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Towards an exposure narrative for metals and arsenic in historically contaminated Ni refinery soils: Relationships between speciation, bioavailability, and bioaccessibility

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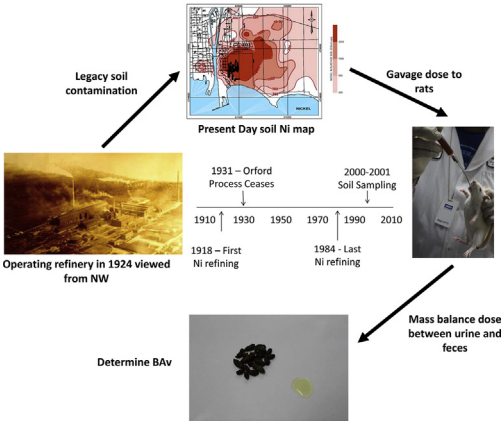
HIGHLIGHTS

- Soil Ni, Cu, and Co are present in solid solution within four Ni mineral species.
- ABA (absolute bioavailability) of Ni from uncontaminated food or $NiSO_4$ was roughly 2%.
- Soil Ni, Co, and As bioaccessibility/bioavailability relations were developed.
- For Ni, these relationships were: ABA = 0.012(BAc)-0.05 and RBA (relative bioaccessibility) = 0.554(BAc)-2.28

GRAPHICAL ABSTRACT



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ABSTRACT

Archived soils contaminated with Ni, Cu, Co, and As from legacy operations of a nickel refinery at Port Colborne, Ontario, Canada were speciated using mineral liberation analysis. Four Ni minerals were identified as fingerprint compounds of the historical refinery emissions. Cu and Co were present in solid solution in these minerals due to their presence in the refinery's feed. The highest concentrations of Ni, Cu, Co, and As in these soils were 18,553, 1915, 196, and 79 mg/kg, respectively, these elevated contaminant concentrations attesting to the importance of incidental soil ingestion to the oral exposure pathway in Port Colborne. The in vitro gastric bioaccessibility (BAc) was determined for these contaminants, as was in vivo oral bioavailability (BAv), using a mass balance approach

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in male Sprague-Dawley rats. In spite of the elevated soil concentrations of Cu, the BAv of this physiologically important metal could not be distinguished from that in commercial rat chow, suggesting low potential for exposure. Co and As also had low apparent BAv (<2%). For Ni, baseline oral BAv of naturally sourced dietary Ni was found to be approximately 2%, as was the oral BAv of Ni from nickel sulfate hexahydrate. The mass balances of $NiSO_4 \cdot 6H_2O$ were fully accounted-for in urine and feces after a single gavage dose, indicating little to no organ incorporation from this highly soluble salt. Therefore, the urinary estimates of Ni BAv for these soils were assumed to represent true BAv despite variable fecal recoveries. The high Ni concentrations enabled BAc-BAv relationships to be developed for these contaminated soils. For absolute bioavailability (ABA) and relative bioavailability (RBA) the relationships were: ABA = 0.0116(BAc)-0.0479 and RBA = 0.5542(BAc)-2.2817. These findings will advance the development of robust exposure narratives for soil metal contamination in Port Colborne and elsewhere.

1. Introduction

The story of nickel, copper, cobalt, and arsenic in soil from Port Colborne, Ontario is an environmental legacy resulting from industrial atmospheric deposition from nickel refinery operations between 1918 and 1984 (Stantec, 2014). The contaminated soils of Port Colborne have been studied for decades, and Vale Canada Limited, the current owner of the former Inco Port Colborne Refinery, has been engaged in a community-based risk assessment (CBRA) process to assess the residual risks to agricultural, ecological, and human receptors resulting from the elevated soil metals existing for several km northeast of the refinery (JWEL, 2004a, 2004b, 2007; Stantec, 2014). The dispersed nature of the contamination has necessitated the use of risk assessment in this community, and the exposure science concepts of bioavailability (BAv) and bioaccessibility (BAc) have been employed to some extent in earlier risk assessment efforts (MOE, 2002, JWEL, 2007, Birmingham and McLaughlin, 2006).

Risk assessment has become the dominant public policy tool for evaluating and protecting human health and the environment (NRC, 2009), and the components of risk assessment, particularly exposure science, have continued to advance, with greater consideration of "upstream and natural factors" and the development of constructs such as the "exposome", which conceptualizes the totality of exposure (NRC, 2012). Within risk assessment, exposure assessments are expected to identify and quantify the exposure of highly exposed and vulnerable sub-populations via all relevant exposure pathways including background exposures from food and water, and should assess BAv, especially when exposures are predominantly via a single route (NRC, 2012). Ni BAv from contaminated soils and baseline exposure from uncontaminated food have not been well characterized to date. Nickel is essential to plants and thus it is normally present in the diet; most animal studies ignore this contribution because the administered doses are much higher. TRVs (toxicity reference values) that are below dietary baseline just because of large assessment factors (AF) should be critically examined and it should be recognized that they should be considered as being "in addition to diet". Having robust oral bioavailability data could allow a refinement of TRV derivation, leading perhaps to a "bioavailable TRV" concept in the future.

At Port Colborne, oral exposure is expected to dominate due to the elevated soil concentrations (Birmingham and McLaughlin, 2006), and potential uptake by vegetation and trophic transfer (in ecological settings). For metals (including the metalloid arsenic), oral BAv refers to the release of metals from the ingested matrix (pure metal compound, contaminated soil, food, fluids) in the gastrointestinal tract and the subsequent absorption into systemic circulation. Bioaccessibility (BAc) refers to the release of metals from matrices under surrogate physiological conditions and, therefore, represents the amount that is "potentially available" for absorption into systemic circulation. Bioelution refers to the in vitro extraction methodologies used to estimate the BAc of metals/metalloids from matrices using artificial biological fluids (Lombaert et al., 2018).

Establishing and validating mathematical relationships between BAv and BAc provides support for the use of bioelution to approximate the oral bioavailable fraction, reducing the overall need for animal testing and enabling the widespread application of oral BAv/BAc correction in exposure assessments and other applications (e.g., grouping and read across). A number of animal models have been used to develop such relationships for Pb (Casteel et al., 2006) and As (Diamond et al., 2016) for a variety of matrices, but similar models do not exist for Ni.

This work comprises four research questions. First, could a mass balance approach using a rat model be used to quantify the baseline bioavailability of the relevant contaminants (Ni, Cu, Co, and As) from food? Second, could this approach also distinguish the bioavailability of these metals from the three contaminated Port Colborne soil types (fill, clay, and organic)? It is now well established that metal bioavailability is very much dependent on the chemical species of the subject metals (Landner and Reuther, 2004), so to help answer this question, MLA (mineral liberation analysis) was used to evaluate the speciation of the metals in these soils to assist in interpreting the subsequent bioavailability measurements for the contaminant metals. Third, for Ni, the major contaminant metal, could the dosing conditions from the key studies used for Ni TRV derivation be replicated to infer the oral bioavailability of Ni in those studies? If so, relative bioavailability (RBA) together with an absorbed dose approach would be available as tools to evaluate oral exposure to Ni at this and other sites. Lastly, was it possible to establish relationships between in vitro BAc and in vivo BAv for metals in these soils that might be broadly applicable for assessing exposure from industrial metal releases, whether fresh (recent) or weathered (older)?

2. Materials and methods

2.1. Soil sample collection

The soils used in this study were in storage since their collection in 2001–2002, when forty-four test pits were dug by backhoe or manually by shovel. Soil sampling occurred from test pit walls at intervals of 2.5 or 5 cm. Soil characteristics (soil classification, mineralogy, metal content, general chemical parameters) are reported elsewhere (JWEL, 2004b).

Thirty-two soil samples from sixteen test pits, air-dried and stored in glass jars, were selected from storage. The primary selection criteria were to have a sufficient sample quantity to meet the requirements for all three components of this research and to have a similar number of each soil type so that the study would reasonably represent the contaminated soil mineralogy near the Port Colborne refinery. It was our expectation that the speciation of the contaminant elements had not been altered due to storage (Blake et al., 2000).

The soils were sieved (250 μm mesh) as per MOE (2002). Clay soils were rock hard and were crushed mechanically before sieving. In addition to the archived soil samples, to establish trophic transfer limits, one organic soil sample was recently collected, as were earthworms from the same location (in a mixed-species sample – as collected). Five

earthworm species are common locally (JWEL, 2004a), but the presence or absence or proportions of these species were not catalogued. The sample represents a population that an insectivorous small mammal might ingest at the sample location.

2.2. Metal speciation methodology

Thirteen samples from seven of the test pits near the Port Colborne refinery site (Fig. S1) were prepared for the MLA (Sylvester, 2012) by mounting rotary-split soil sub-samples in epoxy that was subsequently polished to a mirror finish after hardening. The polished epoxy mounts were prepared in a two-stage process where the sample was first mixed in epoxy that hardened horizontally in 1-inch (2.54 cm) sample cups. The hardened mounts were sectioned transversely in the vertical plane using a diamond-blade rock saw. One of the cut pieces was then mounted in epoxy in a second 1.25-inch (3.18 cm) sample cup with the cut surface down. The resulting mount displays a cross section through the first-stage mount, which was density segregated when it was allowed to harden in the horizontal orientation. The soil mounts were run in the MLA using the mineral grain X-ray mapping (GXMAP) routine (Sylvester, 2012) with pixel by pixel X-ray mapping used to identify all metallic, sulfide and Fe ± Ni oxide and Ni ± Fe oxide phases.

The mineral chemistry of the major Ni-bearing phases was determined by combined quantitative energy and wavelength dispersive spectrometry (EDS/WDS) methods using a JEOL7000 SEM equipped with an Oxford Instruments INCA X-ray analysis system. The SEM accelerating voltage was set to 20 kV with a beam current of 20 nanoamperes. WDS was used to determine the concentration of Fe, Co, Ni and Cu. Count times were 40 s on X-ray peak and 15 s on background. The EDS system was set up for quantitative analysis and was used to determine the concentration of all elements.

2.3. Bioelution methodology

Bioaccessibility estimates were made for 6 fill soils from 2 test pits, 14 clay/mineral soils from 7 test pits, and 12 organic soils from 8 test pits. A number of these samples were duplicates (Table 2). Approximately 2 g of sieved soil samples were transferred to clean aluminum boats and dried in the oven at 60 °C for 48 h, with 1 g assayed for BAc and 0.5 g assayed for total elemental concentrations for the contaminants of interest.

BAc estimates of the contaminant metals used the US EPA (2008) bioelution methodology except that extraction occurred on a shaker incubator in the lab at 37 °C with 132 rpm rotation speed rather than on a rotary extractor. Briefly, 1 g of soil was extracted in 100 mL of 0.4 M (pH 1.5) glycine-HCl buffer for 1 h. Following extraction, approximately 15 mL of the reacted solution was filtered into a clean 15-ml polypropylene centrifuge tube for further analysis using a 0.45 μm cellulose acetate syringe filter. Filtered samples were stored in a refrigerator at 4 °C until analysis.

Soil samples used for BAc analysis were prepared for total metal and As analysis using US EPA method 3051A. For total metals analysis, 9 mL of Trace metal grade HNO_3 and 3 mL of trace metal grade HCl were added to each 0.5 g sample. The samples were digested overnight at room temperature, followed by digestion in a gravity-convection oven at 105 °C for 8 h. After cooling, the digestates were filtered (Whatman Grade 42) and brought up to a total volume of 50 mL in a volumetric flask. The analysis for Ni, Cu, Co, and As in soils and extracts was by ICP–OES. In several cases, where Ni exceeded the calibration limits for ICP_OES, FAAS was used to analyze Ni.

2.4. In vivo bioavailability study methodology

In three preliminary mini-studies (MS1–3) and a main study, oral BAv in rats was estimated by mass-balancing orally dosed contaminant metal substances with renal and fecal outputs. Each treatment group in the three mini-studies and the main study consisted of eight rats. In the mini-studies, urine and feces samples were collected for three 24 h periods, which allowed temporal analysis of metals and As in urine and feces. In the main study, urine and feces were collected daily for 72 h, but were analyzed as pooled cumulative 3-day samples for each rat.

The aim of MS1 and MS2 was to understand the BAv that would have occurred in two reproductive studies that have been most frequently used by regulatory agencies to set oral toxicity benchmarks for assessing nickel health risk (Dutton et al., 2016). In MS1, rats were dosed once by gavage with $NiSO_4 \cdot 6H_20$ in water-for-injection (WFI) at dose levels of 0 (control), 220, 550, 1100, and 2200 μg Ni/kg body weight. This dosing regime and the exposure levels were the same as those of a reproductive study with $NiSO_4 \cdot 6H_2O$ (SLI, 2000). MS2 mimicked the exposure conditions in another reproductive and whole life growth study (Ambrose et al., 1976), in which rats were allowed to feed ad libitum on finely ground rat chow that had been thoroughly mixed with $NiSO_4 \cdot 6H_20$ "fines". Ni concentrations in the diets in the Ambrose study were 0 (control), 100, 1000, and 2500 μg/g chow. For MS2, Harlan Teklad 8728C rat chow was ground in an electrical coffee grinder and sieved to <250 μm size (removing large pieces and the fibrous component of the rat chow). The ground chow was then mixed with $NiSO_4 \cdot 6H_20$ crystals and reground in the coffee grinder to further size reduce and mix the $NiSO_4 \cdot 6H_2O$ particles. The final nominal concentrations were those used in the Ambrose study. For gavage dosing in this study, the spiked, ground food was diluted in 1% methyl cellulose in WFI at a ratio of 4:10 *v*/v. This was found to be the least amount of dilution that could be used because of swelling and high viscosity of this mixture. The measured doses in the MS2 study were 0 (control – unspiked food in WFI), 16, 320, 3250, and 8480 μg Ni/kg body weight. The dose preparation approach in MS2 simulated the dosing methodology used by Ambrose (nickel sulfate fines and food fines mixed together) but it was necessary to provide a defined dose by gavage to enable BAv estimation by mass balance, although this meant that the dose was received all at one time rather than over hours of normal feeding time.

In MS3, samples of each of the three Port Colborne soil types were gavage dosed to rats as a range-finding exercise prior to the main study. Controls (1% methyl cellulose in WFI), a fill soil (TP9 (5–7.5 cm) 13,052 mg Ni/kg), a clay soil (Hruska (5019 mg Ni/kg)), and an organic soil (TP-S (0–2.5 cm) 1980 mg Ni/kg) were orally dosed with a 40:60 (w:w) ratio of soil to 1% methyl cellulose in WFI at a rate of 10 mL/kg. A 250 g rat would receive approximately 1 g of soil by such dosing.

The main study consisted of dosing with sixteen archived soil samples, plus one newly collected organic soil, earthworms collected from the same location as the newly collected soil sample, and controls (WFI). Soil samples were prepared as described for MS3. Earthworms were gut-cleared for 48 h, blended by polytron, and gavaged without dilution. The organic soil and earthworm treatments were included to estimate trophic transfer for the ecological context.

2.5. Animal care

The in vivo studies using male Sprague-Dawley rats (199–302 g) (Charles River, Montreal QC) were conducted by Nucro-Technics, Scarborough, ON. All animals were submitted to an initial general physical examination by a veterinarian or a qualified technician. Teklad Certified Rodent Diet (8728C) and municipal water were provided to the animals ad libitum throughout the 6-day acclimatization and 3-day study periods. During the acclimatization period, rats were housed individually in Nalgene® rat cages, and were moved to metabolic cages for the collection of urine and feces following dosing. The animal room environment was controlled (targeted ranges: temperature 18–26 °C, relative humidity 30–70%, >10 air changes/h) and monitored. The photo-cycle was 12 h light and 12 h dark. Mortality checks were performed twice per day and all animals were inspected twice daily for clinical signs during the course of the studies. The body weight of each rat was recorded

prior to dosing. Each rat was weighed again after the final collection of urine and feces. Food consumption was only recorded for nine of the treatment groups in the main study. These data were used in conjunction with the food consumption data from SLI (2000) to estimate study-wide food consumption and enable bioavailability estimation from the diet in unexposed (control) animals.

The test and control articles were administered orally using a blunt tip gavage needle attached to a syringe. Prior to each individual dose between rats, the gavage needles were wiped clean. Each group of animals were dosed with a new syringe and gavage needle. The dose was adjusted based on the animal's body weight, at a rate of 10 mL/kg body weight, or 4 g/kg on a soil basis.

Urine volumes were measured at the time of collection. Feces sample weights were determined after drying at 60 °C for 18 h. Following the last urine and feces collection, all animals were euthanized by CO_2 overdose and discarded without necropsy examination.

2.6. Metal and arsenic analyses in the in vivo bioavailability studies

Due to concern over potential lack of homogeneity of the food and soil suspensions (which could bias dosing estimates and lead to inaccurate bioavailability estimates), 1 mL aliquots of the food and soil suspensions were collected before and after each animal was dosed with food or soil suspension (MS2, MS3, and main study). Therefore, for each treatment group of 8 animals, 9 samples of dosing suspension were collected, with the analytical values for pre- and post-dose samples being averaged to estimate each of the eight doses per treatment group. The one exception to this was the fill soil TP9 (5–7.5 cm), which only had three aliquots available for analysis. The average value was used as the estimated dose for that treatment.

Samples of food or soil suspensions, urine, and feces were analyzed for Ni, Cu, Co, and As by ICP-MS. Samples (0.25 g) of food/soil suspensions and feces (ground by mortar and pestle after drying) were microwave digested in 12 mL of Aqua-Regia (Hydrochloric and Nitric acid in a 3:1 ratio). One sample per group was used for spike recovery determinations (see supplementary materials for details). Digestates were diluted to 50 mL with water and filtered if necessary, to remove siliceous particles. Subsequent dilutions were performed as necessary depending upon the anticipated concentration of Ni in the digestate. Urine samples were filtered to remove food and other particulates and 0.5 mL aliquots were then diluted to 50 mL with an aqua regia solution prior to analysis. One sample per group was used for spike recovery determination. Twelve sub-samples of Harlan Teklad diet were analyzed to determine the levels of Ni, Cu, Co, and As in the basal diet.

2.7. Data analysis

As per Hurlbert (1984) inferential statistics were not used, as they would not have enhanced the meaningful understanding of our findings. Rather, we have used an estimation approach to identify relationships via confidence interval estimates among treatment groups in the several components of this research (Cumming, 2012). Confidence intervals and regression equations were calculated using Microsoft Excel. Data quality was assessed using a combination of duplicates, certified reference materials, and matrix spikes, as appropriate, for the component studies. Quality assessment results are provided in the supplementary materials.

3. Results and discussion

3.1. Speciation of Ni, Cu, and Co in Port Colborne soils

In Table 1, the metal content in the primary Ni minerals present in the 13 soil samples from 6 test pits are summarized on a test-pit-wise basis based on MLA analysis (Ni, Cu, and Co only, as arsenic levels were too low to be speciated by MLA). Samples are ordered left to

Table 1
Distribution of Ni, Cu, and Co among the major Ni-bearing minerals in the test pit soils from Port Colborne Ontario. Columns sum to 100% of each element. Row data do not sum to 100%. Many other minerals are present in the samples but have not been considered since they did not contain Ni, Cu, or Co.

Test pit	TP9 (fill)				TP206 (clay)				Hruska (clay)				TP-J2 (clay)				TP-S (organic)				TPK2-1 (clay)			
Distance from refinery (km)	0.6				0.6				1.22				1.55				1.3				1.8			
Metal Distribution (%)	Ni	Cu	Co	Grain Size (μm)	Ni	Cu	Co	Grain Size (μm)	Ni	Cu	Co	Grain Size (μm)	Ni	Cu	Co	Grain Size (μm)	Ni	Cu	Co	Grain Size (μm)	Ni	Cu	Co	Grain Size (μm)
Bunsenite (NiO)	35.6	5.2	9.8	19.6	42.1	6.3	14.8	25.2	28.5	0.9	4.7	12.1	33.3	1.3	4	11.8	35.5	0.8	3.6	7.2	25.2	0.6	3.8	12.4
Orford Slag	–	8.8	4.9	38.5	–	16.6	9.9	67.6	32.2	87	76.5	52.7	26.1	84.1	73.2	56.1	43.5	83	88.5	27.8	44.5	90.4	84.4	46.8
Ni Ferrite (trevorite)	55.3	79.5	82.9	6.7	41.7	61.3	68.7	5.2	34.1	11	18.2	21.7	35.2	13.5	22.1	18.4	15.5	3.5	7.1	3.5	26.5	6.4	11.3	8.2
Ni Alloy	6.4	3.5	1.2	14.3	2.6	1.4	0.5	9.5	2.6	0.3	0.2	17.2	3.2	0.5	0.3	12.3	–	0	–	1.1	0.5	–	–	1.9
Ni sulfides	–	0.7	–	–	2.4	2.9	1.6	–	–	0.1	–	–	–	–	–	–	2.1	12	–	–	–	1.9	–	–
Ni clays	1.8	2.3	0.8	4.6	8.6	11.5	4.3	8.8	2.4	0	0.4	2.4	2.1	–	0.4	2.5	3.3	0.7	0.4	5.2	2.9	–	0.4	2.5
Totals	99.1	100	99.6	–	97.4	100	99.8	–	99.8	99.3	100	–	99.9	99.4	100	–	99.9	100	99.6	–	99.6	99.3	99.9	–

right in the table by approximate distance from the historical location of the refinery smoke stack (Fig. S1).

Several characteristic Ni-bearing phases were identified in the soils; bunsenite (NiO), metallic Ni alloy, Ni ferrite spinel (trevorite), Orford slag (alkaline slag – mostly hydroxycancrinite but other phases present) and Ni-bearing clay minerals, the first four being clear indicators of the historical operations at the Port Colborne refinery.

Bunsenite, which is quite rare in nature and can generally be considered an industrial mineral, was identified in all 13 MLA samples, with 9 samples containing minor amounts and 4 samples containing trace amounts. High temperature pyrometallurgical stack processes result in spherical particles, so particle shape is strongly indicative of airborne entrainment and aerial transport from a stack source. MLA images (Fig. 1) show that bunsenite particles generally become smaller and develop a more irregular shape with increasing distance from the refinery, a characteristic of distance-deposition patterns around industrial point sources, with smaller particles being transported farther than larger particles of the same density.

The average elemental and oxide assay values for thirty spot analyses obtained from 10 bunsenite grains shows bunsenite to consist mostly of Ni oxide (~95.5 wt%), with Fe oxide (~1.5 wt%), Cu oxide (~1 wt%) and Co oxide (~1 wt%) in solid solution. Quantitative assay

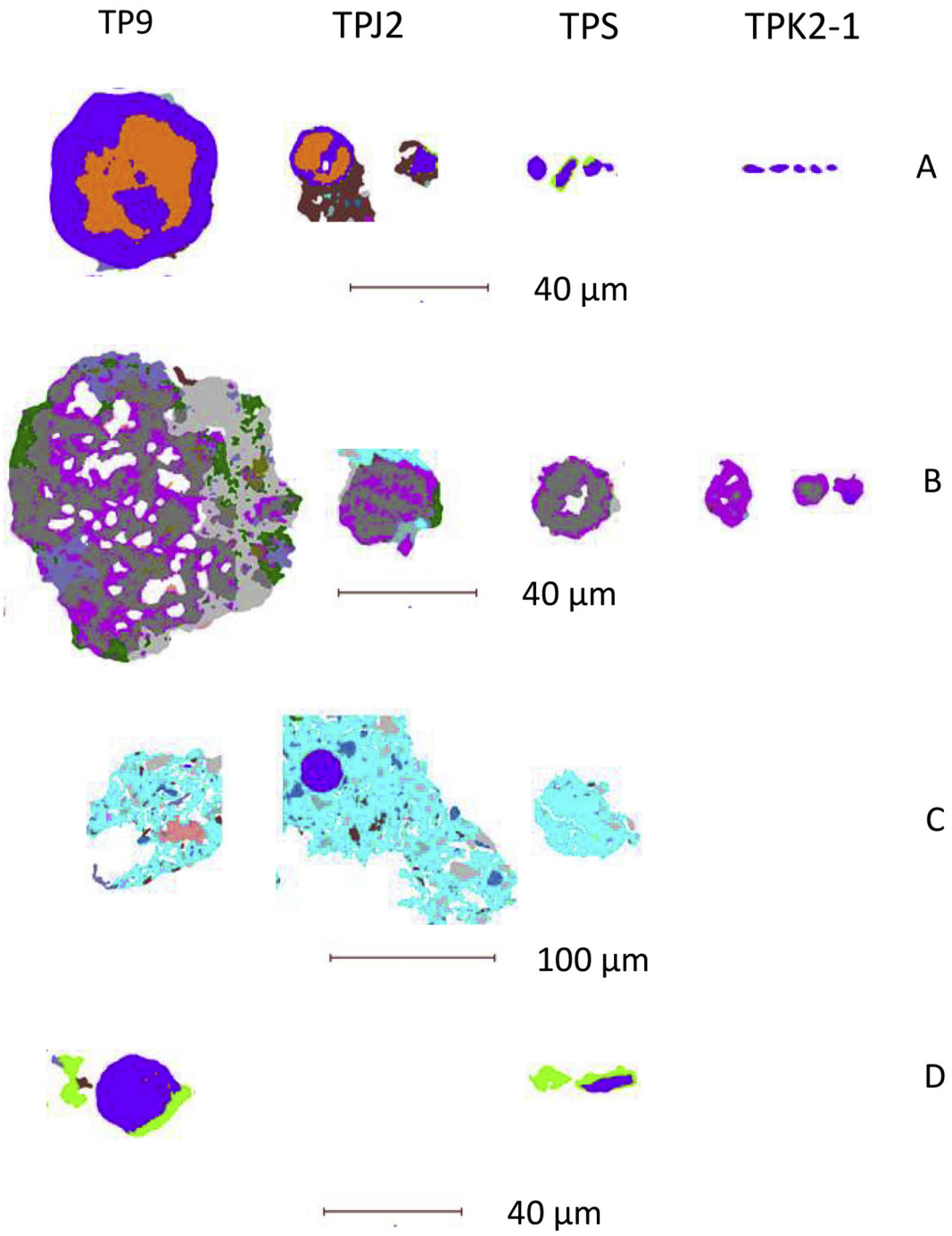

Fig. 1. False color images of primary Ni minerals in Port Colborne soils. MLA images from four test pits – increasing distance from refinery towards the right. (A) Blue bunsenite particles; note the Ni alloy core (orange) in spherical bunsenite particles from TP9 and TPJ2. (B) Trevorite particles (purple) intergrown with the iron spinel mineral magnetite (grey). (C) Orford slag (turquoise). (D) Ni clay (nontronite) rims at edges of bunsenite grains (green). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

totals are 99.1 ± 1.6 wt%, indicating that there are no missing elements from the assay determination, such as hydrogen. This result indicates that the Ni oxide phase in the Port Colborne soil is anhydrous, rather than a Ni oxide/hydroxide phase.

Some bunsenite particles contain "cores" of metallic nickel, present as Ni alloy (Fig. 1) consisting mostly of Ni (~92.5 wt%), with Cu, Fe, and Co in solid solution. The highest proportion of such particles occurs in the soil closest to the Ni Refinery (TP9), where fill was used to reclaim land adjacent to the Welland Canal. Ni-alloy concentrations were lower in the remaining samples outside of the fill area (Table 1). The Ni alloy cores within bunsenite particles would be expected to have low chemical reactivity or biological availability due to the presence of the bunsenite coatings.

Ni-Fe-ferrite is an oxide phase with the mineral name trevorite, and an ideal mineral formula $NiFe^{3}{}_{2}O_{4}$. Trevorite was identified in all thirteen samples that were speciated by MLA, being a major constituent of the two fill samples from TP9 (4–5 wt% - Table 1), a minor constituent in four samples from 3 locations (0.5–1 wt% in TP206, Hruska, and TP-J2), and a trace constituent in the remaining 7 samples from TP-S and TPK2–1 (<0.1 wt%) (Table 1).

MLA particle images show that trevorite is generally intergrown with magnetite (Fig. 1). This is not unusual, since they are both spinel-group minerals with Ni substituting for Fe^{2} in magnetite to form trevorite. As with Ni alloy and bunsenite, trevorite particles tend to become smaller with increasing distance from the Port Colborne refinery.

Average elemental and oxide assay values for 5 spot analyses obtained from 3 trevorite grains show that trevorite consists mostly of ferric oxide (~45 wt%) and nickel oxide (~39 wt%), with minor amounts of Al oxide (~6 wt%), Cu oxide (~4 wt%), Co oxide (~2 wt%) and chromic oxide (~1 wt%), all in solid solution. Quantitative assay totals are 98.9 ± 1.7 wt%, within analytical error of 100 wt%, indicating that there are no missing elements from the assay determination, such as hydrogen.

The Port Colborne soil samples contain an unusual mineral phase with chemistry similar to plagioclase feldspar. The presence of trace amounts of Cu and Ni in solid solution, combined with the porous texture of the particles, identified this phase as hydroxycancrinite, the major mineral phase in the alkaline slag from the Orford Process, a Ni-Cu separation technology used at the Port Colborne refinery before 1931. As such, the Orford slag is an almost century old fingerprint of the pyrometallurgical origin of this mineral in the soils to the north east of the refinery along the primary axis of wind direction.

The hydroxycancrinite (Orford slag) particles are typically larger than other Ni-bearing particles (Ni alloy, bunsenite, and trevorite). The hydroxycancrinite typically occurs in porous particles with abundant trapped gas bubbles (termed vesicles), consistent with formation as a slag. The ideal mineral formula of hydroxycancrinite is $Na_4(AlSiO_4)_3(OH)\cdot(H_2O)$ – consisting of 19% sodium and 5.5 wt% water. WDS X-ray analysis confirms that the hydroxycancrinite contains trace levels of Cu, Ni, and Co in solid solution, averaging 0.24, 0.2 and 0.04 wt% respectively.

Table 2
Total metals and bioaccessibility results for fill, clay/mineral, and organic soils from the Port Colborne area. Values in square brackets are the lower and upper 95% confidence limits. Dashes indicate samples where bioaccessibility could not be determined due to analytes being below detection limits in extracts.

Sample ID	Soil type	Soil [Ni] (mg/kg)	Soil [Cu] (mg/kg)	Soil [Co] (mg/kg)	Soil [As] (mg/kg)	Ni BAc (%)	Cu BAc (%)	Co BAc (%)	As BAc (%)
TP9 (5–7.5 cm)	Fill	14,645	959	192	56	7.2	25.5	22.6	30.0
TP9 (5–7.5 cm) dupl.	Fill	13,052	932	190	57	6.1	21.0	16.7	19.0
TP9 (7.5-10 cm)	Fill	17,420	1227	194	79	7.7	27.3	19.9	24.4
TP9 (10–12.5 cm)	Fill	12,005	732	126	52	7.8	33.0	17.7	37.2
TP9 (12.5–15 cm)	Fill	16,135	998	152	64	5.8	37.4	13.9	37.6
TP17 (10–12.5 cm)	Fill	4288	570	40	24	17.4	58.4	28.6	48.8
	Average - Fill	12,924 [8453, 17,396]	903 [685, 1121]	149 [92, 206]	55 [38, 73]	8.7 [4.5, 12.8]	33.8 [21.0, 46.6]	19.9 [14.9, 24.8]	32.8 [22.6, 43.1]
TP3 (0–2.5 cm)	Clay	8912	822	103	23	9.4	31.8	22.5	–
TP5 (0–5 cm)	Clay	9527	884	108	42	10.2	33.7	22.0	35.42
TP5 (5 cm)	Clay	8686	839	101	33	10.1	32.6	21.8	30.52
TP5 (10–12.5 cm)	Clay	5112	453	59	25	9.1	33.2	18.2	39.39
TP6 (2.5–5 cm)	Clay	18,553	1915	196	67	12.0	43.3	17.6	36.74
TP-J2 (5–10 cm)	Clay	1065	132	27	14	14.5	42.1	–	–
TP-J2 (10–15 cm)	Clay	4582	432	57	26	18.8	46.4	24.6	–
TPK2–1 (5–10 cm) a	Clay	1015	128	29	13	12.2	47.8	–	–
TPK2–1 (5–10 cm) b	Clay	1066	131	26	11	14.4	43.2	–	–
TPK2–1 (5–10 cm) b (dupl.)	Clay	1080	131	29	13	13.3	38.0	–	–
Hruska	Clay	5019	472	59	21	16.4	38.1	21.9	–
TP206 (30–35 cm)	Mineral	12,495	635	102	42	7.9	25.6	16.7	22.36
TP206 (35 cm)	Mineral	7226	324	53	25	9.1	24.1	23.6	–
TP206 (35–40 cm)	Mineral	4436	212	34	19	13.2	26.7	29.7	–
	Average - Clay	6341 [3530, 9152]	537 [268, 805]	71 [44, 97]	27 [18, 35]	12.2 [10.4, 13.9]	36.2 [31.9, 40.5]	21.9 [19.8, 23.9]	32.9 [29.4, 36.4]
Groetlaar	Organic	9754	865	97	48	20.6	30.6	22.0	28.5
Groetlaar (0–15 cm)	Organic	17,088	1353	156	67	21.8	35.5	25.4	34.56
Groetlaar (0–15 cm) dupl.	Organic	16,643	1397	163	73	20.7	27.7	23.8	27.64
SS20	Organic	259	75	14	28	31.9	33.0	64.2	57.06
SS25 V. High Organic	Organic	8125	544	287	60	34.4	25.9	20.3	24.01
SS27 Med Organic	Organic	1640	251	131	19	23.5	26.2	14.0	40.81
Ni 1000 (soil organic)	Organic	2547	286	40	21	15.3	22.8	36.8	–
TP-R4 (10–15 cm)	Organic	2369	398	38	30	33.1	36.8	43.8	62.04
TPS (0–2.5 cm)	Organic	1980	265	35	22	26.0	38.5	44.6	61.54
TP-S (2.5–5 cm)	Organic	1985	268	37	23	27.0	38.6	39.8	59.7
TP-S (10–15 cm) a	Organic	1779	239	32	22	27.8	37.5	45.4	61.31
TP-S (10–15 cm) b	Organic	1868	247	33	25	25.9	36.0	38.0	50.21
	Average - Organic	5503 [1853, 9154]	516 [243, 788]	89 [39, 138]	37 [24, 49]	25.7 [22.2, 29.1]	32.4 [26.2, 43.5]	34.8 [26.2, 43.5]	46.1 [36.8, 55.4]

Among the remaining Ni-rich phases, sulfidic Ni was also found in several of these samples. In addition to being a minor component, the sulfides are found at the cores of spherical particles with coatings of bunsenite, indicating that the sulfides, like the nickel alloy, are trapped within the spheres and unable to interact chemically or biologically unless they are broken open to expose reactive internal surfaces.

A final Ni-bearing phase of interest was a hydrous mica-like phase identified as a Ni clay mineral, most likely belonging to the nontronite group. The Ni clay is present in elongated grains closely associated with bunsenite, typically occurring as rims surrounding the bunsenite grains. It is proposed that these grains have formed in-situ in the soil, as the result of cation exchange between Ni from the bunsenite and other cations present in an unidentified precursor clay mineral. This is not unexpected, given that clay soils are locally common.

The speciation of 13 of the metal-contaminated soils that were also orally dosed to rats helps to provide context to understand the biological uptake of the contaminant metals in a common mammalian model. It is significant that the metals occur in solid solution, confirming that the Ni-bearing phases present in the soil were created at high temperature in the historical pyrometallurgical processes at the refinery. As particle-rich process gases were transported through the flues, exited the stack and carried in the ambient air, the molten particles would have solidified (frozen) with the once-molten metals remaining in place, effectively in "solid solution" (the term common to mineralogy and metallurgy). The chemical reactivity and biological availability of these metals in soil up to a century after their deposition is primarily that of nickel oxide, trevorite, and Orford slag, with Cu and Co being present in solid solution in these phases. All three of these mineral phases are clearly associated with the historical operations at the refinery.

3.2. Oral bioaccessibility of Ni, Cu, Co, and As in Port Colborne soils

In vitro bioelution tests were conducted on 6 fill soil samples, 14 clay/mineral soil samples, and 12 organic soil samples (Table 2). These samples were from 17 test pits (Fig. S1), so for each soil type, some samples were from different depths in the same test pit. Differences in BAc by soil type are seen for Ni, the major contaminant metal at Port Colborne, with BAc being highest in the organic soil (25.7%) and lowest in the fill soil (8.7%). The BAc of Co and As is also highest in organic soil (34.8% Co, 46.1% As), but there were no obvious difference between the fill and clay/mineral soils (~20% Co, 33% As). No difference was seen between soil types for Cu BAc (32–36%). There are few comparable data sets, but using the same bioelution methodology, the Ontario Ministry of the Environment found the BAc of Ni, Cu, Co, and As in fill soils from Port Colborne to be 19, 35, 29, and 35%, respectively (MOE, 2002). These values are comparable to our current results.

3.3. Bioavailability of Ni, Cu, Co, and As from rat chow – baseline urinary and fecal levels in control animals

Among the in vivo studies reported here, we have attempted to estimate the Ni, Cu, Co, and As BAv by mass balancing intake from a single oral gavage dose with urinary and fecal output. Fecal and urinary metals have specificity, with Ni, Co, and As in feces thought to reflect metal that has not been taken up by the test animals, as these metals are not primarily regulated via biliary/fecal excretion. Rather, these elements are primarily regulated via urinary excretion. In contrast, Cu is excreted in bile, so fecal Cu is a relevant measure of both excreted and unabsorbed Cu, with excreted biliary Cu being not just from soil ingestion but from the diet as well. Clearly, this would complicate BAv estimation by fecal mass balance. Cu is not excreted primarily via the kidney and therefore bioavailability cannot be inferred from urinary Cu alone (Fairweather-Tait, 1997).

The background or baseline dietary exposure to the study metals is an important and overlooked area in risk assessment (NRC, 2012). The BAv of dietary metals in risk assessment is often assumed to be 100%. In the absence of isotopic tracers, our approach was to establish baseline urinary and fecal Ni, Cu, Co, and As output in control (unexposed) animals and to distinguish that baseline from the output associated with the gavage doses of these metals (e.g., in soils). Given that the Ni in Harlan Teklad 8728C rat chow is present not as a mineral supplement, but as biologically incorporated components of naturally sourced ingredients (dehulled soybean meal, wheat middlings, flaked corn, ground corn, fish meal, cane molasses, ground wheat, dried whey, soybean oil, brewers dried yeast), these baseline urinary and fecal Ni values allow the BAv of Ni to be inferred from a natural diet in the absence of Ni contamination (Table 3). The same logic also applies to As. In contrast, $CuSO_4$ and $CoCO_3$ were present as mineral supplements to the Teklad diet, with a Cu product specification of 25 mg/kg. Co, Ni, and As specifications were not provided by the manufacturer, but the average values for Cu, Ni, Co, and As from twelve subsamples of the diet in this study were 24.25 mg/kg [95% CI] [16.99, 31.51], 1.94 [1.53, 2.35], 0.78 [0.73, 0.83], and 0.23 [0.21, 0.24], respectively.

Baseline daily urinary Ni mass excretion from control rats in MS1–3 was 1.16 μg Ni, while the estimated daily mass of Ni eaten was 54 μg (Table 3), implying an oral bioavailability of approximately 2.2% from the basal diet. This baseline (background) urinary Ni reflects the excretion of Ni taken up from the normal diet and must be principally due to Ni present in the Harlan Teklad 8728C rodent diet, with City of Toronto drinking water expected to provide no more than 0.05 μg per day (data not shown). The estimated average amount of Ni received by the control animals from food over 72 h was 162.1 μg in this study. The baseline (72-h) mass of Ni recovered in feces was 184.2 μg, with an average daily fecal Ni concentration of 10.3 μg/g. The mass of fecal Ni (185.2 μg) over this period represents 114% (95% CI) [105, 123] of the estimated Ni intake. The amount of nickel present in the diet can be approximately mass-balanced between urine and feces. From these data, the urinary excretion is approximately 2% of the dose received from the diet over the course of the study and this value is the estimated oral BAv of Ni from uncontaminated food containing Ni from natural plant and animal sources. It can be assumed that the baseline urinary Ni excretion in control animals represents an equilibrium physiological

Table 3
Summary mass balance information for Ni mass balance in control animals from three mini-studies and the main study. The sample size of $n = 72$ for MS1–3 in the upper table refers to the fact that samples collected daily for 72 h were analyzed separately. The sample size of $n = 32$ in the lower table refers to the pooled 72-h data, which reduces the sample size by a factor of three. "Food eaten" is estimated from the equation Food eaten (g) = $34{,}326(\text{weight (g)})^{-1.038}$. Values in square brackets are the lower and upper 95% confidence limits.

Study	Variable (units)	n	Mean
MS1,MS2,MS3	24 h Urine Vol. (mL)	72	19.0 [17.7, 20.3]
MS1,MS2,MS3	24 h Urine [Ni] (μg/L)	72	65.9 [59.2, 72.6]
MS1,MS2,MS3	24 h Urinary Ni mass (μg)	72	1.2 [1.1, 1.3]
MS1,MS2,MS3	24 h fecal mass (g)	72	6.2 [6.0, 6.3]
MS1,MS2,MS3	24 h fecal [Ni] (μg/g)	72	10.0 [9.4, 10.6]
MS1,MS2,MS3	24 h Mass Ni eaten (μg)	72	54.0 [53.9, 54.1]
MS1,MS2,MS3	24 h fecal Ni mass (μg)	72	62.0 [57, 67]
MS1,MS2,MS3,Main	72 h Urine Vol. (mL)	32	55.8 [50.7, 60.9]
MS1,MS2,MS3,Main	72 h Urine [Ni] (μg/L)	32	70.3 [61.6, 79.0]
MS1,MS2,MS3,Main	72 h Urinary Ni mass (μg)	32	3.7 [3.3, 4.1]
MS1,MS2,MS3,Main	72 h fecal mass (g)	32	18.5 [17.7, 19.3]
MS1,MS2,MS3,Main	72 h fecal [Ni] (μg/g)	32	10.3 [9.7, 10.9]
MS1,MS2,MS3,Main	72 h fecal Ni mass (μg)	32	184.2 [169.4, 199.0]
MS1,MS2,MS3,Main	72 h food eaten (g)	32	83.5 [83.4, 83.6]
MS1,MS2,MS3,Main	72 h Mass Ni eaten (μg)	32	162.1 [161.9, 162.3]
MS1,MS2,MS3,Main	Urinary Ni recovery (%)	32	2.3 [2.1, 2.5]
MS1,MS2,MS3,Main	Fecal Ni recovery (%)	32	114 [105, 123]
MS1,MS2,MS3,Main	Urinary Cu recovery (%)	32	2.3 [2.1, 2.5]
MS1,MS2,MS3,Main	Fecal Cu recovery (%)	32	66.4 [62.9, 69.9]
MS1,MS2,MS3,Main	Urinary Co recovery (%)	32	2.5 [2.4, 2.6]
MS1,MS2,MS3,Main	Fecal Co recovery (%)	32	78.9 [75.2, 82.6]
MS1,MS2,MS3,Main	Urinary As recovery (%)	32	26.0 [24.6, 27.4]
MS1,MS2,MS3,Main	Fecal As recovery (%)	32	37.9 [33.3, 42.5]

Table 4
Mean urinary and fecal Ni concentrations in MS1, MS2, and MS3 following single gavage dose of Ni in water, food, or soil. Values in square brackets are the lower and upper 95% confidence limits.

		Urinary [Ni] (μg/L)			Fecal [Ni] (mg/kg)		
Study	Treatment	24 h	48 h	72 h	24 h	48 h	72 h
MS1	Control	68.7 [54.1, 83.3]	84.7 [66.3, 103.1]	61.3 [43.6, 79.0]	10.4 [9.6, 11.2]	9.6 [9.2, 10.0]	9.1 [8.2, 10.0]
MS1	0.22 mg Ni/kg	111.7 [82.6, 140.8]	75.5 [39.9, 111.1]	91.0 [60.4, 121.6]	16.6 [16.2, 17.0]	11.4 [8.2, 14.6]	10.7 [9.2, 12.2]
MS1	0.55 mg Ni/kg	299.2 [46.0, 552.4]	97.9 [79.5, 116.3]	61.2 [45.5, 76.9]	34.0 [31.0, 37.0]	12.3 [11.1, 13.5]	11.7 [11.3, 12.1]
MS1	1.1 mg Ni/kg	302.0 [229.4, 374.6]	90.8 [70.3, 111.3]	100.2 [65.4, 135.0]	48.2 [42.2, 54.2]	13.7 [12.3, 15.1]	12.0 [11.2, 12.8]
MS1	2.2 mg Ni/kg	574.7 [457.3, 692.1]	89.8 [56.4, 123.2]	94.7 [71.0, 118.4]	90.7 [82.1, 99.3]	13.1 [11.3, 14.9]	11.3 [10.6, 12.0]
MS2	Control	69.8 [45.9, 93.7]	88.1 [58.0, 118.2]	90.4 [70.1, 110.7]	9.6 [7.6, 11.6]	10.8 [8.0, 13.6]	8.6 [7.2, 10.0]
MS2	100 μg Ni/g food	173.6 [135.4, 211.8]	76.0 [69.4, 82.6]	103.9 [76.9, 130.9]	27.3 [25.7, 28.9]	11.8 [11.2, 12.4]	10.2 [9.5, 10.9]
MS2	1000 μg Ni/g food	1085.9 [718.4, 1453.4]	141.2 [69.6, 212.8]	94.9 [84.4, 105.4]	154.9 [141.1, 168.7]	14.3 [12.0, 16.6]	9.0 [8.4, 9.6]
MS2	2500 μg Ni/g food	2182.3 [1845.8, 2518.8]	142.0 [97.1, 186.9]	60.7 [53.9, 67.5]	370.8 [326.0, 415.6]	20.5 [11.9, 29.1]	12.1 [11.1, 13.1]
MS3	Control	48.6 [34.8, 62.4]	39.7 [26.5, 52.9]	41.5 [28.6, 54.4]	9.9 [8.7, 11.1]	12.0 [7.9, 16.1]	9.9 [9.2, 10.6]
MS3	Soil - TPS 0–0.5 cm	165.4 [130.9, 199.9]	72.2 [52.4, 92.0]	89.0 [69.7, 108.3]	228.2 [191.8, 264.6]	12.8 [10.9, 14.7]	10.9 [8.7, 13.1]
MS3	Soil - TP9 5–7.5 cm	260.5 [196.2, 324.8]	81.0 [54.2, 107.8]	82.2 [52.2, 112.2]	1393 [1149.1, 1636.9]	25.1 [11.3, 38.9]	13.9 [1.4, 26.4]
MS3	Soil - Hruska	433.4 [333.1, 533.7]	99.6 [81.4, 117.8]	99.3 [63.7, 134.9]	412.4 [376.1, 448.7]	17.2 [11.2, 23.2]	10.0 [9.2, 10.8]

state, with essentially constant dietary intake and associated urinary excretion providing a measure of dietary Ni BAv. Risk assessments that assume BAv from a normal uncontaminated diet is 100% will significantly overestimate exposure.

The mass balances for Co from the diet were incomplete. The slightly low Co fecal mass balance indicates a possible systemic cycling ("metabolism") with roughly 19% of the estimated daily Co intake from food (fortified with $CoCO_3$) being unaccounted-for in the urine and feces (Table 3). The As mass balances among the controls were also incomplete, with 36% of the estimated dietary As intake being unaccounted-for in urine and feces. The elevated urinary As mass balance among the controls likely reflects excretion of bioavailable As from arsenobetaine in the fishmeal component of the diet. Since our study only measured urinary and fecal As, it is likely that the unaccounted-for mass of As was present in liver and kidney in the process of being metabolized prior to excretion (Hughes, 2006). Copper homeostasis is known to be regulated primarily in the liver (Ellingson et al., 2015), so in Table 3, urinary Cu is referenced in terms of mass balance rather than inferring BAv from urinary Cu, although they are the same, numerically. The cumulative urinary and fecal recovery of only 68% of the Cu ingested from a $CuSO_4$-supplemented commercial rat chow containing 25 mg Cu/kg likely reflecting Cu incorporation in the increasing biomass of the rats. The unaccounted-for 32% of ingested Cu indicates that a meaningful estimate of copper BAv is not possible from the control data.

3.4. Bioavailability of Ni from nickel sulfate (MS1 and MS2)

Urinary and fecal Ni concentrations in MS1 and MS2 were transiently elevated in treated animals following the gavage dosing and returned to control levels by 72 h (Table 4). The 72-h post-dosing collection period was sufficient to ensure that all dose-related urinary and fecal Ni was measured. In spite of the two different $NiSO_4$ dosing media in MS1 and MS2 (water or spiked food), there was a strong relationship between dose and urinary Ni excretion (Fig. 2). However, when expressed as a percentage of dose, the line has a slope of roughly zero, with the y-intercept of 2.35 essentially reflecting constant urinary

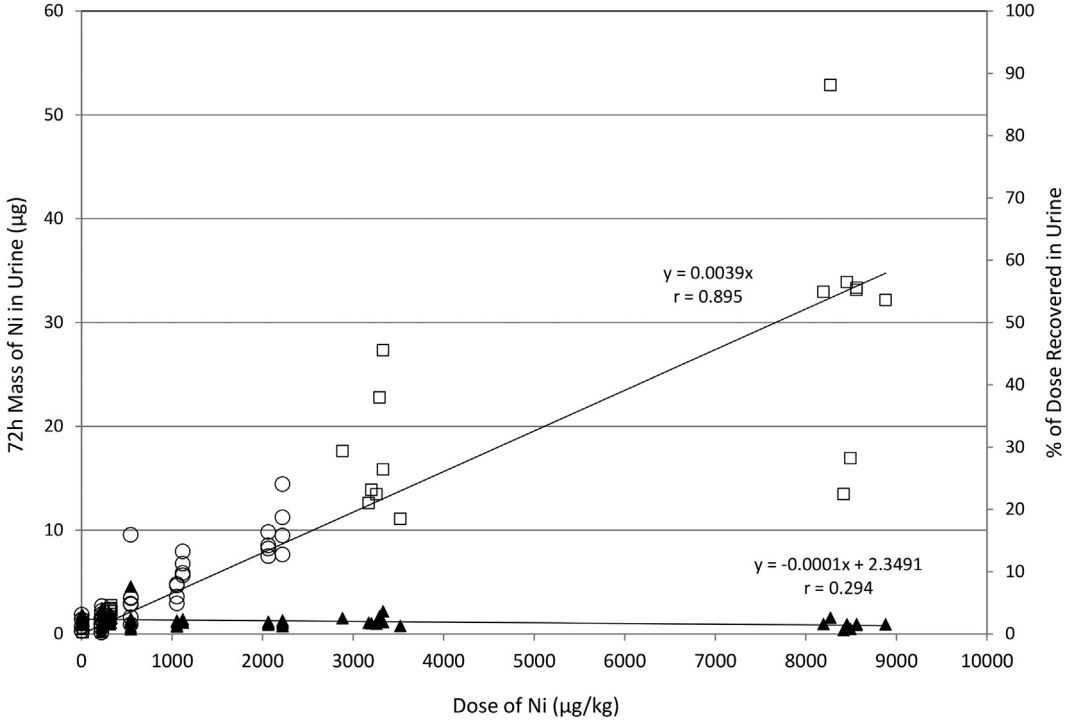

Fig. 2. The baseline-corrected mass of Ni excreted in urine over the 72-h post-dosing period following single gavage dosing. Circles: Dosing with $NiSO_4 \cdot 6H_20$ in water as per SLI (2000) (left y-axis). Squares: Dosing with $NiSO_4 \cdot 6H_20$ in food slurry following from Ambrose et al. (1976) (left y-axis). Filled Triangles: Combined data expressed as a % of the dose (right y-axis).

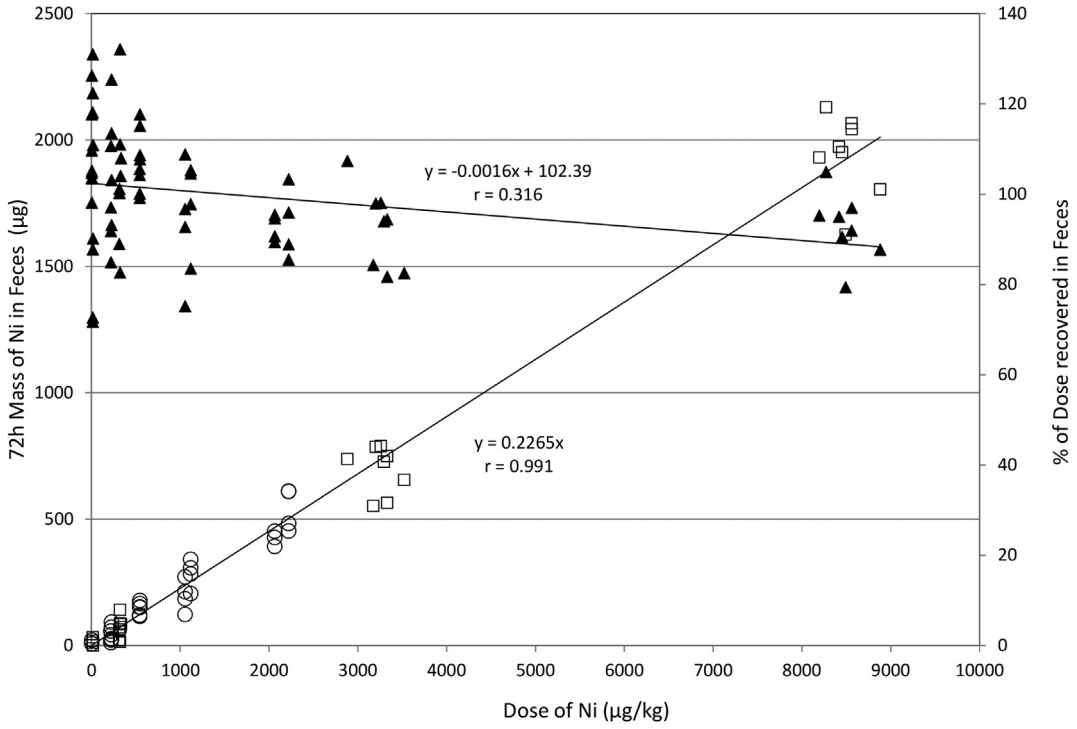

Fig. 3. The baseline-corrected mass of Ni recovered in feces over the 72-h post-dosing period following single gavage dosing. Circles: Dosing with $NiSO_4 \cdot 6H_20$ in water as per SLI (2000) (left y-axis). Squares: Dosing with $NiSO_4 \cdot 6H_20$ in food slurry following from Ambrose et al. (1976) (left y-axis). Filled Triangles: Combined data expressed as a % of the dose (right y-axis).

excretion of approximately 2% over a dose range from background to 8500 µg Ni/kg, regardless of whether the Ni was dosed in water (MS1) or food (MS2).

As with urine, when expressed as a percentage of dose, the recovery of the Ni doses in feces for MS1 and MS2 was essentially constant (zero slope) (Fig. 3) with the exception that the bulk of the dose was

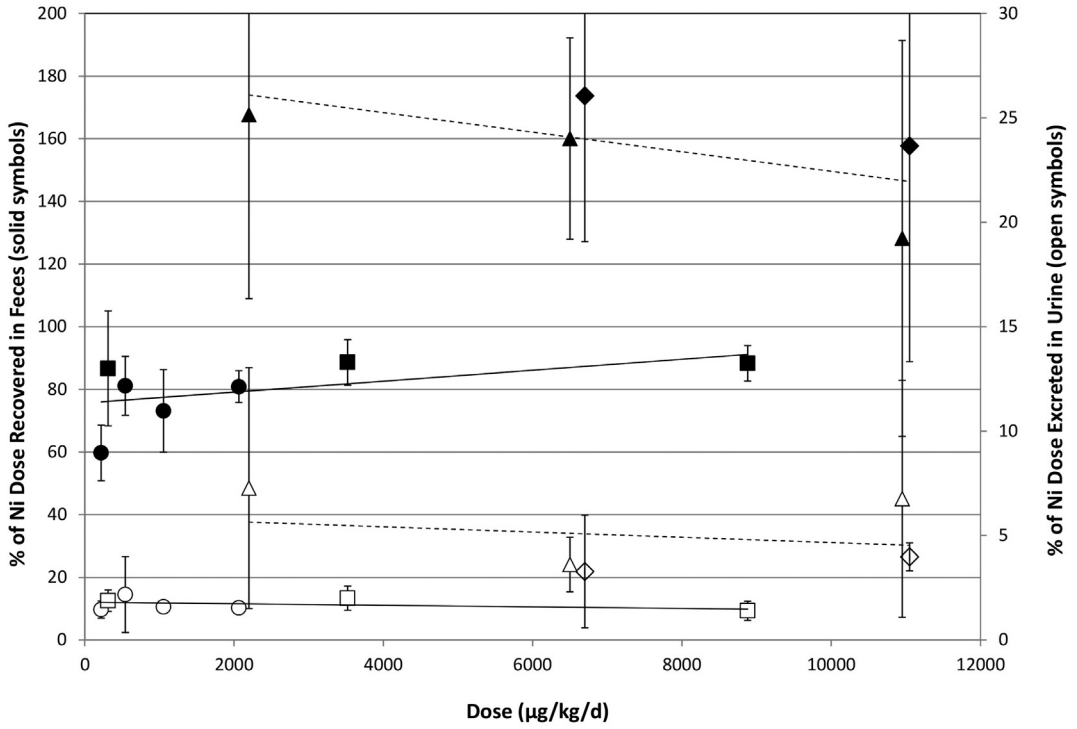

Fig. 4. 24-h urinary and fecal mass balances of Ni (% of gavage dose) for MS1 and MS2 (circles and squares) and the 90-day satellite study (diamonds) and 103 week samples (triangles) from CRL (2005). Error bars are 95% C.I. Urinary mass excretion (% of gavaged dose) is plotted on the right y-axis (open symbols). Fecal recovery (% of gavaged dose) is plotted on the left y-axis (filled symbols). Some x-values are slightly offset for visual clarity. Solid trend lines represent combined data from MS1 and MS2; urine y = −0.000037x 1.81 ($r = 0.384$); feces 0.0017x 75.7 ($r = 0.520$). Dashed trendlines represent combined data from 90-d and 103 week data from CRL (2005); urine y = −0.0013x 5.92 ($r = 0.246$); feces y = −0.0031x 180.8 ($r = 0.654$).

Table 5
Soil Ni, Cu, Co, and As concentrations (reconstructed from dosing solutions) and urinary and fecal mass balances for Port Colborne soils orally dosed by gavage to male Sprague Dawley rats. Values within square brackets are lower and upper 95% confidence limits.

Sample ID	Soil type	[Ni] (mg/kg)	[Cu] (mg/kg)	[Co] (mg/kg)	[As] (mg/kg)	Urinary Ni mass balance (%)	Fecal Ni mass balance (%)	Urinary Cu mass balance (%)	Fecal Cu mass balance (%)	Urinary Co mass balance (%)	Fecal Co mass balance (%)	Urinary As mass balance (%)	Fecal As mass balance (%)
TP9 (5–7.5 cm)	Fill	11,515	810	196	53	0.045 [0.041,0.04]	93.7 [87.1, 100.3]	−1.7 [−2.0, −1.4]	108 [87, 129]	0.88 [0.47, 1.28]	52.2 [27.0, 77.3]	0.2 [−1.1, 1.5]	100.2 [94.6, 105.8]
TP9 (7.5-10 cm)	Fill	16,739	818	246	88	0.04 [0.03, 0.05]	68.0 [2.1, 133.9]	−0.3 [−1.1, 0.6]	109 [24, 193]	0.96 [0.42, 1.49]	57.2 [6.4108.1]	0.2 [−0.2, 0.5]	42.4 [−1.9, 86.7]
TP9 (10–12.5 cm)	Fill	8575	239	115	55	0.05 [0.03, 0.07]	142.3 [106.8, 177.8]	4.9 [−11.3, 20.9]	562 [−140, 1265]	1.06 [0.58, 1.54]	149.7 [116.6, 182.8]	−0.7 [−1.6, 0.3]	53.9 [38.2, 69.6]
TP9 (12.5–15 cm)	Fill	8289	828	110	57	0.08 [0.05, 0.10]	233.4 [177.2, 289.6]	−0.4 [−1.1, 0.2]	146 [110, 183]	1.10 [0.77, 1.42]	200.0 [151.3, 248.7]	0.8 [0.3, 1.3]	131.2 [97.2, 165.2]
TP17 (10–12.5 cm)	Fill	3462	384	37	23	0.23 [0.16,0.29]	110.7 [13.9, 207.4]	−1.3 [−2.5, −0.1]	108 [37, 179]	2.04 [1.24, 2.84]	83.0 [24.6, 141.5]	0.7 [−1.3, 2.7]	32.0 [−19.1, 83.1]
Average - Fill		9716	616	141	55	0.09	129.6	0.23	207	1.2	108.4	0.3	72
TP6 (2.5–5 cm)	Clay	13,756	1535	186	61	0.13 [0.11, 0.14]	107.7 [60.1, 155.3]	−0.4 [−0.7, −0.1]	98 [51, 144]	2.19 [1.56, 2.82]	93.2 [54.8, 131.6]	1.0 [0.2, 1.9]	76.0 [41.6, 110.3]
TP-J2 (5–10 cm)	Clay	5861	779	103	29	0.06 [0.04,0.08]	71.9 [47.5, 96.3]	−0.4 [−0.5, −0.2]	70 [45, 95]	0.65 [0.43, 0.87]	65.3 [43.9, 86.7]	−0.3 [−0.7, 0.1]	55.7 [32.2, 79.2]
TP-J2 (10–15 cm)	Clay	3356	420	48	21	0.07 [0.06, 0.08]	88.7 [61.5, 115.9]	−0.7 [−1.1, −0.3]	87 [59, 114]	0.89 [0.72, 1.06]	84.3 [60.3, 108.3]	−0.1 [−0.5, 0.3]	65.8 [43.3, 88.2]
TPK2–1 (5–10 cm) a	Clay	972	113	22	10	0.05 [0.02, 0.07]	90.8 [62.1, 119.4]	−3.2 [−4.9, −1.5]	32 [−102, 166]	0.73 [0.19, 1.27]	78.3 [48.1, 108.5]	−3.4 [−5.4, −1.4]	49.1 [7.9, 90.3]
TPK2–1 (5–10 cm) b	Clay	914	118	20	10	0.01 [−0.01, 0.03]	112.5 [81.0, 143.9]	−4.1 [−5.1, −3.2]	287 [43, 530]	0.04 [−0.23, 0.32]	128.6 [91.6, 165.6]	−3.7 [−4.8, −2.6]	104.3 [50.5, 158.2]
Hruska	Clay	4942	455	69	15	0.14 [0.13, 0.15]	72.0 [68.5, 75.5]	−4.3 [−5.2, −3.3]	107 [89, 126]	1.48 [0.52, 2.45]	77.0 [72.8, 81.1]	−3.7 [−8.8, 1.4]	125.0 [112.5, 137.4]
TP206 (35 cm)	Mineral	5558	333	48	28	0.08 [0.07, 0.10]	55.1 [23.7, 86.6]	−3.7 [−5.7, −1.7]	−196 [−311, −81]	1.27 [0.77, 1.77]	4.4 [−44.7, 53.5]	−2.1 [−3.3, −0.9]	15.6 [−7.5, 38.6]
TP206 (35–40 cm)	Mineral	7136	518	97	33	0.05 [0.04, 0.06]	15.2 [−2.2, 32.6]	−5.0 [−5.9, −4.1]	−63 [−146, 20]	0.38 [0.13, 0.63]	3.7 [−19.3, 26.8]	−4.8 [−6.0, −3.5]	−1.2 [−12.9, 10.5]
Average - Clay		5312	534	74	26	0.07	76.7	−2.7	53	0.95	66.9	−2.1	61.3
Groetlaar (0–15 cm)	Organic	8136	822	101	42	0.45 [0.24, 0.67]	83.6 [57.5, 109.6]	−2.8 [−3.4, −2.2]	67 [33, 101]	1.67 [1.10, 2.24]	74.1 [50.0, 98.2]	−2.0 [−3.9, −0.2]	55.1 [32.9, 77.4]
SS20	Organic	240	61	10	24	0.38 [0.20,0.57]	56.1 [32.2, 79.9]	−23.2 [−33.9, −12.5]	−245 [−463, −26]	−1.00 [−2.55, 0.55]	25.4 [−34.8, 85.6]	−3.7 [−5.6, −1.7]	52.9 [38.6, 67.3]
TPS (0–2.5 cm)	Organic	2022	226	33	19	0.19 [0.17, 0.21]	81.3 [72.6, 90.1]	−5.1 [−6.0, −4.2]	170 [128, 211]	1.40 [0.42, 2.38]	94.0 [79.6, 108.4]	4.0 [1.9, 6.1]	85.9 [69.0, 102.8]
TP-S (2.5–5 cm)	Organic	1527	196	26	17	0.33 [0.17, 0.50]	57.8 [28.7, 86.9]	−6.8 [−11.3, −2.2]	25 [−14, 64]	3.98 [0.18, 7.79]	59.8 [35.7, 83.9]	−1.4 [−11.2, 8.4]	30.6 [3.7, 57.6]
TP-S (10–15 cm) a	Organic	1609	210	27	19	0.09 [0.03, 0.13]	37.0 [19.7, 54.2]	−6.3 [−9.4, −3.1]	−44 [−87, −2]	1.21 [0.20, 2.21]	22.3 [0.7, 43.8]	−5.3 [−8.1, −2.6]	18.8 [2.8, 34.8]
TP-S (10–15 cm) b	Organic	1563	70	27	20	0.29 [0.21, 0.37]	55.8 [32.9, 78.6]	−30.1 [52.8, −7.4]	−106 [−313, 102]	3.39 [2.10, 4.68]	31.1 [7.7, 54.6]	−4.2 [−7.7, −0.7]	25.8 [6.8, 44.7]
Earthworm Soil	Organic	2971	122	46	27	0.37 [0.22, 0.52]	31.7 [14.3, 49.0]	−17.5 [−24.2, −10.7]	−555 [−852, −258]	1.59 [0.89, 2.29]	−18.3 [−49.9, 13.3]	−3.9 [−6.3, −1.6]	2.1 [−11.9, 16.2]
Average - Organic		2581	244	38	24	0.30	57.6	−13.9	−98.4	1.75	41.2	−2.4	38.8
Earthworms	Tissue	6.9	3.7	1	1	6.87 [2.27, 11.48]	–	–	–	−3.99 [−16.82, 8.85]	–	–	–

recovered in the feces. The considerable variability in fecal recovery seen around the low doses likely reflects variation in food consumption, which can be a significant fraction of the exposure at low applied doses. The recovery of the Ni applied dose from the pooled MS1 and MS2 data was 99.6% (95% CI) [96.6; 102.6] in the feces and 2.1% [2.0; 2.2] in the urine, indicating an essentially complete closed Ni mass balance in these two mini-studies, with the 2.1% urinary Ni excretion representing absolute Ni BAv (Ni ABA). These results suggest that 2.1% BAv was likely to have been present in the two reproductive studies which form the basis for many jurisdictional oral Ni TRVs, namely SLI (2000) and Ambrose et al. (1976).

The two mini-studies justify the mass balance approach for evaluating the BAv of Ni and the other contaminant elements present in Port Colborne soils and enable the adjustment of the applied doses of Ni in Ambrose et al. (1976) and SLI (2000) to an absorbed-dose basis for assessment of risk. For comparison, our 24-h urinary and fecal Ni data were superimposed on measurements after 90 days and 103 weeks of continuous daily gavage doses of 10, 30, and 50 mg/kg body weight (as $NiSO_4 \cdot 6H_2O$) from a two-year oral carcinogenicity study (Heim et al., 2007) (Fig. 4). Even after receiving high doses of Ni for the previous 90 days or 103 weeks, the urinary Ni intercept (5.92% of dose) implies BAv of approximately 6%. Part of the apparent urinary Ni excretion would certainly have included carry-over from previous daily doses. Thus, the apparent BAv of Ni after either a single or repeated dose was likely closer to the 2% observed in MS1 and MS2. The CRL fecal recoveries were well over 100% (y-axis intercept of 181% of dose), which also very likely reflects that the animals had been dosed continually, with the fecal mass of Ni reflecting multiple prior daily doses. These data support our current findings for the BAv of nickel sulfate and, because there is no carry-over from previous doses, confirms that single oral doses are most suitable for estimating BAv using a mass balance approach. The data demonstrate a close to first-order kinetics for Ni absorption over a broad range of concentrations and indicate that systemic Ni BAv estimated with single exposures can be extrapolated to systemic BAv after repeated exposures.

The literature on the oral BAv of Ni from the sulfate salt in rats generally agrees with our findings although there are discrepancies in study methodologies that complicate comparison. Ishimatsu et al. (1995) found the BAv of nickel sulfate (estimated from Ni in blood, urine, and selected organs) to be 11% after 24 h. Ishimatsu did not evaluate fecal Ni, so total mass balance was not possible. In contrast, Vasiluk et al. (2011) reported BAv of nickel sulfate 24 h after dosing to be 39% by fecal mass balance. For comparison, if BAv were estimated as the difference between applied dose and Ni recovered in feces, the equivalent pooled 24-h BAv estimate across the four aqueous nickel sulfate doses in MS1 was 26% (95% C.I. [12.4%, 40.1%]), which is not dissimilar to the Vasiluk et al. value. Fecal or urinary mass balance in isolation from each other provide an incomplete estimate of Ni BAv. Total urinary and fecal mass balance over 72 h is preferred.

3.5. Oral bioavailability of Ni, Cu, Co, and As from Port Colborne soils

The daily urinary and fecal Ni, Cu, Co, and As mass balance data in MS3 were summed over 72-h and reported with the 72-h totals from the main study (Table 5). The soil metal concentrations reconstructed from the dosing solutions are generally similar to the values from the subsamples used for BAc testing (Table 2), but sufficiently different to remind us of the particulate nature of the metal contamination in these soils, with differences existing in particle density and particle size among the metal-bearing particles in the soils, the proportions of which can vary considerably even within sub-samples from the same sampling location.

For Ni, the urinary mass balance (ABA) values ranged from 0.01 to 0.45%, somewhat less than the 2.1% seen for the highly soluble nickel sulfate salt in MS1 and MS2. The low apparent oral BAv of the Ni in these soils reflects the presence of poorly soluble species such as bunsenite, Ni ferrite, and Orford slag, the primary Ni species in the contaminated soil. Given the low percentage of the Ni dose being present in the urine, it would be expected that the majority of the dose be present in the fecal mass balance. However, although the confidence intervals for fecal Ni mass balance did encompass 100% for the fill and clay/mineral soils (i.e. closed mass balance), there was considerable variability seen between the soils, and the fecal recoveries tended towards not being complete, particularly in the organic soils (Table 5). The reasons for this variability are unclear. Tracers of gastrointestinal transport could potentially be used to identify the reasons for these discrepancies.

The mass balances in Table 5 were calculated after subtracting the baseline urinary and fecal Ni, Cu, Co, and As levels observed in the pooled controls (Table 3). For Ni and Co, the confidence intervals for the small urinary mass recoveries were non-zero, indicating BAv. Negative values for baseline-corrected urinary Cu, with confidence intervals that bracket zero, should be thought of as “zeroes”, reflecting that Cu is not regulated in the kidney and that urinary excretion is not a good measure of bioavailable Cu. Estimating Cu BAv from the soils was confounded by the Cu supplementation of the rat chow. The approximate dose of Cu from the soil containing the highest Cu concentration (TP6 (2.5–5 cm) – 1535 mg/kg) would be 613 μg, a mere 60 μg less than from the $CuSO_4$-supplemented rat chow. The highly variable fecal mass balances demonstrate that the study could not distinguish between Cu from highly-contaminated Port Colborne soils and that in the diet (Table 5), suggesting the likely absence of oral risk for Cu from these soils.

Co, which is primarily excreted via the kidney, showed small incremental increases in urinary excretion following dosing, but the fecal mass balances were variable, in some cases being relatively low (Table 5). Arsenic, which is also excreted from the kidney, could not be distinguished from baseline urinary As in the main study. Fecal mass balances for As were also quite variable. After gavage dosing of As-contaminated Port Colborne soils, there was no conclusive observation of increased urinary As output that would indicate bioavailable As had been ingested in excess of the baseline dietary exposure. For the fill and clay soils as groups, the confidence intervals for the fecal mass balances of As bracketed 100%, but the fecal mass balance of As in the organic soils could not be closed. This could reflect a pool of As undergoing detoxification in the liver prior transport to the kidney before urinary excretion.

3.6. BAv/BAc relationships for Ni, Co, and As in Port Colborne soils

Oral BAc testing provides a bridge in the understanding between total metal concentrations in soil and the proportion of which is truly bioavailable. Here, 19 of the soils that underwent BAc testing were also gavage-dosed to rats for BAv estimation. Fig. 5 presents in vivo oral ABA estimates as a function of the Ni, Co, and As BAc estimates for these 19 samples. The linear regressions for Co and As have small correlation coefficients because the slope of the BAv/BAc lines for these contaminants are flat, with BAc being large multiples of the corresponding BAv for the same soils. In contrast, the BAv/BAc relationship for Ni has a relatively large (0.716) correlation coefficient (Fig. 5).

Having generated BAv data for the soluble nickel sulfate in MS1 and MS2, the ABA data for Ni in Port Colborne soils can also be expressed as RBA, in which ABA values of the soils are divided by the ABA (roughly 2.1%) of $NiSO_4 \cdot 6H_2O$ (Fig. 6). From this comparison, it can be seen that nickel BAc represents a very conservative estimate of Ni ABA and is a good predictor of Ni RBA.

3.7. Trophic transfer of metals in Port Colborne soils

An organic soil having Ni, Cu, Co, and As concentrations of 2971, 122, 46, and 27 mg/kg, respectively, and a sample of co-located mixed population of gut-cleared earthworms containing 6.9, 3.7, 0.5, and 0.6 mg/kg (fresh weight), respectively, were orally dosed to rats to simulate

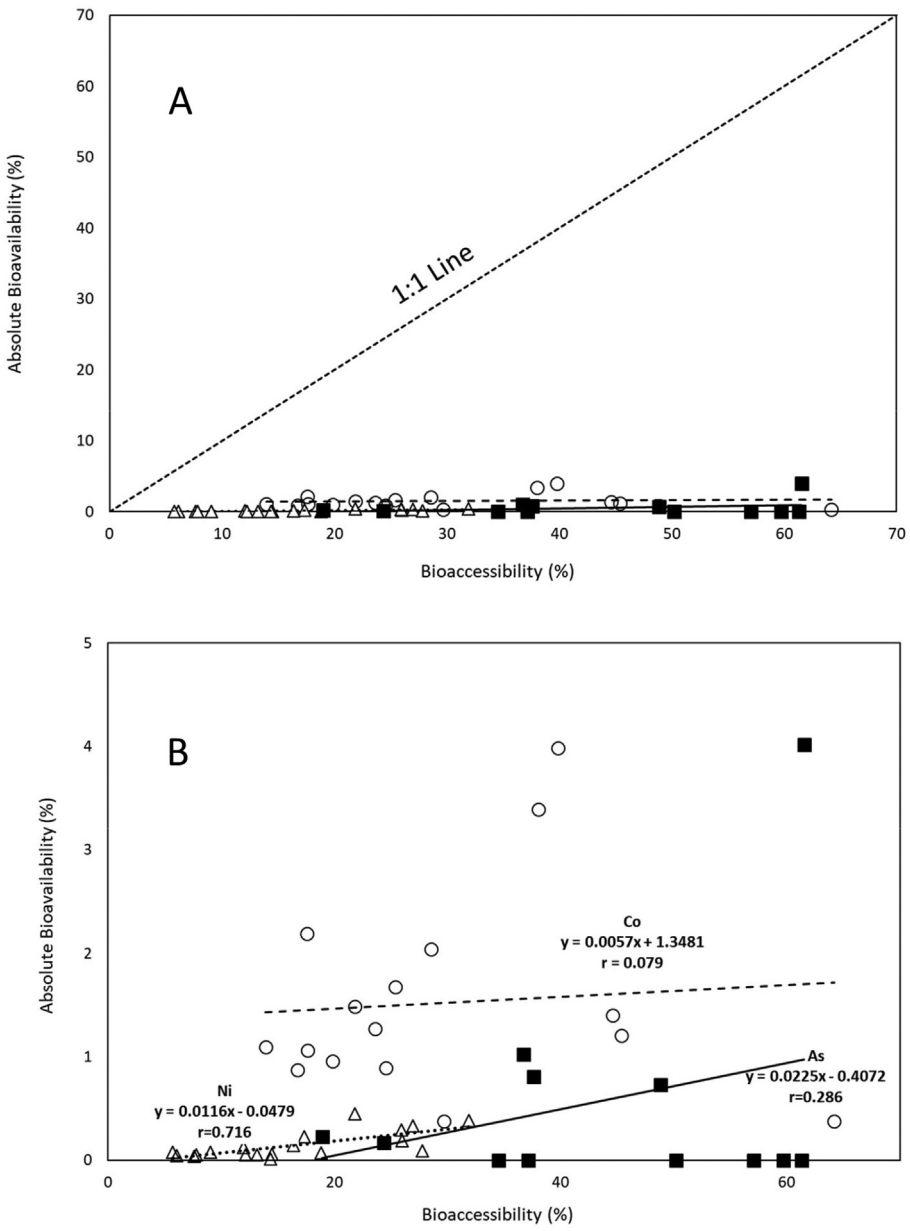

Fig. 5. Absolute bioavailability (urinary mass balance (%)) of Ni, Co, and As in relation to in vitro bioaccessibility (%) for Port Colborne soil samples. Triangles – Ni; Circles – Co; Squares – As. (A). Perspective showing the 1:1 line. (B). Close-up view of the same data.

trophic transfer from soil to soil invertebrate to rodent (Table 5). The biota-soil accumulation factors (BSAF) were 0.002 (Ni), 0.028 (Cu), 0.011 (Co), and 0.022 (As). Due to the low levels of the study metals in the earthworms, it was not possible to close the mass balances from the in vivo dosing of rats, and urinary mass balances were only seen for Ni and Co. For Ni, 6.9% of the dose was excreted in the urine (95% C.I. [2.27, 11.48]). The confidence band for BAv of Ni from earthworms overlaps with that of nickel sulfate, but the upper limit suggests slightly higher BAv for the Ni in earthworm than for nickel sulfate. This could be due to the presence of Ni metallothionein in the earthworms as seen in smelter-impacted soils elsewhere (Mustonen et al., 2014), which could account for somewhat altered BAv from that of the Ni salt. The 95% confidence interval for Co urinary mass balance was 12.8%, which when considered with the negative mass balance (−4%) identifies that Co excretion after dosing with earthworms was not distinguishable from baseline urinary Co output. Cu and As mass balances were also not measurable from earthworm oral dosing. Together, the BSAFs and BAv findings suggest little potential for trophic transfer of the metals from the contaminated soils of Port Colborne.

3.8. Conclusions

A mass balance approach was used to successfully quantify the BAv of Ni in the key studies upon which most regulatory Ni TRVs are based. The essentially complete mass balance shows orally dosed Ni from a highly soluble salt can be completely mass balanced, with roughly 2% of the applied dose in urine indicating 2% ABA. By extension, urinary excretion of Ni after oral exposure to other media (uncontaminated diet or contaminated soil) represents the oral BAv. Unaccounted-for Ni due to poor fecal recovery should not be assumed to indicate greater bioavailability than for a soluble Ni salt. Instead, other explanations should be sought; mass balancing conservative tracer elements could help understand the poor fecal recoveries after soil ingestion. For Ni, the baseline ABA from uncontaminated diet was also roughly 2%, while for the

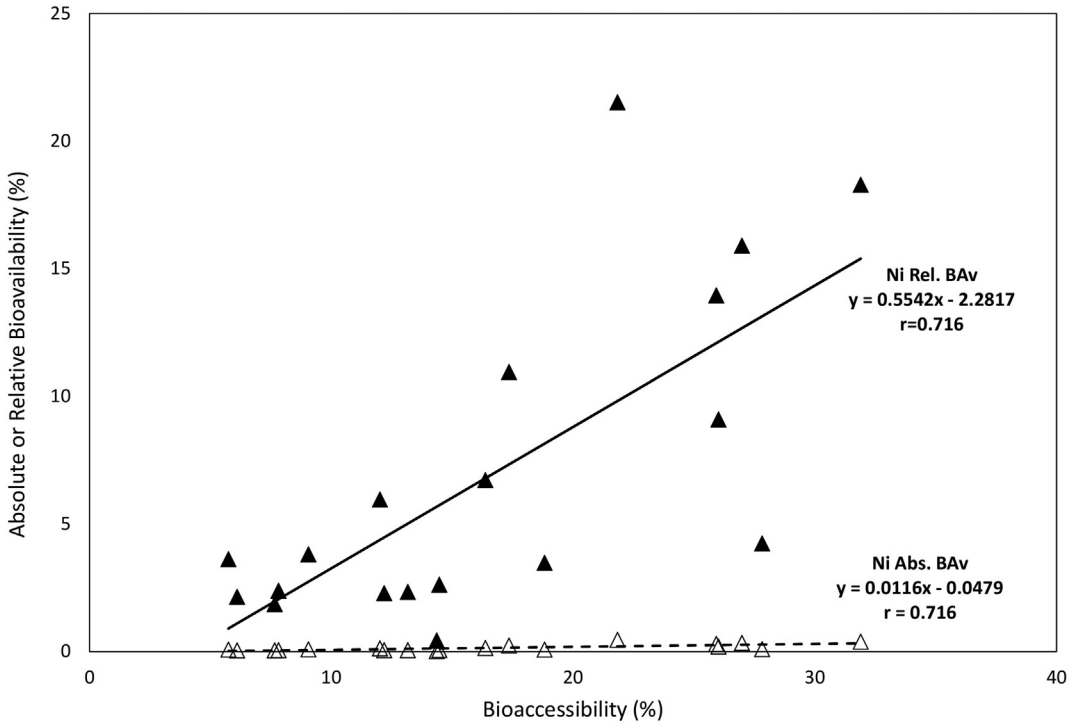

Fig. 6. Absolute and relative bioavailability (urinary mass balance – %) of Ni in relation to in vitro bioaccessibility (%) for 19 Port Colborne soil samples. Open triangles – Ni absolute bioavailability; Filled triangles – Ni relative bioavailability.

contaminated soils the ABA ranged from 0.07% in clay soil to 0.3% in organic soil.

For Co and As, which, like Ni, are excreted in urine, the same logic should apply – that the urinary mass excretion reflects oral BAv. However, our findings are difficult to interpret due to dietary Co supplementation in feed. It is likely that our urinary As BAv estimates reflect true ABA, in spite of the complicated As metabolism (hepatic methylation followed by urinary excretion). Further study is required for these two elements.

For Cu, which is primarily excreted via bile, dietary Cu supplementation confounded fecal mass balances, with very large confidence intervals for all soil samples. What can be said is that it was not possible to demonstrate enhanced uptake due to ingestion of the Cu-contaminated Port Colborne soils or earthworms living in Cu-contaminated soil. It is likely that risk due to Cu in these soils is quite small.

BAc measurements provide upper limit estimates of oral BAv that can be incorporated in risk assessment, and reflect the potential for contaminants to be solubilized in the gastrointestinal tract. However, bioelution methods currently cannot mimic all key components of BAv (competitive inhibition of uptake, absorption), which explains in part why, for these soils, BAc was roughly 100 times higher than the BAv measured in vivo. The relationship between RBA and BAc derived for these soils is a useful advance for developing exposure narratives for these and other contaminated soils. The Ni RBA-BAc relationship enables BAv extrapolation to be used in evaluating oral exposure and risk in other situations where BAc estimates are available but BAv estimates are not.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.05.164.

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