability (St. Fergus > Brechin > Insch > Culbin; Table 4).

Copper was significantly ($p < 0.05$) more bioavailable in soils sieved to <180 µm than soils sieved to <2 mm (Tables 3, 5 and 6). The finer particle size fraction contained a higher metal concentration because silt and sand size fractions sorb cations more effectively than the sand fraction. Analysis of the bulk density of the fractions confirmed that there was no significant difference between the soil types or the fractions (data not shown) as Meunier et al. [28]. Many researchers have used soil particle size fraction of less than 250 µm to study the bioavailability of metal contamination.[7,19,27,29–31] The selection of such a particle size fraction was because it is most likely to be the fraction that adheres to fingers of children exposed to soils. Ni bioavailability was not significantly different ($p = 0.26$) between soils sieved to <2 mm and <180 µm particle size fractions (Tables 4 and 6). A similar finding was previously reported by Morman et al. [31].

3.3. Intrinsic soil properties

Standard physiochemical properties of soils amended with Cu and Ni are summarised in Table 1. The pH values ranged from 4.2 to 6.3 for Cu and Ni amended soils (Table 1) and from 5.2 to 6.0 for CASS soil (Table 2). The binding effectiveness (as defined by K_d value) of the soil varied with soil texture and pH (Tables 1). Copper was more strongly sorbed by the amended soils than Ni. This result was in agreement with results reported for Cu and Ni sorption by Covelo et al. [32]. A significant decrease in the pH values with increasing Cu amendment was measured in Culbin and St. Fergus soils (Table 1). The effect of Ni amendments on soil pH was less pronounced (Table 1). In a similar investigation, Oorts et al. [10] reported a decrease in soil pH value of up to 0.75 units. This pH change is a consequence of the metal salt amendment [13]. Furthermore the predominantly sandy texture of these soils would offer little alkalinity to buffer against this decline in the pH value.

The range of the calculated K_d values for Cu and Ni (Tables 1) was comparable to the field-based partitioning

Table 1. Physiochemical properties of soils amended with copper and nickel.

	[a]pH				[b]K_d			
	Brechin	Culbin	Insch	St. Fergus	Brechin	Culbin	Insch	St. Fergus
Cu amendment dose (mg/kg)								
0	6.0 ± 0.1	4.8 ± 0.1	4.8 ± 0.0	6.1 ± 0.0	454.1 ± 118.8	1103.2 ± 251.0	202.6 ± 15.7	137.0 ± 68.1
50	6.0 ± 0.0	4.9 ± 0.0	4.8 ± 0.0	5.8 ± 0.0	2679.1 ± 187.0	852.7 ± 176.0	4134.6 ± 643.0	1061.8 ± 549.0
250	5.9 ± 0.0	4.6 ± 0.1	4.6 ± 0.1	5.9 ± 0.1	3219.9 ± 70.2	254.0 ± 40.6	820.9 ± 23.7	1288.9 ± 219.0
1000	5.8 ± 0.0	4.7 ± 0.0	4.3 ± 0.0	5.6 ± 0.0	486.9 ± 30.4	168.9 ± 21.2	76.6 ± 3.0	1788.7 ± 230.0
2500	5.0 ± 0.0	4.8 ± 0.1	4.2 ± 0.0	5.5 ± 0.1	63.3 ± 26.0	131.7 ± 15.5	32.7 ± 2.6	916.2 ± 345.8
Ni amendment dose (mg/kg)								
0	6.3 ± 0.1	5.1 ± 0.1	4.8 ± 0.0	6.2 ± 0.0	532.4 ± 242.4	136.3 ± 51.0	1565.9 ± 730.0	695.8 ± 488.4
25	6.2 ± 0.0	5.1 ± 0.1	4.8 ± 0.0	6.2 ± 0.0	796.1 ± 106.8	53.2 ± 7.9	170.1 ± 49.6	720.2 ± 592.8
250	6.2 ± 0.0	4.7 ± 0.1	4.6 ± 0.1	4.9 ± 0.5	354.7 ± 23.1	18.1 ± 1.1	16.9 ± 1.1	341.3 ± 10.3
1000	5.7 ± 0.1	4.5 ± 0.0	4.3 ± 0.0	4.7 ± 0.3	77.6 ± 6.9	8.7 ± 1.6	8.1 ± 0.4	88.4 ± 8.0
2500	5.1 ± 0.1	4.9 ± 0.1	4.2 ± 0.0	5.5 ± 0.2	15.8 ± 3.4	13.8 ± 1.6	7.2 ± 1.4	173.2 ± 29.6

[a]The pH values of soils amended with Cu and Ni were significantly different; LSD was not calculated as the non-parametric (Kruskal–Wallis) test was used.
[b]There were significant differences between the K_d values of soils amended with Cu and Ni, LSD at ($p = 0.05$) was 0.2.

Table 2. Physiochemical properties of CASS soils.

Field no.	[a]pH	[b]K_d
5	6.0 ± 0.1	548.6 ± 34.4
12	5.2 ± 0.0	1278.4 ± 147.5
14	5.8 ± 0.1	1017.7 ± 149.2
16	5.7 ± 0.0	1067.2 ± 242.2
18	5.6 ± 0.3	1685.4 ± 228.4

[a]The pH values of the five field soils were significantly different, LSD at ($p = 0.05$) was 0.2.
[b]The K_d values of the five field soils were significantly different, LSD at ($p = 0.05$) was 248.9.

Table 3. Total copper concentrations, bioavailability and percentages of bioavailability for soils sieved to <2 mm and <180 µm particle size fractions.

[a]Cu amendment dose (mg/kg)	[b]Total Cu concentrations (mg/kg)				[c]Bioavailable Cu concentrations (mg/kg)				% Bioavailable Cu concentrations			
	Brechin	Culbin	Insch	St. Fergus	Brechin	Culbin	Insch	St. Fergus	Brechin	Culbin	Insch	St. Fergus
<2 mm particle size fraction												
0	11.0 ± 1.8	72.8 ± 13.5	13.5 ± 1.0	7.2 ± 1.3	7.8 ± 0.0	21.4 ± 0.9	4.7 ± 0.0	0.8 ± 0.0	70.5 ± 0.0	29.4 ± 1.3	34.4 ± 0.0	10.8 ± 0.0
50	85.2 ± 2.2	57.3 ± 9.5	141.5 ± 5.6	56.7 ± 7.5	42.9 ± 0.3	19.1 ± 0.9	47.3 ± 0.4	21.9 ± 0.2	50.4 ± 0.3	33.3 ± 1.6	33.4 ± 0.3	38.8 ± 0.5
250	285.5 ± 7.8	136.3 ± 3.8	259.2 ± 7.2	270.2 ± 9.3	159.9 ± 2.2	68.5 ± 6.6	132.3 ± 2.2	49.9 ± 1.8	56.0 ± 0.8	50.2 ± 4.8	51.0 ± 0.8	18.5 ± 0.7
1000	1209.3 ± 182.0	264.8 ± 6.6	1054.5 ± 25.0	1121.5 ± 79.7	620.0 ± 4.8	149.3 ± 8.3	480.2 ± 7.7	449.8 ± 4.5	51.3 ± 0.4	56.4 ± 3.1	45.5 ± 0.7	40.1 ± 0.4
2500	2521.7 ± 99.9	313.7 ± 13.2	2269.0 ± 51.5	1923.3 ± 395.1	1392.4 ± 17.8	151.6 ± 11.0	883.8 ± 8.7	930.3 ± 51.0	55.2 ± 0.7	48.3 ± 3.6	39.0 ± 0.4	48.4 ± 2.7
<180 µm particle size fraction												
0	22.8 ± 1.3	189.2 ± 2.6	24.8 ± 2.7	24.3 ± 0.8	12.4 ± 0.4	61.0 ± 1.8	9.6 ± 0.3	10.2 ± 0.2	54.3 ± 1.9	32.2 ± 0.9	38.5 ± 1.0	42.1 ± 0.7
50	95.7 ± 2.9	97.2 ± 9.9	273.0 ± 1.3	412.0 ± 4.5	69.2 ± 0.7	96.0 ± 7.8	47.0 ± 0.3	55.9 ± 0.7	72.4 ± 0.7	98.8 ± 8.0	17.2 ± 0.1	13.6 ± 0.2
250	366.5 ± 6.8	331.5 ± 5.2	359.5 ± 10.7	1667.0 ± 48.0	260.7 ± 1.4	222.8 ± 0.3	163.3 ± 0.7	284.8 ± 9.3	71.1 ± 0.4	67.2 ± 0.1	45.4 ± 0.2	17.1 ± 0.6
1000	1400.2 ± 33.6	873.2 ± 28.0	1385.3 ± 32.6	7356.7 ± 282.0	855.3 ± 1.9	427.0 ± 3.1	619.5 ± 1.4	2326.8 ± 12.0	61.1 ± 0.1	48.9 ± 0.3	44.7 ± 0.1	31.6 ± 0.2
2500	3353.7 ± 65.6	891.2 ± 38.0	3076.3 ± 105.0	10983.3 ± 303	1958.9 ± 2.0	484.1 ± 16.2	1338.2 ± 3.0	3587.1 ± 8.0	58.4 ± 0.1	54.3 ± 1.8	43.5 ± 0.1	32.7 ± 0.1

[a]Using different doses of Cu to amend the soils had a significant effect ($p < 0.05$) on the total Cu concentrations pos-amendments. In addition, different Cu doses significantly influenced Cu bioavailability of Brechin, Culbin, Insch and St. Fergus soils.

[b]Total Cu concentrations significantly differed ($p < 0.05$) between Brechin, Culbin, Insch and St. Fergus soils (the LSD was 0.5 mg/kg for < 2 mm particle size fraction, and 0.9 mg/kg for < 180 µm particle size fraction).

[c]The bioavailable Cu concentrations were significantly different ($p < 0.01$) between Brechin, Culbin, Insch and St. Fergus soils, the LSD was not calculated as the non-parametric test (Kruskal–Wallis) was used for the two particle size fractions.

Table 4. Total nickel concentrations, bioavailability and percentages of bioavailability for soils sieved to <2 mm and <180 µm particle size fractions.

[a]Ni amendment dose (mg/kg)	[b]Total Ni concentrations (mg/kg)				[c]Bioavailable Ni concentrations (mg/kg)				% Bioavailable Ni concentrations			
	Brechin	Culbin	Insch	St. Fergus	Brechin	Culbin	Insch	St. Fergus	Brechin	Culbin	Insch	St. Fergus
<2 mm particle size fraction												
0	23.3 ± 4.4	5.0 ± 0.0	31.7 ± 9.3	11.7 ± 1.7	19.6 ± 0.3	8.3 ± 0.5	5.9 ± 0.7	10.6 ± 1.3	84.2 ± 1.1	83.2 ± 4.7	18.7 ± 0.2	91.0 ± 11.3
25	66.7 ± 9.3	25.0 ± 2.9	106.7 ± 29.5	31.7 ± 6.0	19.8 ± 1.1	19.9 ± 0.4	26.9 ± 1.1	24.6 ± 4.1	29.7 ± 1.7	79.9 ± 1.5	25.3 ± 1.0	77.8 ± 12.9
250	295.0 ± 18.0	76.7 ± 3.3	200.0 ± 10.4	290.0 ± 21.8	122.6 ± 1.8	60.8 ± 6.5	88.0 ± 5.7	168.8 ± 0.6	41.6 ± 0.6	79.3 ± 8.4	44.0 ± 2.8	58.2 ± 0.2
1000	891.7 ± 29.2	100.0 ± 2.9	491.7 ± 17.6	658.3 ± 13.6	440.1 ± 2.8	97.7 ± 4.5	247.4 ± 9.6	428.4 ± 9.7	49.4 ± 0.3	97.7 ± 4.5	50.3 ± 1.9	65.1 ± 1.5
2500	1770.0 ± 121.9	150.0 ± 12.6	866.7 ± 14.8	1108.3 ± 119.0	837.8 ± 5.4	139.8 ± 1.4	442.7 ± 6.3	544.3 ± 2.8	47.3 ± 0.3	93.2 ± 9.1	51.1 ± 0.7	49.1 ± 2.5
<180 µm particle size fraction												
0	18.3 ± 1.7	21.7 ± 1.0	30.2 ± 8.2	55.2 ± 3.4	14.0 ± 0.0	9.3 ± 0.4	6.2 ± 0.0	6.2 ± 0.0	76.1 ± 0.0	42.9 ± 2.1	20.6 ± 0.0	11.2 ± 0.1
25	57.8 ± 6.8	58.2 ± 6.1	104.3 ± 14.4	273.2 ± 9.4	22.2 ± 0.3	29.5 ± 0.8	21.7 ± 0.8	66.1 ± 1.4	38.4 ± 0.5	50.6 ± 1.3	20.8 ± 0.7	24.2 ± 0.5
250	391.3 ± 1.7	210.0 ± 11.9	274.7 ± 11.9	1761.8 ± 41.0	210.3 ± 0.7	110.6 ± 0.7	98.7 ± 1.0	523.4 ± 0.7	53.7 ± 0.2	52.7 ± 0.3	35.9 ± 0.4	29.7 ± 0.0
1000	1318.8 ± 25.1	294.7 ± 15.0	675.2 ± 20.4	5901.7 ± 35.0	658.8 ± 0.9	184.7 ± 4.7	295.8 ± 1.4	1717.9 ± 1.0	50.0 ± 0.1	62.7 ± 1.6	43.8 ± 0.2	29.1 ± 0.2
2500	2285.0 ± 152.9	274.3 ± 12.0	1212.8 ± 36.5	7626.7 ± 40.0	1168.7 ± 3.2	226.3 ± 8.5	503.8 ± 5.0	2521.3 ± 1.0	51.2 ± 0.1	82.5 ± 3.1	41.5 ± 0.4	33.1 ± 0.2

[a]Using different doses of Ni to amend the soils had a significant effect ($p < 0.05$) on the total and the bioavailable Ni concentrations of Brechin, Culbin, Insch and St. Fergus soils sieved to the two particle size fractions.
[b]Total Ni concentrations significantly differed ($p < 0.05$) between Brechin, Culbin, Insch and St. Fergus soils, the LSD was not calculated as the non-parametric test (Kruskal–Wallis) was used for the two particle size fractions.
[c]The bioavailable Ni concentrations were significantly different ($p < 0.05$) between Brechin, Culbin, Insch and St. Fergus soils, the LSD was not calculated as the non-parametric test (Kruskal–Wallis) was used.

Table 5. Total copper concentrations, bioavailability and percentages of bioavailability for CASS soils sieved to <2 mm and <180 µm particle size fractions.

Field no	[a]Total Cu concentrations (mg/kg)	[b]Bioavailable Cu concentrations (mg/kg)	% Bioavailable Cu concentrations
<2 mm particle size fraction			
5	69.0 ± 0.3	33.3 ± 0.8	48.3 ± 1.0
12	102.0 ± 13.3	40.6 ± 1.6	40.7 ± 3.5
14	100.8 ± 9.3	43.4 ± 0.4	43.8 ± 4.2
16	55.3 ± 2.6	30.7 ± 1.4	55.7 ± 2.5
18	109.7 ± 7.8	59.9 ± 0.3	55.3 ± 4.4
<180 µm particle size fraction			
5	267.7 ± 43.3	39.9 ± 0.1	14.9 ± 0.1
12	144.4 ± 3.6	61.3 ± 2.1	42.5 ± 1.5
14	159.3 ± 16.4	58.6 ± 1.2	36.8 ± 0.8
16	84.2 ± 3.9	40.4 ± 0.1	48.0 ± 0.1
18	121.8 ± 4.8	72.9 ± 0.8	59.8 ± 0.7

[a]Total Cu concentrations significantly differed ($p < 0.05$) between CASS soils (the LSD was 11.5 mg/kg for <2 mm particle size fraction, and 0.9 mg/kg for <180 µm particle size fraction).
[b]There was significant difference ($p < 0.05$) in Cu bioavailability between the two particle size fractions (the LSD was 1.5 mg/kg for <2 mm particle size fraction, and it was not calculated for <180 µm particle size fraction as the non-parametric test Kruskal–Wallis was used).

Table 6. Total copper concentrations, bioavailability and percentages of bioavailability for SS soils sieved to <2 mm and <180 µm particle size fractions.

Field no	Element	[1]Total concentrations (mg/kg)	[2]Bioavailable concentrations (mg/kg)	% Bioavailable concentration
<2 mm particle size fraction				
1	Cu	27299 ± 6594	13292 ± 321	48.69 ± 1.2
2	Cu	39831 ± 1675	14888 ± 502	37.38 ± 1.3
1	Ni	3619 ± 830	1663 ± 97	45.95 ± 2.7
2	Ni	10533 ± 2861	318 ± 13	3.03 ± 0.1
<180 µm particle size fraction				
1	Cu	34881.44 ± 359.1	14093.38 ± 412.8	40.40 ± 1.18
2	Cu	25852.42 ± 244.8	13698.13 ± 437.8	52.99 ± 1.69
1	Ni	5363.74 ± 184.6	1969.79 ± 56.9	36.72 ± 1.06
2	Ni	1696.05 ± 35.2	265.15 ± 11.4	15.63 ± 0.67

[1]Total Cu and Ni concentrations significantly differed ($p < 0.05$) between SS soils (the LSD was not calculated as the non-parametric test Kruskal–Wallis was used.
[2]There was significant difference ($p < 0.05$) in Cu and Ni bioavailability between the two particle size fractions (the LSD was not calculated as the non-parametric test Kruskal–Wallis was used).

data calculated by Janssen et al. [33], and Sauvé et al. [24]. The results confirmed that the K_d values decreased with increasing dose of amended Cu and Ni (Tables 1) until a critical threshold value was reached. This was because the greater the proportion of the metal remaining in the aqueous phase, the lower the calculated K_d value.[33,34] A key limitation in the use of the K_d value for characterising soil is that there is not a linear response but that it varies according to the metal burden imposed upon the soil. While an estimation of the mid-range K_d value can be predicted, the K_d values at extreme (low or high) metal loadings do not follow such a trend.[9]

3.4. Influence of soil pH and partitioning coefficients (K_d) on bioavailability

Copper and Ni bioavailability concentrations correlated with soil pH ($r_s = -0.92, -0.94, 0.96, -0.88$, $p < 0.05$ for soils amended with Cu, $r_s = -0.84, -0.93, -0.74, -0.95$, $p < 0.01$ for soils amended with Ni and $r_s = -0.84$, $p < 0.05$ for CASS soil). These results were for soils sieved to <2 mm and <180 µm particle size fractions (Tables 3–5). The greatest bioavailable concentrations of Cu and Ni were associated with those soils with the lowest pH values. Metals became less sorbed and bioavailable as the pH value increased.[1] The metal bioavailability in the stomach phase of the extraction was higher than in the intestinal phase on account of the lower pH value of the gastric juice. The higher pH value in the intestine resulted in the re-adsorption and precipitation of metals after dissolution into the stomach.[7] There was no significant correlation between the pH values and Cu and Ni bioavailability of SS soils ($p = 0.94$ and 0.72 for Cu, 0.94 and 0.63 for Ni; Table 6).

Similar to the response to the pH, there was a correlation between Cu and Ni gastrointestinal bioavailability (mg/kg) and the partitioning coefficients (K_d) values of Culbin, Brechin and Insch soils ($r_s = -0.83, -0.75, -0.98$, $p < 0.05$ for Cu amended soils, $r = -0.92$, $r_s = -0.89, -0.93$, $p < 0.05$ for Ni amended soils and $r = -0.67$, $p < 0.05$ for CASS soil). This was not observed for St. Fergus soils as there was no correlation between the K_d values of St. Fergus soils and Cu and Ni bioavailability ($r_s = -0.45$ and $p = 0.17$ and $r_s = -0.36$ and $p = 0.19$ for St. Fergus soils amended with Cu and Ni respectively; Tables 3 and 4). The higher pH values of St. Fergus soils and the commensurate increase in the K_d values confirmed this observation. This finding

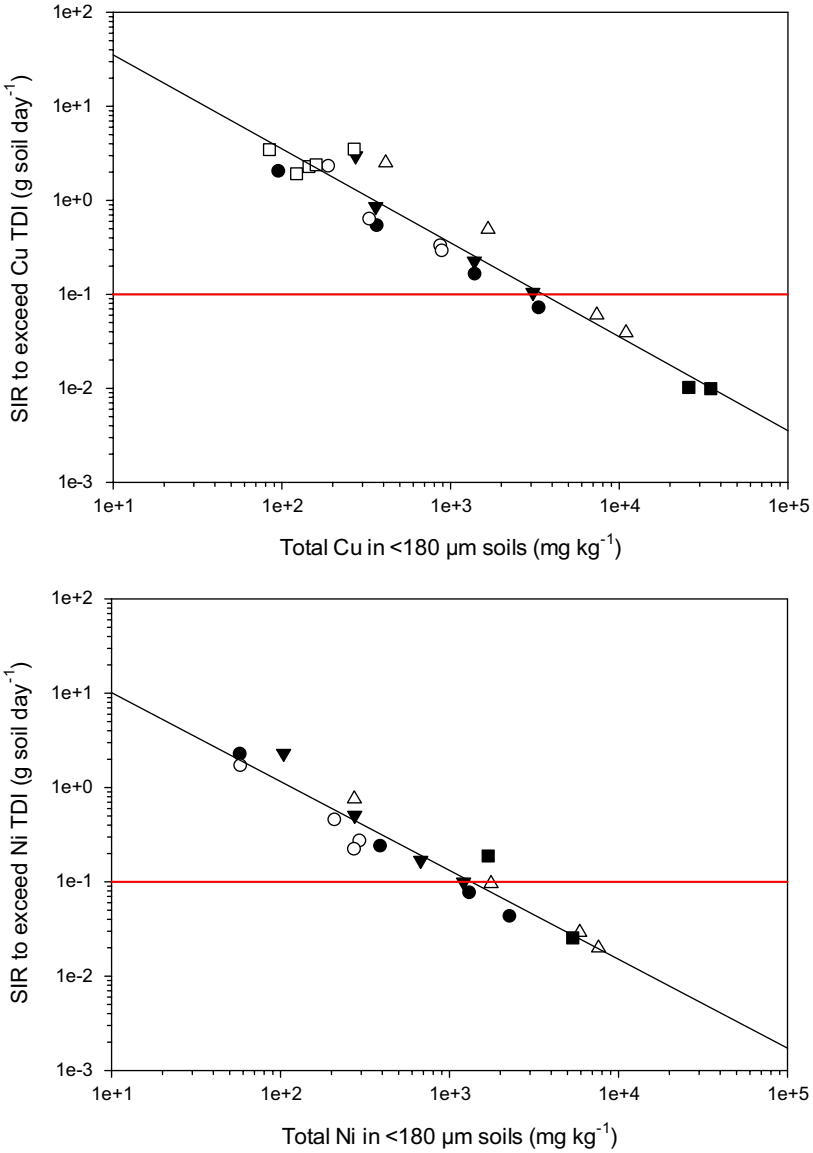

Figure 1. Hazard associated with an involuntary ingestion of Cu and Ni soils Brechin (●), Culbin (○), Insch (▲), St Fergus (Δ), SS soils (■) and CASS soils (□). Data represent a mean value based on three replicate soils. The SIR threshold (—) signifies a SIR of 0.1 g soil day^{-1} associated with an involuntary ingestion of soil by children.

was in agreement with results reported by Buchter et al. [35], who acknowledge that soil pH was the most important soil property that affected K_d. The results in this study revealed that while the K_d is a key driver in the consideration of ecological soil aspects and water protection, it also seems to have a value when assessing bioavailability quantification.

3.5. Hazard characterization

The ultimate aim of bioaccessibility testing is to identify a hazard posed to human health, especially for young children, who may come into contact with contaminated soil through play and are prone to hand-to-mouth transfer of soil and its ingestion.[36,37] An estimate of the relative hazard posed by the contaminated soils can be obtained from the rate of ingestion of a particular contaminated soil that would exceed a metal specific TDI value.[37] The hazard posed by the contaminated soils assessed in this study is provided in Figures 1 and 2. Based on the soil ingestion rate (SIR) for involuntary intake (0.1 g d^{-1}) of <180 µm soil fractions [1,19,36] the data presented confirm that most of the contaminated soils do not pose hazard to human health (Figure 1). The greatest exposure was associated with the Cu contamination of the SS soils, where as little as 0.01 g of soil would exceed the Cu TDI. Nickel posed a hazard in the SS soils, followed by conditioned St. Fergus and Brechin soils. In contrast, when considering a deliberate (1 g d^{-1}) intake of Ni contaminated soils, only Ni contamination associated with the conditioned soils did not pose hazard to human health (Figure 2), which is comparable to hazard highlighted by Ni guidance value of 180 mg kg^{-1}.[38] The Cu contamination of soils under the same scenario was of a lesser concern, but indicated a higher exposure than hazard based on Cu guidance value.

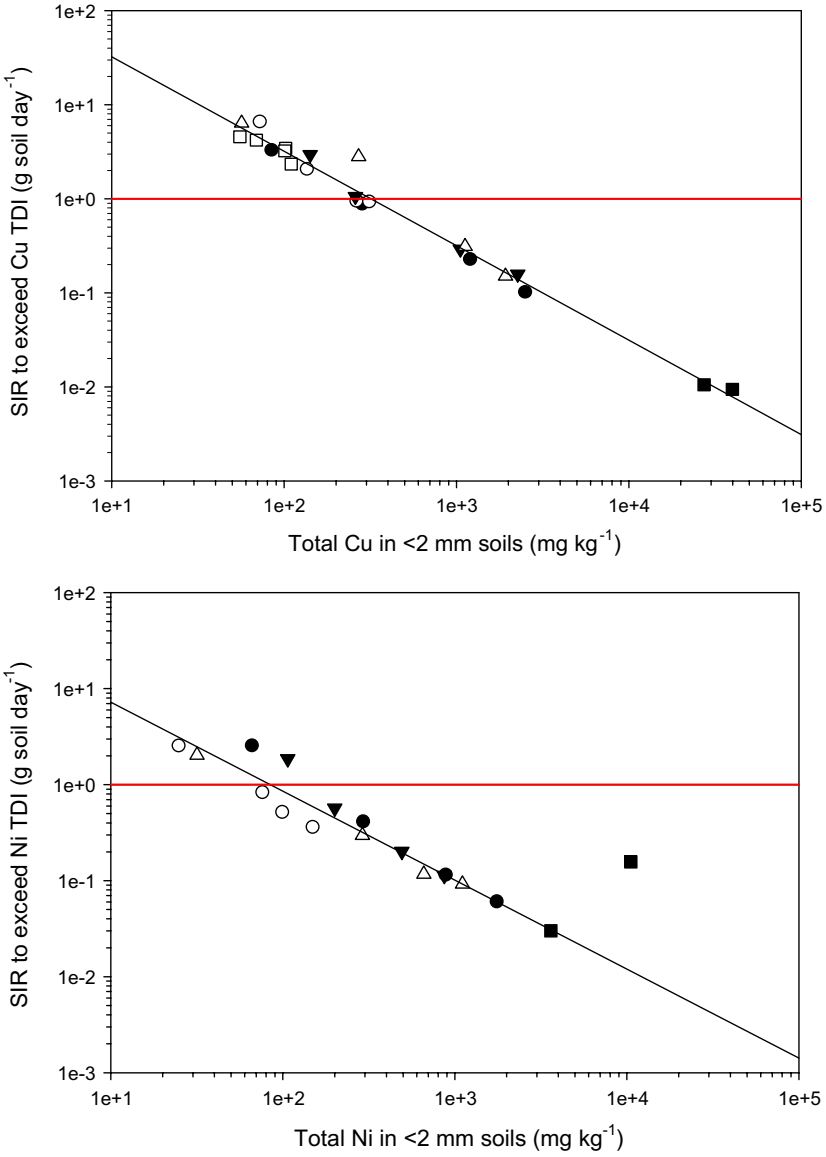

Figure 2. Hazard associated with a deliberate ingestion of Cu and Ni soils Brechin (●), Culbin (○), Insch (▲), St Fergus (Δ), SS soils (■) and CASS soils (□). Data represent a mean value based on three replicate soils. The SIR threshold (—) signifies a SIR of 1 g soil day^{-1} associated with a deliberate ingestion of soil by children.

4. Conclusion

Complexity and heterogeneity of soils affect metal bioavailability and mobility. This study substantiated bioavailability and mobility of Cu and Ni in laboratory conditioned and field contaminated soils. Cu and Ni bioavailability was negatively correlated with soil pH and the K_d values (i.e. a high pH = high K_d = low bioavailability). These findings confirmed that the low pH value of the simulated *in vitro* digestion assay allowed more metal mobility from the soil matrix, which increased metal bioavailability in the human gastrointestinal tract. The total contaminant background concentration had a key role on the human bioavailable fraction as it was positively correlated with the concentration of the bioavailable fraction. The assessment of exposure based on two particle size fractions reflecting a deliberate (<2 mm) and involuntary (<180 µm) ingestion of soil indicated higher concentrations of Cu and Ni in finer soil particles (<180 µm). However, the bioavailability values of both metals in both particle fractions were determined by the number of high quality binding sites. Further, a simplified hazard assessment based on the SIR values also emphasized the importance of receptors behavior. Although Cu and Ni were classified as non-mutagens, their bioavailable concentrations reported in this study significantly exceeded the TDI values.

The optimised *in vitro* digestion assay was able to offer a better insight for the effect of the ingested soil contaminant on oral bioavailability. Furthermore, it enabled the development of a more accurate estimate of contaminant bioavailability for the use in human health hazard assessment in a rapid and cost effective manner. While this study demonstrates the usefulness of bioavailability consideration in human health risk assessment, the assessment of exposure based on oral bioavailability needs to be further expanded to a wider range of metals, source of contamination and soils.

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