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Bioaccessibility of metals in fish, shellfish, wild game, and seaweed harvested in British Columbia, Canada

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ABSTRACT

Fish, shellfish, wild game, and seaweed are important traditional foods that are essential to the physical and cultural well-being of Indigenous peoples in Canada. The goal of this study was to measure the concentration and bioaccessibility of As, Cd, Hg, Se, Cu and Mn in 45 commonly consumed traditional foods collected by harvested by the First Nations Food, Nutrition, and Environment Study (FNFNES) from 21 First Nations communities in British Columbia, Canada, in 2008–2009. A significant and negative correlation was observed between Hg concentration and Hg bioaccessibility. Metal bioaccessibility tended to be high; median values ranging between 52% (Mn) and 83% (Cu). The notable exceptions were observed for As in wild game organs (7–19%) and rabbit meat (4%) as well as Hg in salmon eggs (10%). Results of Principal Components Analysis confirmed the unique pattern of bioaccessibility of As and Hg in traditional foods, suggesting that, unlike other metals, As and Hg bioaccessibility are not simply controlled by food digestibility under the operating conditions of the *in vitro* model. These data provide useful information for dietary contaminant risk assessment and intake assessments of essential trace elements.

1. Introduction

Traditional foods such as moose, deer, and salmon are rich sources of essential trace elements (ETEs) and serve as the foundation of a healthy diet for thousands of First Nation peoples in the province of British Columbia (Chan et al., 2011). However, a dietary transition within First Nations communities has seen a decline in the consumption of traditional foods and an increase in processed foods high in sodium, carbohydrates, and fat (Chan and Receuver, 2000; Kuhnlein et al., 2004). Not only does this dietary transition result in cultural loss, but also increased prevalence of obesity, diabetes, cardiovascular disease, gall bladder disease, anemia, and may be linked to increases in some types of cancer (Kuhnlein and Receuver, 1996). Research has documented that the nutritional value of the diets of First Nations peoples could be improved by increasing consumption of traditional foods (Chan et al., 2006; Downs et al., 2009; Kuhnlein et al., 2002); therefore, better communication regarding which foods are safe and which foods are unhealthy is of utmost importance. This conclusion has resulted in initiatives at the local, provincial, and federal levels communicating the healthfulness of traditional foods. For example, Canada's Food Guide for First Nations, Inuit, and Métis recommends: daily consumption of traditional meats, weekly consumption of fish and shellfish, and the use of traditional fats (e.g. ooligan grease) as all or part of the daily allotted 30–45 mL of unsaturated fat (Health Canada, 2007).

Global transport of environmental pollutants and ever improving analytical techniques make the detection of Contaminants of Potential Concern (COPC) in traditional foods commonplace, even in pristine environments without point-source contamination from resource development. For example, Pb and Cd can be found in the edible tissues of waterfowl and game birds (Belinsky and Kuhnlein, 2000; Braune and Malone, 2006), Hg can be found in the flesh and eggs of fish (Belinsky et al., 1996; Bureau of Chemical Safety, 2007), As and Cd can be found in fish, shellfish and the kidneys of terrestrial biota (Bragigand et al., 2004; Gamberg et al., 2005; Koch et al., 2007; Laparra et al., 2007). However, the detection of environmental contaminants in traditional foods does not necessarily denote the presence of health risk. Instead, health risk is a function of the food consumption pattern of the receptor, the concentration and oral bioavailability of the COPC in the food items, and the sensitivity of the receptor to the COPC (Health Canada, 2004).

Oral bioavailability refers to the fraction of an ingested contaminant that reaches systemic circulation and is typically measured according to post-exposure blood concentrations using *in vivo* models (Versantvoort et al., 2004). *In vitro* gastrointestinal (GI) models offer an affordable and reproducible approach for the calculation of bioaccessibility (Intawongse and Dean, 2006; Moreda-Pineiro et al., 2011), which refers to the fraction of an ingested

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COPC solubilized in simulated GI fluids (Laparra, 2003). Since dissolution is a necessary precursor to absorption, bioaccessibility may be used as a surrogate for oral bioavailability in exposure assessments (Versantvoort et al., 2004). Although operational parameters (e.g. enzyme concentration, fluid composition, Liquid:-Solid ratio, etc.) vary from one model to another (Van de Wiele et al., 2007), each model simulates a subset of physiochemical and enzymatic conditions of the human GI tract (Adenugba et al., 2008; Cabanero et al., 2007; He et al., 2010; Laird et al., 2009; Mateo et al., 2011).

Previous studies demonstrate that the percent bioaccessibility of As, Hg and other trace elements only occasionally approach 100% (Cabanero et al., 2004, 2007; Moreda-Pineiro et al., 2012a,b; Torres-Escribano et al., 2011a,b). For example, HgT (methylmercury + inorganic Hg^{II}) bioaccessibility from seaweed and lobster hepatopancreas were 3% and 4%, respectively (Torres-Escribano et al., 2011a). Similarly, the bioaccessibility of HgT in Tuna ranged between 13% and 19% (Torres-Escribano et al., 2011b) and the HgT bioaccessibility for sardines, tuna, and swordfish ranged between 9% and 17% (Cabanero et al., 2004). Arsenic bioaccessibility, on the other hand, has been seen to be substantially higher, ranging between 83% and 99% in whitefish, cold water fish, and molluscs (Moreda-Pineiro et al., 2012a). Thus, the assumption that 100% of ingested COPC and trace elements are bioaccessible (Health Canada, 2004) may overestimate exposure in some circumstances but not in others.

Prior to the work described in this article, little to no information was available describing the bioaccessibility of metals in the traditional foods of First Nations in British Columbia. The primary objective of this work was to measure the concentration and *in vitro* bioaccessibility of ETE (Cu, Se, Mn) and COPC (As, Cd, Hg) in a diverse array of traditional foods collected in British Columbia. We hypothesized that: (i) the bioaccessibility of trace elements would tend to be less than 100%, (ii) bioaccessibility would differ between trace elements and food items, and (iii) bioaccessibility of trace elements would be highly correlated with each other.

2. Materials and methods

2.1. Sample collection

Traditional foods were collected in 2008 and 2009 from 21 on-reserve British Columbia (BC) First Nations communities as part of the FNFNES (Fig. 1). Sampling proceeded using an Ecozone and Culture Area framework to effectively capture the variability in traditional food use between geographic regions in the province of BC. Up to 30 uncooked composite samples (with each sample combining tissues from five organisms) were collected from each of the 21 participating communities for a total of 427 composite traditional food samples. Of these 427 composite samples, 45 were selected for bioaccessibility analysis to include the most commonly consumed traditional food in BC. These foods included Chinook salmon ($n = 4$), butter clams ($n = 4$), salmon eggs ($n = 6$), sockeye salmon ($n = 5$), deer liver ($n = 3$), moose kidneys ($n = 5$), moose liver ($n = 7$), deer meat ($n = 3$), moose meat ($n = 3$), rabbit meat ($n = 3$), and laver seaweed ($n = 2$).

2.2. Total metals

Uncooked composite food samples were digested using an open vessel with concentrated nitric acid and hydrogen peroxide using methods based upon those described in EPA Method 200.3. Total concentrations of As, Cd, Mn, Se, Pb, and Cu were measured using ICP-MS whereas total Hg was measured using CVAFS. Each concentration was expressed in terms of wet weight. Each batch of samples included blanks, duplicates, and standard reference materials. Percent recovery of certified reference materials ranged between 83% (Mn) and 118% (Cd). The Limit of Detection (LOD) for As, Cd, Mn, Se, Cu, Pb, and Hg were 20, 4, 20, 20, 20, and 4 ng g^{-1}, respectively.

2.3. In vitro extraction

The operating conditions of the *in vitro* GI model were designed, with modifications, from those previously used for the digestion of Inuit traditional foods (Laird et al., 2009). Each uncooked food item (2 g) was macerated and transferred into a 150 mL glass serum bottle. Thereafter, each sample was subjected to simulated stomach (2 h; 130 rpm; 37 °C; Liquid:Solid Ratio 15:1) and simulated small intestinal (3 h; 130 rpm; 37 °C; Liquid:Solid Ratio 25:1) solutions. The previously described GI solutions (Laird et al., 2009) were modified to improve *in vitro* digestion of the food items. Specifically, the simulated stomach solution was modified to contain pH 2.0 HCl, pepsin (0.6%; Sigma Aldrich) and NaCl (0.85%; VWR). Further, the previously described small intestinal phase was modified to include a 5 mL aliquot of a saturated $NaHCO_3$ (VWR) solution plus a 15 mL aliquot of an enzyme solution containing $NaHCO_3$ (12.5 g L^{-1}; VWR), Oxgall (6.0 g L^{-1}; Sigma Aldrich), and porcine pancreatin (3.0 g L^{-1}; Sigma Aldrich).

The bioaccessible fraction of the *in vitro* extracted food samples was separated via centrifugation (5000 g; 20 min). An aliquot (10 mL) of the supernatant was acidified with nitric acid prior to microwave-assisted digestion and elemental analysis via ICP-MS. Aliquots (2 mL) of the acidified *in vitro* extracts were acid digested with 1 mL of concentrated nitric acid in a UltraWave Single Reaction Chamber (SRC) microwave (Milestone) at 220 °C for 5 min (16 min ramp; 12.3 °C min^{-1}). The digested sample were diluted to 15 mL with MQ water and the concentrations of 23 elements were measured in the *in vitro* extracts, including Mn, Cu, As, Se, Cd, Pb, and Hg. Percent bioaccessibility was blank corrected by subtracting each respective metal concentration in the blanks (i.e. simulated GI fluid in the absence of traditional food).

2.4. Statistics

Summary statistical analysis, Pearson correlation, and Principal Components Analysis (PCA) were conducted using SPSS Statistics (IBM, Version 20). For foods where either the bioaccessible or total concentrations were below the Limit of Detection (LOD), half the LOD was used. Samples where both the bioaccessible and total analyte concentrations were below the LOD were excluded from subsequent analysis. Pearson correlations were declared significant when $P < 0.05$. PCA was used to identify the extent to which the bioaccessibility of metals varied with one another. This enabled us to evaluate whether foods that showed high bioaccessibility for one metal also showed high bioaccessibility for other metals. To ensure that $n_{observations} - n_{variables} > 4$, two PCA were run. The first PCA studied the intercorrelations between As, Cd, Mn, Se, and Cu while the second PCA studied the intercorrelations between As, Mn, Se, and Hg. The correlation matrices were checked for singularity and Bartlett's Test of Sphericity was confirmed ($P < 0.001$). The Kaiser–Meyer–Olkin Measure of Sampling Adequacy was 0.642. Principal components were extracted based upon eigenvalues greater than 1.0 with convergence within 25 iterations and Varimax rotation was employed.

3. Results and discussion

3.1. Metal concentrations in food samples

The concentrations of metals that were consistently detected (As, Cd, Hg, Se, Mn, and Cu) in fish, shellfish, wild game, and seaweed are reported in Table 1. In contrast, Pb concentrations tended to be at or below the LOD (20 ng g^{-1}). Exceptions to this trend included (mean ± SD): butter clams (0.065 ± 0.070 μg g^{-1} Pb), rabbit meat (0.25 ± 0.31 μg g^{-1} Pb) and laver seaweed (0.70 ± 0.71 μg g^{-1} Pb). In addition, two of the five moose kidney samples exceeded the LOD with Pb concentrations of 0.85 and 0.15 μg g^{-1}.

Overall, the BC country foods evaluated in this study were of high nutritional quality with few contaminant-related concerns (Chan et al., 2011). For example, Hg concentrations in fish such as sockeye and Chinook salmon were below the Health Canada guidelines of 0.5 mg kg^{-1} (Health Canada, 2012). Arsenic concentrations in butter clams were within the normal background concentrations of clams (Edmonds and Francesconi, 1993; Koch et al., 2007). As observed elsewhere (Almela et al., 2002; Laparra, 2003), total As in laver seaweed was approximately 10-times the 3 mg kg^{-1} inorganic As guideline used in France and the USA (Mabeau and Fleurence, 1993). However, previous work has shown that only a small fraction of As in *Porphyra* sp. is inorganic and is instead predominately arsenosugar (Almela et al., 2002; Llorente-Mirandes et al., 2011). Therefore, the health risk from the As within these seaweeds may be negligible. The nutrient content of the BC traditional foods tended to meet or exceed the Se, Cu, and Mn values reported for similar foods (e.g. clams, Whitefish eggs, Moose liver, Deer meat, Laver seaweed) in the USDA SR23 database (NDB

Fig. 1. Map showing the 21 British Columbia First Nations Communities that participated in the First Nations, Food, Nutrition, and Environment Study. Sampling proceeded according to the Ecozone/Culture Area framework illustrated in the figure legend.

numbers: 15,157, 35,158, 35,051, 17,343, and 11,446). Consequently, 24-h recall data from the FNFNES showed that the nutrient (energy, protein, fiber, Ca, Fe, Zn, Mg, Cu, K, and P) intake of study participants was higher on days with traditional foods than on days without (Chan et al., 2011).

3.2. Contaminant and nutrient bioaccessibility in BC foods

3.2.1. Overview

Since the Pb concentrations in the majority of the studied traditional foods were below the detection limit, the Pb results were excluded from the bioaccessibility analyses. The bioaccessibility of

Table 1
Mean percent *in vitro* bioaccessibility (standard deviation) of As, Cd, HgT, Se, Mn, and Cu from select BC First Nations traditional foods.

Traditional food	Scientific name	Arsenic		Cadmium		Mercury			Selenium		Manganese		Copper	
		Total ($\mu g\ g^{-1}$)	IVBA (%)	Total ($\mu g\ g^{-1}$)	IVBA (%)	Total ($\mu g\ g^{-1}$)	MeHg[c]	IVBA (%)	Total ($\mu g\ g^{-1}$)	IVBA (%)	Total ($\mu g\ g^{-1}$)	IVBA (%)	Total ($\mu g\ g^{-1}$)	IVBA (%)
Shellfish														
Butter clams ($n = 4$)	*Saxidomus giganteus*	4.50 (*0.48*)	108 (*21.9*)	0.087 (*0.032*)	107 (*20.2*)	0.012 (*0.012*)	50 (*29*)	50 (*28.9*)	0.43 (*0.05*)	98 (*16.6*)	1.43 (*0.33*)	102 (*17.4*)	1.79 (*0.57*)	117 (*16.7*)
Fish & fish eggs														
Salmon eggs ($n = 6$)	NA[d]	0.35 (*0.09*)	73 (*19.7*)	0.005 (*0.003*)	74 (*60.4*)	0.045 (*0.070*)	30 (*49*)	10 (*7.6*)	2.44 (*0.62*)	76 (*18.0*)	0.88 (*0.49*)	96 (*22.3*)	17.0 (*14.0*)	106 (*18.5*)
Chinook salmon ($n = 4$)	*Oncorhynchus tshawytscha*	0.85 (*0.19*)	57 (*15.8*)	0.007 (*0.006*)	18 (*19.8*)	0.088 (*0.077*)	59 (*10*)	49 (*22.1*)	0.48 (*0.17*)	52 (*15.8*)	0.92 (*0.84*)	38 (*21.3*)	0.86 (*0.24*)	64 (*34.6*)
Sockeye salmon ($n = 4$)	*Oncorhynchus nerka*	0.64 (*0.23*)	68 (*4.9*)	0.011 (*0.006*)	61 (*58.4*)	0.077 (*0.028*)	63 (*20*)	46 (*21.3*)	0.48 (*0.17*)	50 (*10.6*)	0.46 (*0.38*)	48 (*20.6*)	1.36 (*1.32*)	63 (*23.7*)
Wild game organs														
Deer liver ($n = 3$)	*Odocoileus virginianus*	0.06 (*0.03*)	10 (*10.3*)	0.18 (*0.15*)	79 (*8.5*)	0.014 (*0.019*)	25	138 (*5.1*)	0.93 (*0.65*)	52 (*6.5*)	2.62 (*2.64*)	81 (*16.0*)	31.7 (*28.3*)	90 (*17.7*)
Moose kidneys ($n = 5$)	*Alces alces*	0.03 (*0.02*)	19 (*16.0*)	14.1 (*10.5*)	68 (*9.1*)	0.015 (*0.016*)	19 (*12*)	63 (*27.1*)	0.77 (*0.32*)	62 (*7.4*)	2.20 (*0.92*)	84 (*23.6*)	3.45 (*0.45*)	88 (*14.1*)
Moose liver ($n = 7$)	*Alces alces*	0.03 (*0.01*)	7 (*6.1*)	2.85 (*2.70*)	48 (*15.5*)	0.004 (*0.001*)	43 (*12*)	100 (*65.8*)	0.90 (*0.52*)	35 (*15.8*)	2.85 (*1.24*)	66 (*17.1*)	43.7 (*31.3*)	79 (*21.3*)
Wild game meat														
Rabbit meat ($n = 3$)	*Lepus townsendii*	0.02 (*0.01*)	4 (*0.1*)	0.80 (*1.38*)	94 (*23.3*)	0.008 (*0.009*)	11	106 (*40.7*)	0.29 (*0.28*)	45 (*17.0*)	0.84 (*0.58*)	27 (*39.4*)	2.05 (*0.85*)	57 (*27.3*)
Deer meat ($n = 3$)	*Odocoileus virginianus*	0.01[a]	NA[b]	0.005 (*0.006*)	NA[b]	0.003 (*0.006*)	50	NA[b]	0.16 (*0.06*)	37 (*18.0*)	0.39 (*0.28*)	30 (*18.2*)	2.06 (*0.19*)	56 (*15.0*)
Moose meat ($n = 3$)	*Alces alces*	0.01[a]	59 (*13.3*)	0.03 (*0.02*)	40 (*11.3*)	0.003[a]	-	NA[b]	0.15 (*0.07*)	29 (*21.4*)	0.55 (*0.25*)	41 (*19.9*)	1.52 (*0.20*)	59 (*16.4*)
Seaweed														
Laver ($n = 2$)	*Porphyra abottae*	33.05 (*2.90*)	79 (*7.4*)	5.58 (*0.25*)	64 (*4.6*)	0.001[a]	-	NA[b]	0.30 (*0.00*)	65 (*25.7*)	22.7 (*2.1*)	86 (*8.4*)	2.90 (*0.42*)	59 (*10.1*)

[a] Total analyte concentration in the food item is below the Limit of Detection.
[b] Not available. Both the bioaccessible and total analyte concentrations were below the Limit of Detection.
[c] Percent of total mercury as methylmercury.
[d] Not available. Salmon species not identified.

COPC and ETE tended to be high, with averages ranging between 54% (selenium) and 83% (manganese) (Table 1). Table 2 describes the upper limit of the 95th confidence interval for the bioaccessibility of each ETE and COPC. Given the variability in bioaccessibility within food types, metal bioaccessibility often approached or was equivalent to 100% (Table 2). Therefore, these results indicate that bioaccessibility will generally have little effect on dietary exposure estimates from traditional foods for BC First Nations. The notable exceptions to this trend were observed for As in wild game organs and rabbit meat as well as Hg in salmon eggs (Table 2).

Butter clams showed the highest percent bioaccessibility (ranging between 98% and 117%) for five of the six analytes of interest (As, Cd, Se, Mn, and Cu) (Table 1). In contrast, the highest Hg bioaccessibility was observed for deer liver (138%). Bioaccessibility values in excess of 100% were occasionally observed due to heterogeneity of samples. Despite the high percent bioaccessibility of trace elements in butter clams, low total concentrations dictated that the maximal bioaccessible quantities were observed in laver seaweed (As), moose kidneys (Cd), salmon eggs (Se), laver seaweed (Mn), and moose liver (Cu). Wild game organs such as deer liver (10%), moose kidneys (19%), and moose liver (7%) showed the lowest As bioaccessibility values while the minimum Se, Mn, and Cu bioaccessibility values were observed for moose meat (29%), deer meat (30%), and deer meat (56%), respectively. It should be noted though that, for two of the three deer liver samples, both the bioaccessible and total HgT concentrations were below the LOD. This indicates that, if present, Hg in deer liver may be maximally bioaccessible but that Hg is only occasionally detected in the livers of deer in BC.

It is important to note that the bioaccessibility values reported herein represent those observed in uncooked foods. However, of the 11 types of foods described in this article, only salmon eggs and seaweed are commonly consumed raw. Limited quantities of the 45 traditional food samples used in this work precluded reanalysis using cooked foods. Previous studies have occasionally shown that cooking can affect metal bioaccessibility. For example, baking *Porphyra* sp. increased As bioaccessibility from 87% to 100% (Almela et al., 2005). Se bioaccessibility in cooked cod was 61% (Crews et al., 1996) similar to the Se bioaccessibility in BC salmon (Chinook 52%; Sockeye 50%). Additionally, research has shown that cooking occasionally decreases the bioaccessibility As, Cd, Cu, Se, and Hg in fish (He et al., 2010; Torres-Escribano et al., 2011b). As previously noted (Torres-Escribano et al., 2011b), the use of bioaccessibility data from raw foods within exposure assessments could potentially overestimate risks resulting from contaminants in foods.

3.2.2. Comparison to previous bioaccessibility reports

Differences in trace element bioaccessibility between studies are difficult to directly compare due to the lack of a generally accepted *in vitro* extraction protocol. Of the metals described in this article, As has the most robust database of previously reported food bioaccessibility values (He et al., 2010; Koch et al., 2007; Laparra, 2003; Laparra et al., 2007, 2004; Moreda-Pineiro et al., 2012a, 2011; Torres-Escribano et al., 2011a; Williams et al., 2009). However, most of this data is specific to As in fish and seaweed. To our knowledge, this is the first study to report the bioaccessibility of As in terrestrial mammal organs such as deer liver and moose kidney. The bioaccessibility of As in laver seaweed was similar to reports from previous studies (i.e., range between 32% and 79%, depending upon seaweed species and *in vitro* model) (Koch et al., 2007; Laparra, 2003; Laparra et al., 2004; Torres-Escribano et al., 2011a). Arsenic bioaccessibility values for fish and shellfish are typically high and often approach 100% (He et al., 2010; Laparra et al., 2007; Moreda-Pineiro et al., 2012a). As such, the maximal As bioaccessibility observed in butter clams collected by BC First

Table 2
Trace element bioaccessibility for each food grouped according to the upper limit of the 95th confidence interval of the mean.

UL[a]	Contaminant of potential concern			Essential trace element		
	As	Cd	Hg	Se	Mn	Cu
⩽33%	Deer liver Moose liver Rabbit meat		Salmon eggs			
34–66%	Moose kidneys	Chinook salmon Moose liver Moose meat		Sockeye salmon Deer liver Moose liver Moose meat	Chinook salmon Deer meat	
66–99%	Salmon eggs Chinook salmon Sockeye salmon Moose meat	Deer liver Moose kidneys Laver seaweed	Butter clams Chinook salmon Sockeye salmon Moose kidney	Salmon eggs Chinook salmon Moose kidneys Rabbit meat Deer meat	Sockeye salmon Moose liver Rabbit meat Moose meat	Sockeye salmon Moose liver Deer meat Moose meat
100%	Butter Clams Laver seaweed	Butter clams Salmon eggs Sockeye salmon Rabbit meat	Deer liver Moose liver Rabbit meat	Butter clams Laver seaweed	Butter clams Salmon eggs Deer liver Moose kidneys Laver seaweed	Butter clams Salmon eggs Chinook salmon Deer liver Moose kidneys Rabbit meat Laver seaweed

[a] Upper limit of the 95th confidence interval of the bioaccessibility of each contaminant of potential concern (As, Cd, Hg) and essential trace element (Se, Mn, Cu).

Nations is consistent with those reported in the literature. In contrast, the As bioaccessibility values observed for Sockeye (64–73%) and Chinook (36–76%) appear to be somewhat lower than previous reports for cold water fish. It is unknown whether these differences are due to varying *in vitro* extraction operating conditions or due to differences in food composition.

Large variation in HgT bioaccessibility has been observed between different types of foods. For example, generally low bioaccessibility (i.e. 9–17%) was observed for tuna, sardines, and swordfish (Cabanero et al., 2004, 2007). But, in another study, the percent bioaccessibility of HgT in swordfish was up to 87% (Torres-Escribano et al., 2011b) and HgT bioaccessibility in mackerel ranged between 75% and 90% depending upon the amount of fish tissue added to the extraction (Hwang and Shim, 2008). HgT bioaccessibility data from Sockeye and Chinook salmon closely resembled HgT bioaccessibility data generated from Arctic char (33–94%) harvested in Northern Canada (Laird et al., 2009). Surprisingly, HgT bioaccessibility from moose kidney (63%) may far exceed that was observed in a caribou kidney collected in the Canadian Arctic (0.7%) (Laird et al., 2009).

Unlike HgT, Se bioaccessibility appears to be remarkably consistent between species of marine fish. For example, the bioaccessibility of Se in Sockeye and Chinook salmon (Table 1) is consistent to values observed for tuna, sardines, swordfish, cod, seabass, and red seabream (Cabanero et al., 2004, 2007; Crews et al., 1996; He et al., 2010). Additionally, Mn, Cu, and Cd bioaccessibility of laver seaweed from BC (Table 1) was consistent with previous reports for *Porphyra* sp. (Moreda-Pineiro et al., 2012a). In contrast, Mn and Cu bioaccessibility results for the mollusc and salmon species tested in this article are greater than the results recently published for mussels, white fish, and cold water fish (Moreda-Pineiro et al., 2012a; Navarro et al., 2008). Large variation in Cd bioaccessibility between different composite samples of the same food type precluded effective comparisons of Cd bioaccessibility.

3.3. Intercorrelations of concentrations & bioaccessibility

No correlation was observed between the total concentrations of HgT and Se (Table 3). In contrast, significant and positive correlations were observed between the total concentrations of As and

Table 3
Pearson correlation coefficients (two-tailed significance denoted by asterisks) for the concentration and percent bioaccessibility of As, Cd, Mn, Se, and Cu.

	Total concentration ($\mu g\ g^{-1}$)						% HgT as MeHg	Percent bioaccessibility					
	As	Cd	Mn	Se	Cu	HgT		As	Cd	Mn	Se	Cu	HgT
Total concentration ($\mu g\ g^{-1}$)													
As	1.000	0.091	0.943***	−0.163	−0.139	−0.126	0.079	0.307**	0.088	0.190*	0.184	−0.090	−0.376**
Cd	–	1.000	0.218**	0.081	−0.006	−0.176*	−0.372***	−0.378***	0.005	0.267**	0.012	0.076	0.341*
Mn	–	–	1.000	−0.103	0.071	−0.167*	−0.280**	0.044	−0.003	0.192*	0.040	−0.130	0.588***
Se	–	–	–	1.000	0.389***	0.176	−0.221**	−0.034	−0.182	0.481***	0.292***	0.457***	−0.230
Cu	–	–	–	–	1.000	−0.183*	−0.009	−0.460***	−0.238*	0.163*	−0.229**	0.098	0.344*
HgT						1.000	−0.347***	0.263**	−0.292**	0.027	0.136	0.043	−0.544***
%HgT as MeHg	–	–	–	–	–	–	1.000	0.096	−0.052	−0.369***	−0.275**	−0.269**	0.144
Percent bioaccessibility													
As	–	–	–	–	–		–	1.000	0.259*	0.224**	0.692***	0.266**	−0.659***
Cd	–	–	–	–	–		–	–	1.000	0.458***	0.407***	0.367***	0.181
Mn	–	–	–	–	–		–	–	–	1.000	0.648***	0.723***	−0.118
Se	–	–	–	–	–		–	–	–	–	1.000	0.672***	−0.182
Cu	–	–	–	–	–		–	–	–	–	–	1.000	−0.035
HgT	–	–	–	–	–	–	–	–	–	–	–	–	1.000

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

Mn ($r = 0.943$), Cd and Mn ($r = 0.218$), and Se and Cu ($r = 0.389$). Additionally, weak but significantly negative correlations were observed between As and Se ($r = -0.163$), Cd and HgT ($r = -0.176$), Mn and HgT ($r = -0.167$), and Cu and HgT ($r = -0.183$). However, several of these correlations were driven by seaweed samples. When seaweed was excluded from the analysis, the correlations between: As and Mn ($r = -0.073$), Cd and HgT ($r = -0.167$), and Mn and HgT ($r = -0.147$) were all non-significant. Interestingly, the percent of HgT present as MeHg was negatively correlated with the total concentrations of Cd ($r = -0.372$), Mn ($r = -0.280$), Se ($r = -0.221$), and HgT ($r = -0.347$). The correlations were also present when evaluated using dry weight concentrations.

The correlation between the total concentration of a trace element and its respective percent bioaccessibility tended to be either non-significant (e.g. Cd, Cu) or positive (e.g. As, Mn, Se) (Table 3). Similarly, As bioaccessibility from marine fish was strongly correlated to concentrations of As and Cu (He et al., 2010). In contrast to our results, He et al. (2010) observed no correlation between Se bioaccessibility and concentration but was able to detect a correlation for Cu. The percent bioaccessibility of HgT was significantly and negatively correlated ($r = -0.544$) with HgT concentration. This is especially of interest since it suggests that HgT exposure due to the consumption of BC country foods containing higher HgT concentrations may be somewhat offset by a lower HgT percent bioaccessibility. These HgT results are contrary to previous observations for Inuit country foods where no association was observed between HgT concentration and bioaccessibility (Laird et al., 2009).

The consistent positive correlations between the percent bioaccessibility of As, Cd, Mn, Se, and Cu may highlight, in part, the role of food type and food digestibility in controlling the solubilization of trace elements from foods (Table 3). Therefore, these correlations potentially reflect artifacts of the operating conditions of the *in vitro* model employed in this study. When PCA was conducted on the total concentrations and percent bioaccessibility of As, Cd, Se, Cu, and Mn, four components (Eigenvalue > 1.0) explained 83% of the variance (Table 4). This showed that Se, Cu, and Mn bioaccessibility were all major contributors to Component 1 (Eigenvalue 3.14). Consequently, Component 1 may reflect the relative digestibility of different food items under the operating conditions of the GI model employed in the study. This observation is consistent with those from a PCA bi-plot of bioaccessibility results from mussels that showed that relatively bioaccessible metals clustered together (Navarro et al., 2008). Interestingly, As bioaccessibility was a relatively minor contributor to Component 1. This may suggest that a factor independent of food digestibility influences the bioaccessibility of As in traditional First Nations foods. In support of this hypothesis, low As bioaccessibility was observed in wild game organs (e.g. deer liver, moose kidney, moose liver) despite the high bioaccessibility of Se, Mn, and Cu. Similarly, PCA showed that HgT bioaccessibility may also be controlled by a factor other than food digestibility (Table 5). These trends observed for As and Hg could be due to complexation with suspended organic matter in the simulated GI fluids; however, speciation analysis of the bioaccessible fractions of these elements will be necessary before it is possible to adequately test this hypothesis. Furthermore, it should be noted though that *in vitro* validation studies are necessary to determine whether or not these correlations are artifacts of the *in vitro* method or whether they reflect underlying processes influencing dietary exposure.

Table 4
Principal Component Analysis of the concentrations and percent bioaccessibilities of As, Cd, Mn, Se, and Cu (missing values excluded pairwise).

Component	Total	% of Variance	Cumulative%	
1	3.138	31.382	31.382	
2	2.171	21.713	53.094	
3	1.869	18.690	71.785	
4	1.141	11.413	83.198	
	1	2	3	4
Total As ($\mu g\ g^{-1}$)	0.062	0.980	0.128	−0.067
Total Cd ($\mu g\ g^{-1}$)	0.161	0.155	0.044	0.849
Total Mn ($\mu g\ g^{-1}$)	−0.013	0.979	0.050	0.135
Total Se ($\mu g\ g^{-1}$)	0.498	−0.119	0.717	−0.037
Total Cu ($\mu g\ g^{-1}$)	−0.011	−0.012	0.819	0.193
IVBA As (%)	0.438	0.203	−0.377	−0.722
IVBA Cd (%)	0.541	−0.030	−0.575	0.150
IVBA Mn (%)	0.891	0.179	0.140	0.195
IVBA Se (%)	0.854	0.114	−0.176	−0.293
IVBA Cu (%)	0.878	−0.152	0.127	−0.001

Table 5
Principal Component Analysis of the concentrations and percent bioaccessibility of As, Mn, Se, and HgT (i.e., inorganic Hg^{II} + methylmercury). Missing values were excluded pairwise.

Component	Total	% of Variance	Cumulative%
1	3.430	38.108	38.108
2	2.92	32.440	70.548
3	1.086	12.071	82.618
	1	2	3
Total As ($\mu g\ g^{-1}$)	−0.524	0.332	0.668
Total Mn ($\mu g\ g^{-1}$)	0.196	−0.903	−0.147
Total Se ($\mu g\ g^{-1}$)	0.810	0.168	0.125
Total HgT ($\mu g\ g^{-1}$)	0.206	0.093	0.930
% HgT as MeHg	−0.848	0.335	−0.097
IVBA As (%)	0.276	0.875	0.244
IVBA Mn (%)	0.908	0.005	0.068
IVBA Se (%)	0.791	0.422	−0.097
IVBA Hg (%)	−0.154	−0.562	−0.608

4. Conclusion

In vitro extraction procedures such as the technique employed in this study facilitate the quantification of contaminant and nutrient bioaccessibility for exposure and human health risk assessment. Previous studies, largely conducted using market-purchased seafood, have shown that the trace element bioaccessibility to vary both between metals and between foods. However, little data was available describing the bioaccessibility of contaminants and nutrients in the traditional foods consumed by Indigenous peoples. We have reported, for the first time, that the bioaccessibility of As in wild game organs may be lower than values observed for more commonly reported food items (seaweed, molluscs, fish). This information will assist ongoing exposure assessments considering the health risks of metal contamination in the traditional foods of First Nations people due to global resource development. PCA demonstrated that the bioaccessibility of As and HgT may be controlled through processes other than food digestibility.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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References

Adenugba, A.A., McMartin, D.W., Beck, A.J., 2008. In vitro approaches to assess bioavailability and human gastrointestinal mobilization of food-borne polychlorinated biphenyls (PCBs). J. Environ. Sci. Health B 43, 410–421.

Almela, C., Algora, S., Benito, V., Clemente, M.J., Devesa, V., Suner, M.A., Velez, D., Montoro, R., 2002. Heavy metal, total arsenic, and inorganic arsenic contents of algae food products. J. Agric. Food Chem. 50, 918–923.

Almela, C., Laparra, J.M., Velez, D., Barbera, R., Farre, R., Montoro, R., 2005. Arsenosugars in raw and cooked edible seaweed: characterization and bioaccessibility. J. Agric. Food Chem. 53, 7344–7351.

Belinsky, D.L., Kuhnlein, H.V., 2000. Macronutrient, mineral, and fatty acid composition of Canada goose (*Branta canadensis*): an important traditional food resource of the Eastern James Bay Cree of Quebec. J. Food Compost. Anal. 13, 101–115.

Belinsky, D.L., Kuhnlein, H.V., Yeboah, F., Penn, A.F., Chan, H.M., 1996. Composition of fish consumed by the James Bay Cree. J. Food Compost. Anal. 9, 148–162.

Bragigand, V., Berthet, B., Amiard, J.C., Rainbow, P.S., 2004. Estimates of trace metal bioavailability to humans ingesting contaminated oysters. Food Chem. Toxicol. 42, 1893–1902.

Braune, B.M., Malone, B.J., 2006. Organochlorines and trace elements in upland game birds harvested in Canada. Sci. Total Environ. 363, 60–69.

Bureau of Chemical Safety, 2007. Human Health Risk Assessment of Mercury in Fish and Health Benefits of Fish Consumption. Ministry of Health, Ottawa, pp. 1–76.

Cabanero, A.I., Madrid, Y., Camara, C., 2004. Selenium and mercury bioaccessibility in fish samples: an in vitro digestion method. Anal. Chim. Acta 526, 51–61.

Cabanero, A.I., Madrid, Y., Camara, C., 2007. Mercury–selenium species ratio in representative fish samples and their bioaccessibility by an in vitro digestion method. Biol. Trace Elem. Res. 119, 195–211.

Chan, H.M., Receuver, O., 2000. Mercury in the traditional diet of Indigenous peoples in Canada. Environ. Pollut. 110, 1–2.

Chan, H.M., Fediuk, K., Hamilton, S., Rostas, L., Caughey, A., Kuhnlein, H., Egeland, G., Loring, E., 2006. Food security in Nunavut, Canada: barriers and recommendations. Int. J. Circumpolar Health 65, 416–431.

Chan, H.M., Receveur, O., Sharp, D., Schwartz, H., Ing, A., Tikhonov, C., 2011. First Nations Food, Nutrition, and Environment Study (FNFNES): results from British Columbia (2008/2009). University of Northern British Columbia, Prince George.

Crews, H.M., Clarke, P.A., Lewis, D.J., Owen, L.M., Strutt, P.R., Izquierdo, A., 1996. Investigation of selenium speciation in in vitro gastrointestinal extracts of cooked cod by high-performance liquid chromatography inductively coupled plasma mass spectrometry and electrospray mass spectrometry. J. Anal. At. Spectrom. 11, 1177–1182.

Downs, S.M., Arnold, A., Marshall, D., McCargar, L.J., Raine, K.D., Willows, N.D., 2009. Associations among the food environment, diet quality and weight status in Cree children in Quebec. Public Health Nutr. 12, 1504–1511.

Edmonds, J.S., Francesconi, K.A., 1993. Arsenic in seafoods – human health aspects and regulations. Mar. Pollut. Bull. 26, 665–674.

Gamberg, M., Braune, B., Davey, E., Elkin, B., Hoekstra, P.F., Kennedy, D., Macdonald, C., Muir, D., Nirwal, A., Wayland, M., Zeeb, B., 2005. Spatial and temporal trends of contaminants in terrestrial biota from the Canadian Arctic. Sci. Total Environ. 351, 148–164.

He, M., Ke, C.H., Wang, W.X., 2010. Effects of cooking and subcellular distribution on the bioaccessibility of trace elements in two marine fish species. J. Agric. Food Chem. 58, 3517–3523.

Health Canada, 2004. Federal Contaminated Site Risk Assessment in Canada. Part I. Guidance on Human Health Preliminary Quantitative Risk Assessment.. Ministry of Health, Ottawa, pp. 1–41.

Health Canada, 2007. Canada's Food Guide: First Nations, Inuit, and Metis. Ministry of Health, Ottawa, pp. 1–3.

Health Canada, 2012, Canadian standards (maximum levels) for various chemicals contaminants in foods at: http://www.hc-sc.gc.ca/fn-an/securit/chem-chim/contaminants-guidelines-directives-eng.php.

Hwang, H.J., Shim, S.M., 2008. Impact of sodium copper chlorophyllin on mercury absorption using an in vitro digestion with human intestinal cell model. Food Sci. Biotechnol. 17, 564–568.

Intawongse, M., Dean, J.R., 2006. In vitro testing for assessing oral bioaccessibility of trace metals in soil and food samples. Trends Anal. Chem. 25, 876–886.

Koch, I., McPherson, K., Smith, P., Easton, L., Doe, K.G., Reimer, K.J., 2007. Arsenic bioaccessibility and speciation in clams and seaweed from a contaminated marine environment. Mar. Pollut. Bull. 54, 586–594.

Kuhnlein, H.V., Receuver, O., 1996. Dietary change and traditional food systems of Indigenous peoples. Annu. Rev. Nutr. 16, 417–442.

Kuhnlein, H.V., Chan, H.M., Legee, D., Barthet, V., 2002. Macronutrient, mineral and fatty acid composition of Canadian arctic traditional food. J. Food Compost. Anal. 15, 545–566.

Kuhnlein, H.V., Receuver, O., Soueida, R., Egeland, G., 2004. Arctic Indigenous peoples experience the nutrition transition with changing dietary patterns and obesity. J. Nutr. 124, 1447–1453.

Laird, B.D., Shade, C.W., Gantner, N., Chan, H.M., Siciliano, S.D., 2009. Bioaccessibility of mercury from traditional northern country foods measured using an in vitro gastrointestinal model is independent on mercury concentration. Sci. Total Environ. 407, 6003–6008.

Laparra, J.M., 2003. Estimation of arsenic bioaccessibility in edible seaweed by an in vitro digestion method. J. Agric. Food Chem. 51, 6085.

Laparra, J.M., Velez, D., Montoro, R., Barbera, R., Farre, R., 2004. Bioaccessibility of inorganic arsenic species in raw and cooked Hizikia fusiforme seaweed. Appl. Organomet. Chem. 18, 662–669.

Laparra, J.M., Velez, D., Barbera, R., Montoro, R., Farre, R., 2007. Bioaccessibility and transport by Caco-2 cells of organoarsenical species in seafood. J. Agric. Food Chem. 44, 5892–5897.

Llorente-Mirandes, T., Ruiz-Chancho, M.J., Barbero, M., Rubio, R., Lopez-Sanchez, J.F., 2011. Determination of water-soluble arsenic compounds in commercial edible seaweed by LC–ICPMS. J. Agric. Food Chem. 59, 12963–12968.

Mabeau, S., Fleurence, J., 1993. Seaweed in food products – biochemical and nutritional aspects. Trends Food Sci. Technol. 4, 103–107.

Mateo, R., Baos, A.R., Vidal, D., Camarero, P.R., Martinez-Haro, M., Taggart, M.A., 2011. Bioaccessibility of Pb from ammunition in game meat is affected by cooking treatment. PLoS One 6.

Moreda-Pineiro, J., Moreda-Pineiro, A., Romaris-Hortas, V., Moscoso-Perez, C., Lopez-Mahia, P., Muniategui-Lorenzo, S., Bermejo-Barrera, P., Prada-Rodriguez, D., 2011. In vivo and in vitro testing to assess the bioaccessibility and the bioavailability of arsenic, selenium and mercury species in food samples. Trends Anal. Chem. 30, 324–345.

Moreda-Pineiro, J., Alonso-Rodriguez, E., Romaris-Hortas, V., Moreda-Pineiro, A., Lopez-Mahia, P., Muniategui-Lorenzo, S., Prada-Rodriguez, D., Bermejo-Barrera, P., 2012a. Assessment of the bioavailability of toxic and non-toxic arsenic species in seafood samples. Food Chem. 130, 552–560.

Moreda-Pineiro, J., Moreda-Pineiro, A., Romaris-Hortas, V., Dominguez-Gonzalez, R., Alonso-Rodriguez, E., Lopez-Mahia, P., Muniategui-Lorenzo, S., Prada-Rodriguez, D., Bermejo-Barrera, P., 2012b. Trace metals in marine foodstuff: bioavailability estimation and effect of major food constituents. Food Chem. 134, 339–345.

Navarro, R., Arana, G., Etxebarria, N., Dean, J.R., 2008. Evaluation of the physiologically based extraction test as an indicator of metal toxicity in mussel tissue. Anal. Chim. Acta 622, 126–132.

Torres-Escribano, S., Denis, S., Blanquet-Diot, S., Calatayud, M., Barrios, L., Vélez, D., Alric, M., Montoro, R., 2011a. Comparison of a static and a dynamic in vitro model to estimate the bioaccessibility of As, Cd, Pb and Hg from food reference materials *Fucus* sp. (IAEA-140/TM) and lobster hepatopancreas (TORT-2). Sci. Total Environ. 409, 604–611.

Torres-Escribano, S.T.-E.S., Ruiz, A., Barrios, L., Velez, D., Montoro, R., 2011b. Influence of mercury bioaccessibility on exposure assessment associated with consumption of cooked predatory fish in Spain. J. Sci. Food Agric. 91, 981–986.

Van de Wiele, T.R., Oomen, A.G., Wragg, J., Cave, M., Minekus, M., Hack, A., Cornelis, C., Rompelberg, C.J.M., De Zwart, L.L., Klinck, B., Van Wijnen, J., Verstraete, W., Sips, A.J.A.M., 2007. Comparison of five *in vitro* digestion model to *in vivo* experimental results: lead bioaccessibility in the human gastrointestinal tract. J. Environ. Sci. Health A Tox. Hazard. Subst. Environ. Eng. 42, 1203–1211.

Versantvoort, C.H.M., van de Kamp, E., Rompelberg, C.J.M., 2004. Development and applicability of an in vitro digestion model in assessing the bioaccessibility of contaminants from food. RIVM, Bilthoven, pp. 1–87.

Williams, G., West, J.M., Koch, I., Reimer, K.J., Snow, E.T., 2009. Arsenic speciation in the freshwater crayfish, *Cherax destructor* Clark. Sci. Total Environ. 407, 2650–2658.