DOI:10.1021/jf103480y

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Metal Bioavailability and Risk Assessment from Edible Brown Alga *Laminaria japonica*, Using Biomimetic Digestion and Absorption System and Determination by ICP-MS

Shun-Xing Li,*[,†,§] Lu-Xiu Lin,[†] Feng-Ying Zheng,[†,§] and Qing-Xiang Wang[†,§]

[†]Department of Chemistry and Environmental Science, Zhangzhou Normal University, Zhangzhou, China 363000, and [§]Fujian Province University Key Laboratory of Analytical Science (Zhangzhou Normal University), Zhangzhou, China 363000

A new biomimetic digestion and absorption system, including in vitro bionic digestion and biomimetic membrane extraction, was used for the first time for the pretreatment of edible *Laminaria japonica*. After bionic digestion, 11 species of trace metals (V, Cr, Mn, Fe, Ni, Cu, Zn, Se, As, Cd, and Pb) in the resulting chyme were transformed into their final coordinated complexes and then absorbed by the biomembrane. Similar to the biomembrane between gastrointestinal tract and blood vessels, monolayer liposome was used for the first time as a biomembrane model. Affinity-monolayer liposome metals (AMLMs) were separated, determined by ICP-MS, and then used for the metal bioavailability assessment as the bioassimilated part. The action of gastrointestinal acidity and components (including digestive enzymes) was assessed on the basis of the concentration of AMLMs; the safe dosage and tolerable upper intake level of *L. japonica* for adults were proposed as 33.3 and 230.8 g/day, respectively.

KEYWORDS: Edible seaweeds; bioavailability; risk assessment; trace metals; sample pretreatment

INTRODUCTION

Edible marine algae are an important food source in several East Asian countries. Consumption of seaweeds in Europe and North America has steadily increased following the spread of Japanese and Chinese cuisine and of health foods throughout the world (*1*, *2*). The beneficial nutrients are rich in seaweed species, containing vitamins (*3*), amino acids (*4*), dihomo-γ-linolenic acid (*4*), polysaccharides essential (*4*), dietary fiber (*5*), and trace elements (*6*). Dietary *Laminaria japonica* is a important food source in many parts of the world, has been reported to have antioxidant and antitumor activities, and could reduce the risk of intestinal or mammary cancer (*7*, *8*).

The elemental compositions of edible seaweed are now frequently studied, but most of the research has been limited to the determination of total content (*9*−*12*) or to the concentration of inorganic/organic (*13*), dissolved/particulate, or exchangeable/nonexchangeable trace metals (*14*). This resulted in inaccuracies in guiding the proper use of edible seaweeds because the results do not indicate whether or not such trace metals can be introduced into the digestion system and be absorbed by the cells in the intestine, thus influencing metal bioavailability. Because not all species of trace metals in seaweeds are bioavailable, it is necessary to understand exactly what species are present. Furthermore, the influence of gastrointestinal digestion on the metal species must be considered. With respect to potential toxicity, it is necessary to plan the dose of seaweeds to avoid metal overload. All of these problems should be given appropriate attention in the bioavailability and risk assessment of seaweeds. The design of valid methods for speciation analysis and bioavailability assessment of trace metals in seaweeds is key.

Bioavailability studies should ideally be determined in vivo. Human in vivo studies, however, are costly and time-consuming, are rather complicated to perform, and sometimes yield quite variable results. Laboratory animal in vivo studies are less expensive but limited by uncertainties with regard to the differences in metabolism between animals and man. As an alternative to human and animal in vivo studies, bioavailability of trace metals has also been estimated through simple, rapid, and inexpensive in vitro enzymatic methods (*15*−*21*). Metal bioavailability was based on soluble metals extracted by in vitro bionic digestion procedure. A Caco-2 cell monolayer model was used for in vitro studies (*17*), but this method is also time-consuming and complicated.

The major obstacle to rapidly identifying metal bioavailability and risk in foods is the lack of experimental model systems that can replace costly and time-consuming animal studies. A new biomimetic system, including in vitro bionic digestion (i.e., solubility study) and biomimetic membrane extraction (i.e., absorption study), was used in this paper for metal bioavailability in seaweeds. After in vitro bionic digestion of the seaweeds, trace metals were transformed into their final coordinated complexes and then absorbed or excreted. Because a monolayer liposome is the basic backbone of cell membranes and similar to the biomembrane between the gastrointestinal tract and blood vessels, the content of affinity-monolayer liposome metals (AMLMs) could be an important and accurate criterion to assess the metal bioavailability in the chyme because they display higher bioavailability than

*Corresponding author (e-mail lishunxing@fjzs.edu.cn; phone +86 596 2523251; fax +86 596 2520035).

 Published on Web 01/05/2011

Table 1. Components of Saliva, Gastric Juice, Duodenal, and Bile[a]

	saliva	gastric juice	duodenal	bile
inorganic materials	10 mL of 189.6 g/L KCl 10 mL of KSCN 20 g/L 10 mL of NaH_2PO_4 88.8 g/L 10 mL of Na_3PO_4 57 g/L 1.7 mL of NaCl 175.3 g/L 1.8 mL of NaOH 40 g/L	15.7 mL of NaCl 175.3 g/L 3.0 mL of NaH_2PO_4 88.8 g/L 9.2 mL of KCl 89.6 g 18 mL of $CaCl_2 \cdot 2H_2O$ 22.2 g/L 10 mL of NH_4Cl 30.6 g/L 8.3 mL of HCl 37% (g/g)	40 mL of NaCl 175.3 g/L 40 mL of $NaHCO_3$ 84.7 g/L 10 mL of KH_2PO_4 8 g/L 6.3 mL of KCl 89.6 g/L 10 mL of $MgCl_2$ 5 g/L 0.18 mL of HCl 37% (g/g) 9 mL of $CaCl_2.2H_2O$ 22.2 g/L	30 mL of NaCl 175.3 g/L 68.3 mL of $NaHCO_3$ 4.7 g/L 4.2 mL of KCl 89.6 g/L 0.2 mL of HCl 37%g/g 10 mL of $CaCl_2 \cdot 2H_2O_2$ 2.2 g/L
organic materials	8 mL of urea 25 g/L	10 mL of glucose 65 g/L 10 mL of glucuronic acid 2 g/L 3.4 mL of urea 25 g/L 10 mL of glucoseamine hydrochloride 33 g/L	4 mL of urea 25 g/L	
bioenzymes	145 mg of α-amylase 15 mg of uric acid 50 mg of mucin	1 g of bovine serum albumin 1 g of pepsin 3 g of mucin	1 g of bovine serum albumin 3 g of pancreatin 0.5 g of lipase	1.8 g of bovine serum albumin 6 g of bile
pH	6.5 ± 0.2	1.30 ± 0.07	7.8 ± 0.2	8.0 ± 0.2

[a] pH values of all the solutions were adjusted by HCl or NaOH, the total volume of the digest solution was diluted to 500 mL with ultrapure water and be stored at 4 °C.

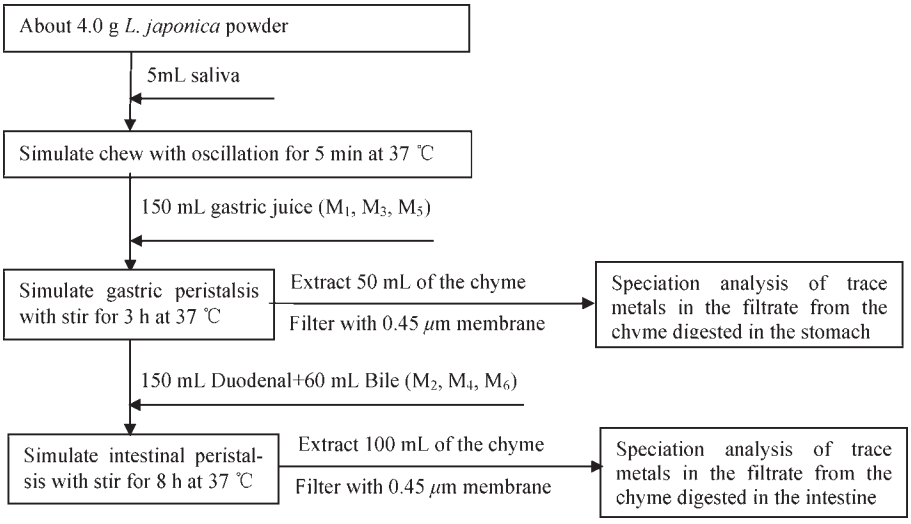

Figure 1. Schematic diagram of in vitro bionic digestion of *L. japonica* powder. The pairs of M_1 and M_2, M_3 and M_4, and M_5 and M_6 are used for the simulation of gastric or intestine acidity, gastrointestinal acidity with gastrointestinal inorganic and organic compositions (excluding the digestive enzymes), and gastrointestinal acidity with gastrointestinal inorganic and organic compositions (including the digestive enzymes), respectively.

water-soluble metals (WSMs). The action of gastrointestinal acidity, inorganic or organic components (including digestive enzymes) was assessed on the basis of the concentration of AMLMs, the main absorption site (stomach or intestine) for trace metals was studied, and the safe dosage and tolerable upper intake level of *L. japonica* per day were established.

MATERIALS AND METHODS

Apparatus. An RE-52 rotator evaporator (Ya Rong Biochemical Instrument Factory, China), an SHA-B temperature-consistent oscillating water bath (GuoHua Co., China), an MK-III microwave digestion system (Sineo Microwave Chemistry Technology Co., China), and an Agilent 7500cx series inductively coupled plasma mass spectrometer (Agilent Technologies Co., USA) was used for metal analytical determinations. The pH values were measured using a Mettler Toledo 320-S pH-meter (Mettler Toledo Co., China) with a combined electrode. Milli-Q purified water was obtained from a Milli-Q purified water apparatus (Millipore Co., USA). Other pieces of equipment were used, including an 86C ULT ultra low temperature freezer (Thermo Electron Co., USA), a DM LB2 microscope (Leica Microwsystems Wetzlar GmbH, Germany), and an agate mortar.

Chemicals. Concentrated nitric acid, 69−70% (Merck KGaA, Germany), and hydrogen peroxide, 30% (Merck KGaA), were used for digestion of seaweed samples. Certified reference materials NIES-03 (green algae, *Chlorella kessleri*) and NIES-09 (*Sargasso*) were supplied from the Japanese National Institute of Environmental Studies (Ibaraki, Japan). Biological chemicals such as uric acid, mucin, albumin bovine, pepsin, pancreatin, lipase, bile, and lecithin were all of analytical grade and purchased from Sigma (St. Louis, MO). According to the literature (*22*, *23*), the compositions and amounts of inorganic and organic compositions, including digestive enzymes, were designed on the basis of human physiology. The detailed components are listed in **Table 1**. Milli-Q purified water (18.2 MΩ) was used for all sample preparations. To avoid metal contamination, all glassware and plastic ware were washed and kept for 48 h in 10% (v/v) nitric acid and then rinsed several times with ultrapure water before use.

Edible Seaweed Samples. *L. japonica* was purchased from Zhongmin Supermarket in Zhangzhou, Fujian, China, and it was identified by Professor Chen Yulin. The *L. japonica* was rinsed rapidly with Milli-Q purified water three times, was heated at 80 °C to constant weight, and then powdered carefully in an agate mortar.

Experimental Design of in Vitro Bionic Digestion Procedure. The gastrointestinal tract is simulated for the mouth, stomach, and small

intestine, as these compartments are likely to determine the metal bioavailability. A schematic representation of the in vitro digestion model is presented in **Figure 1**. The pairs M_1 and M_2, M_3 and M_4, and M_5 and M_6 are used for the simulation of gastric or intestinal acidity, gastrointestinal acidity with gastrointestinal inorganic and organic compositions (excluding digestive enzymes, i.e., semibionic digestion), and gastrointestinal acidity with gastrointestinal inorganic and organic compositions (including digestive enzymes, i.e., whole bionic digestion), respectively. With each set of digested samples, a blank sample (i.e., including digestion juice and excluding *L. japonica* powder) was obtained by using the same digestion procedure, and its metal contents were determined and then used for correction of analytical signals.

Metal Species Distribution in the Chyme from *L. japonica*. The liposome monolayer was prepared according to the following procedure: 0.25 mg of egg-derived lecithin was exactly weighed, dissolved in 5 mL of chloroform, and then transferred to a rotatory evaporator to be evaporated for 10 min to form a multilayer liposome. Twenty-five milliliters of chyme was mixed with multilayer liposome to a homogeneous liposome suspension, which was vibrated at 37 °C in a N_2 atmosphere to make the multilayer liposome totally enter into this filtrate. The filtrate from the chyme with multilayer liposome was frozen at −71 °C in a superlow freezer for 20 min and then thawed at 37 °C, which was repeated three times to form monolayer liposome and to improve the metal species distribution in the chyme in the monolayer liposome−water system. The magnified morphology of the multilayer and monolayer liposome is shown in **Figure 2**. Because the size of the monolayer liposome was 0.3−0.35 μm, AMLMs and WSMs can be separated using suction filtration with 0.45 and 0.22 μm membranes, respectively.

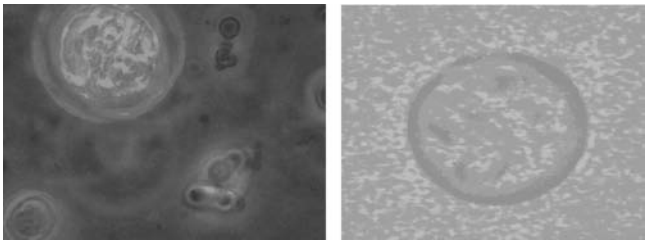

Figure 2. Morphologies of multilayer (left) and monolayer liposome (right) after amplifying 4000 times.

Determination of Metal Concentration in *L. japonica* Powder and Its Chyme and Metal Species Distribution in the Chyme. The decomposition method of the *L. japonica* powder and the filtrates of the chyme was: (a) the sample amount was about 300 mg for *L. japonica* powder and 5 mL for the filtrates of the chyme and corresponding control experiments, all AMLMs, and WSMs; (b) in a Teflon digestion vessel, the sample was added to 4.0 mL of concentrated HNO_3 and 2.0 mL of H_2O_2 (30%) and was decomposed under microwaves for 7 min under a pressure of 15 atm; and (c) after being cooled naturally to room temperature, the decomposed solution was diluted to 25 mL for determination of the concentration of trace metals in the sample by ICP-MS.

RESULTS AND DISCUSSION

Accuracy of This Method. Analysis of the proposed methods was evaluated by analyzing certified reference materials (CRMs) NIES-03 (green algae, *Chlorella kessleri*) and NIES-09 (*Sargasso*). Results of these analyses are shown in **Table 2**, and good agreement with certified concentration in both CRMs was

Table 2. Analysis of Certified Reference Materials after Using the Microwave-Assisted Acid Digestion with HNO_3/H_2O_2 (n = 3)

	NIES-CRM-03 (*Chlorella*)		NIES-CRM-09 (*Sargasso*)	
	certified value (μg/g)	found value (μg/g)	certified value (μg/g)	found value (μg/g)
V	—[a]	<0.50	1.0 ± 0.1	0.7 ± 0.1
Cr	—[a]	—[b]	0.20 ± 0.01	0.21 ± 0.01
Mn	69 ± 5	70 ± 1	21.2 ± 1.1	20.6 ± 0.8
Fe	1850 ± 92	1824 ± 23	187 ± 6	181 ± 3
Ni	—[a]	0.5 ± 0.1	—[a]	0.65 ± 0.02
Cu	3.5 ± 0.3	3.4 ± 0.2	4.9 ± 0.2	4.8 ± 0.1
Zn	20.5 ± 1.0	20.1 ± 0.5	15.6 ± 1.2	14.9 ± 0.4
Se	0.014	0.015 ± 0.002	—[a]	—[b]
As	—[a]	6.3 ± 0.4	115 ± 9	112 ± 3
Cd	0.026	0.027 ± 0.002	0.15 ± 0.02	0.15 ± 0.01
Pb	0.6	0.5 ± 0.1	1.35 ± 0.05	1.28 ± 0.05

[a] No certified values. [b] <LOQ.

Table 3. Metal Contents in *L. japonica* Powder or Its Chyme and the Metal Solubility Ratio (Percent) (n = 3)

		chyme[a]											
		M_1		M_2		M_3		M_4		M_5		M_6	
metal	MTs[b] (μg/g)	μg/g	%	μg/g	%	μg/g	%	μg/g	%	μg/g	%	μg/g	%
V	0.82	0.27	32.93	0.35	42.68	0.22	26.83	0.75	91.46	0.74	90.24	0.76	92.68
Cr	0.47	0.24	51.06	0.28	59.57	0.29	61.70	0.30	63.83	0.23	48.94	0.38	80.85
Mn	4.03	0.85	21.09	1.03	25.56	3.12	77.42	1.30	32.26	3.44	85.36	3.73	92.55
Fe	75.47	14.32	18.97	16.09	21.32	12.75	16.89	16.97	22.49	14.69	19.46	28.76	38.11
Ni	0.32	0.19	59.38	0.14	43.75	0.27	84.38	0.24	75.00	0.21	65.63	0.23	71.88
Cu	1.18	0.90	76.27	0.83	70.34	0.37	31.36	0.68	57.63	0.48	40.68	0.95	80.51
Zn	14.65	12.31	84.03	12.27	83.75	12.91	88.12	10.22	69.76	13.20	90.10	13.60	92.83
Se	0.28	0.18	64.29	0.09	32.14	0.20	71.43	0.19	67.86	0.15	53.57	0.14	50.00
As	3.18	1.57	49.37	1.60	50.31	0.97	30.50	1.38	43.40	0.68	21.38	0.86	27.04
Cd	0.23	0.12	52.17	0.21	91.30	0.22	95.65	0.19	82.61	0.20	86.96	0.18	78.26
Pb	0.36	0.17	47.22	0.34	94.44	0.13	36.11	0.16	44.44	0.06	16.67	0.18	50.00

[a] M_1, simulation of gastric acidity; M_2, simulation of intestinal acidity; M_3, M_1 plus organic and inorganic compositions in stomach (semibionic digestion); M_4, M_2 plus organic and inorganic compositions in intestine (semi-bionic digestion); M_5, M_3 plus digestive enzymes in stomach (whole-bionic digestion); M_6, M_4 plus digestive enzymes in intestine (whole-bionic digestion). [b] MTs, metal total content in *L. japonica* powder.

Table 4. Contents of Affinity-Monolayer Liposome Metals (AMLMs) and Water-Soluble (WSMs) Metals in the Filtrate of the Chyme from the Digestion of *L. japonica* in the Stomach or Intestine on Gastric Acidity, Intestinal Acidity, Semibionic or Whole Bionic Conditions, and the Detailed D_{aw} Values ($n = 3$)

Section A

	digestion on gastric acidity			digestion on intestinal acidity		
metal	AMLMs (μg/g)	WSMs (μg/g)	D_{aw}	AMLMs (μg/g)	WSMs (μg/g)	D_{aw}
V	0.09	0.18	0.50	0.30	0.05	6.00
Cr	0.11	0.13	0.85	0.07	0.21	0.33
Mn	0.36	0.49	0.73	0.82	0.21	3.90
Fe	9.72	4.60	2.11	12.42	3.67	3.38
Ni	0.08	0.11	0.73	0.04	0.10	0.40
Cu	0.62	0.28	2.21	0.60	0.23	2.61
Zn	7.12	5.19	1.37	4.34	7.93	0.55
Se	0.05	0.13	0.38	0.02	0.07	0.29
As	0.58	0.99	0.59	1.44	0.16	9.00
Cd	0.03	0.09	0.33	0.17	0.04	4.25
Pb	0.05	0.12	0.42	0.20	0.14	1.43

Section B

	semibionic digestion in stomach			semibionic digestion in intestine		
metal	AMLMs (μg/g)	WSMs (μg/g)	D_{aw}	AMLMs (μg/g)	WSMs (μg/g)	D_{aw}
V	0.11	0.11	1.00	0.59	0.16	3.69
Cr	0.12	0.17	0.71	0.11	0.19	0.58
Mn	2.07	1.05	1.97	1.14	0.16	7.13
Fe	7.85	4.90	1.60	8.38	8.59	0.98
Ni	0.09	0.18	0.50	0.08	0.15	0.53
Cu	0.13	0.24	0.54	0.54	0.14	3.86
Zn	8.19	4.72	3.47	8.70	1.52	5.72
Se	0.11	0.09	1.22	0.04	0.15	0.27
As	0.30	0.67	0.45	1.14	0.24	4.75
Cd	0.04	0.18	0.223	0.12	0.07	1.71
Pb	0.04	0.09	0.44	0.04	0.12	0.33

Section C

	whole bionic digestion in stomach			whole bionic digestion in intestine		
metal	AMLMs (μg/g)	WSMs (μg/g)	D_{aw}	AMLMs (μg/g)	WSMs (μg/g)	D_{aw}
V	0.24	0.50	0.48	0.30	0.46	0.65
Cr	0.07	0.16	0.44	0.26	0.12	2.17
Mn	1.74	1.70	1.02	1.10	2.63	0.42
Fe	10.17	4.52	2.25	20.51	8.25	2.49
Ni	0.05	0.16	0.31	0.10	0.13	0.77
Cu	0.11	0.37	0.30	0.44	0.51	0.86
Zn	5.69	7.51	0.76	9.97	3.63	2.75
Se	0.07	0.08	0.88	0.12	0.02	6.00
As	0.52	0.16	3.25	0.26	0.60	0.43
Cd	0.11	0.09	1.22	0.03	0.15	0.20
Pb	0.03	0.03	1.00	0.16	0.02	8.00

found. The method described was applicable for the determination of low levels (ng/g) of trace metals (V, Cr, Mn, Fe, Ni, Cu, Zn, Se, As, Cd, and Pb) in *L. japonica* powder and its chyme from bionic digestion and metal distribution of AMLMs and WSMs in the chyme.

Metal Total Concentration in *L. japonica* Powder and Its Chyme. Eight essential elements (V, Cr, Mn, Fe, Ni, Cu, Zn, and Se) and three toxic elements (As, Cd, and Pb) in the *L. japonica* powder and its chyme could be found, and the results are shown in **Table 3**. In *L. japonica* powder, the essential trace elements (Mn, Fe, Cu, and Zn) and toxic As are rich at the level of micrograms per gram and the concentrations of V, Cr, Ni, Se, Cd, and Pb are 0.23−0.82 μg/g. The arsenical concentration was low in the seawater but was high in *L. japonica*; that is, arsenic could be accumulated by *L. japonica*. This result is similar to those of previous studies (*24*−*26*). Dimethylarsinoylribosides (arsenosugars) are a dominant species in marine algae. Although arsenosugars are not acutely toxic and small amounts of arsenic are essential to the human body, there is a possibility of arsenosugars having slight chronic toxicity (*26*).

By the digestion of *L. japonica* powder with six different types of in vitro digestive juice, most metals could be dissolved into the chyme. The metal solubility was different for different metal species and the type of digestive juice. When *L. japonica* was digested by the same types of digestive juice, the total organic and inorganic compositions in its chyme were the same, but the metal ligands were different for different metal species due to the difference of metal ion potential and coordination capacity. When *L. japonica* was digested by different types of digestive juice, the organic and inorganic substances in its chyme were different and then the metal coordinated complexes were different, too. Therefore, the metal concentration in chyme was dependent on the species of metal coordinated complexes.

The action of gastrointestinal components and digestive enzymes on the metal solubility could be assessed by the contrast of metal contents in the chyme between M_1 and M_3, between M_2 and M_4, between M_3 and M_5, and between M_4 and M_6. The influence of the addition of inorganic and organic components on the metal solubility in the stomach and in the intestine was the same for Cr, Mn, Ni, Cu, Se, As, and Pb and was different for V, Fe, Zn, and Cd; in the stomach, the solubility was increased for six metals (Cr, Mn, Ni, Zn, Se, and Cd) and was decreased for five metals (V, Fe, Cu, As, and Pb); in the intestine, the solubility of six metals (V, Cr, Mn, Fe, Ni, and Se) was enhanced, and the metal solubility of Cu, Zn, As, Cd, and Pb was inhibited. The action of digestive enzymes on metal solubility was different for the different metal species and the gastrointestinal tract; the metal solubility was enhanced for V, Fe, Cu, and Zn in the stomach and for V, Cr, Mn, Fe, Cu, Zn, As, and Pb in the intestine and was inhibited by the other situation; the influence of the addition of digestive enzymes was obvious for the solubility of V and Pb in the stomach and for the solubility of Mn in the intestine. When *L. japonica* was whole-bionic digested from the stomach to the intestine, most of the metal solubilities, except for two metals (Se and Cd), were increased. According to the results in **Table 3**, the metal solubility in the chyme was controlled by (a) gastrointestinal components, including acidity, inorganic/organic components, and digestive enzymes, and (b) the metal species.

Metal Species Distribution and Bioavailability in *L. japonica*. After being digested in the stomach or intestine, the metals in the filtrate of the chyme from *L. japonica* powder (i.e., released metal complexes) were transformed into their final coordinated complexes before absorption by the gastrointestinal biomembrane. However, only the biomembrane-affinity metal species in the released metal complexes could be used as the final criterion for the assessment of metal bioavailability. For this reason, the affinities of the released metal complexes were studied using the monolayer liposome and the parameter, which could accurately show the metal distribution in the stomach and intestine, and need to be defined. This parameter was defined as D_{aw}, given by the ratio of the concentrations of AMLMs to WSMs. A higher D_{aw}, that is, the higher proportion of AMLM, could indicate higher bioavailability because it means that much more metal could be absorbed by the biomembrane. After digestion of the *L. japonica* powder by gastrointestinal acidity and semibionic or whole bionic digestion, the amounts of AMLMs and WSMs, that is, the metal species distribution in the chyme in a monolayer liposome−water system, and the detailed metal D_{aw} values are shown in **Table 4**, sections **A**, **B**, and **C**, respectively. The effects of

Table 5. Ratio of Affinity-Monolayer Liposome Metals in the Total Metal from the *L. japonica* Powder (R_{TM}, %) and in the Released Metal Complexes from the Filtrate of the Chyme (R_{SM}, %)

	chyme[a]											
	M_1		M_2		M_3		M_4		M_5		M_6	
metal	R_{TM}	R_{SM}	R_{TM}	R_{SM}	R_{TM}	R_{SM}	R_{TM}	R_{SM}	R_{TM}	R_{SM}	R_{TM}	R_{SM}
V	10.98	33.33	36.59	50.00	13.41	85.71	71.95	78.67	29.27	32.43	36.59	39.47
Cr	23.40	45.83	14.89	41.38	25.53	25.00	23.40	36.67	14.89	30.43	55.32	68.42
Mn	8.93	42.35	20.35	66.35	51.36	79.61	28.29	87.69	43.18	50.58	27.30	29.49
Fe	12.88	67.88	16.46	61.57	10.40	77.19	11.10	49.38	13.48	69.23	27.18	71.31
Ni	25.00	42.10	12.50	33.33	28.13	28.57	25.00	33.33	15.63	23.81	31.25	43.48
Cu	52.54	68.89	50.85	35.14	11.02	72.28	45.76	79.41	9.32	22.92	37.29	46.31
Zn	48.60	57.84	29.62	63.44	55.90	35.37	59.39	85.13	38.84	43.11	68.05	73.31
Se	17.86	27.78	7.14	55.00	39.29	22.22	14.29	21.05	25.00	46.67	42.86	85.71
As	18.24	36.94	45.28	30.93	9.43	90.00	35.85	82.61	16.35	76.47	8.18	30.23
Cd	13.04	25.00	73.91	18.18	17.39	80.95	52.17	63.16	47.83	55.00	13.04	16.67
Pb	13.89	29.41	55.56	30.77	11.11	58.82	11.11	25.00	8.33	50.00	44.44	88.88

[a] M_1, simulation of gastric acidity; M_2, simulation of intestinal acidity; M_3, M_1 plus organic and inorganic compositions in stomach (semibionic digestion); M_4, M_2plus organic and inorganic compositions in intestine (semibionic digestion); M_5, M_3 plus digestive enzymes in stomach (whole bionic digestion); M_6, M_4 plus digestive enzymes in intestine (whole bionic digestion).

gastrointestinal acidity, and inorganic and organic components (including digestive enzymes), on the metal bioavailability are discussed.

After digestion of the *L. japonica* powder on gastric acidity, eight metal AMLMs (V, Cr, Mn, Ni, Se, As, Cd, and Pb) in the chyme were less than their WSMs, their D_{aw} values were < 1.0. Only three metal D_{aw} values (Fe, Cu, and Zn) were > 1.0; that is, these three metal AMLMs were grteater than their WSMs. When *L. japonica* powder was digested on intestinal acidity, the metal bioavailability was varied; seven metal D_{aw} values (V, Mn, Fe, Cu, As, Cd, and Pb) were > 1.0, suggesting that their AMLMs were far greater than their WSMs, and the other four metal D_{aw} values (Cr, Ni, Zn, and Se) were < 1.0; the toxic metal D_{as} values for As, Cd, and Pb were 9.00, 4.25, and 1.43, respectively.

When *L. japonica* powder was digested by semibionic digestion juice in the stomach, the amounts of AMLMs (Cr, Ni, Cu, As, Cd, and Pb) were less than the concentration of WSMs, except for V, Mn, Fe, Zn, and Se. After digestion of *L. japonica* powder by semibionic digestion juice in the intestine, the D_{aw} values of V, Mn, Cu, Zn, As, and Cd were far greater than 1.0 and the situations of Cr, Fe, Ni, Se, and Pb were the opposite.

The main factors of the human digestive system were simulated with in vitro whole bionic digestion, including normal human body temperature, the addition of saliva, the acidity, and inorganic and organic materials (including digestive enzymes) in the stomach or intestine. The content of AMLMs in the stomach and intestine could be a more accurate criterion to assess metal bioavailability in *L. japonica* powder. After being digested by whole bionic digestion in the stomach, the D_{aw} values of V, Cr, Ni, Cu, Zn, and Se were all < 1.0, and the contents of AMLMs were less than those of WSMs, indicating lower bioavailability, but the essential metals Mn and Fe and toxic metals As, Cd, and Pb mainly existed in the species of AMLMs in stomach. When the chyme was digested continually into the intestine, species of Cr, Fe, Zn, Se, and Pb mainly existed as AMLM species; their detailed D_{aw} values were 2.06, 2.49, 2.74, 4.14, and 7.40, respectively.

The metal AMLMs were the metal bioavailable species. The ratios of AMLM in the total metal from the *L. japonica* powder (R_{TM}) and in the released metal complexes from the filtrate of the chyme (R_{SM}) were calculated and are shown in **Table 5**. The metal R_{TM} and R_{SM} values were in the range of 8.18−68.05 and 16.67−88.88%, respectively, so the total metal from the *L. japonica* powder or the released metal complexes from the filtrate of its chyme could not be used for the metal bioavailability and risk assessment directly. The influence of the addition of inorganic and organic components on the 11 species of metal bioavailability in the stomach and in the intestine was same; six species of metal R_{TM} and R_{SM} (V, Cr, Mn, Ni, Zn, and Se) were enhanced and the other five species of metal R_{TM} and R_{SM} (Fe, Cu, As, Cd, and Pb) were inhibited. When the digestive enzymes were added into the digestive juice, only three species of metal R_{TM} and R_{SM} (V, Fe, and As) in the stomach and four species of metal R_{TM} and R_{SM} (Cr, Fe, Ni, and Zn) in the intestine were increased and the inhibition effect was seen in the other situations. When the *L. japonica* powder was digested by whole bionic digestion from the stomach into the intestine, only three metal bioavailabilities (Fe, As, and Cd) were decreased and the other eight metal bioavailabilities were increased.

As discussed above, metal bioavailability in *L. japonica* was affected by the metal species, the gastrointestinal acidity, and the compositions in the stomach and intestine, including inorganic and organic components, and digestive enzymes.

Safe Dosage and Risk Assessment Based on the Level of AMLMs of Trace Metals. Risk assessment of trace metals has examined two ends of the toxicity spectrum: (1) that associated with intakes that are too high and the resulting toxicity and (2) that associated with intakes that are too low and the resulting nutritional problems (*27*−*30*).

After whole bionic digestion of *L. japonica* powder, the level of metal AMLMs in the intestine could form the basis of dosage design to avoid metal overload. Several metal safety baseline levels for human consumption have been drawn as follows (*31*−*36*): Recommended Dietary Allowances (RDA) are set to meet the needs of almost all (97−98%) individuals in a group; Adequate Daily Dietary Intake (ADDI) is believed to cover the needs of all individuals in a group, but lack of data prevents specification, with confidence, of the percentage of individuals covered by this intake; Tolerable Upper Intake Level (UL) and Maximum Level of Daily Intake without Detriment to Health (ML) are likely to pose no risk of adverse effect.

Safe dosage values of *L. japonica* used as powder could be calculated by the ratio of AMLMs to metal RDA or ADDI values, and the maximum values of *L. japonica* were calculated by the ratio of AMLMs in the intestine to metal UL or ML values. The results are shown in **Table 6**. Because trace metals coexist in *L. japonica*, the minimum metal RDA and ADDI values should be adopted to avoid the overload of each metal. Therefore, the

Table 6. Reference Data of Trace Metals for Adults Acquired from the Literature, Safe Dosage, and Maximum Values of *Laminaria japonica* Compared to Recommended Values[a]

metal	metal RDA/ADDI values (μg/day)	metal UL/ML values (mg/day)	metal AMLMs in intestine after whole bionic digestion of *L. japonica* (μg/g)	safe dosage values of *L. japonica* (g/day)	maximum values of *L. japonica* (g/day)
V	10−100	1.8	0.30	33.3	6000.0
Cr	20−35	0.06	0.26	76.9	230.8
Mn	1800−2300	11.0	1.10	1636.4	10000.0
Fe	8000−18000	45.0	20.51	390.0	2194.1
Ni	50−799	1.0	0.10	500.0	10000.0
Cu	900	10.0	0.44	2045.4	22727.3
Zn	8000−11000	40.0	9.97	802.4	4012.0
Se	40−70	0.4	0.12	333.3	3333.3
As			0.26		
Cd		0.3	0.03		10000.0
Pb	34−440	0.3	0.16	212.5	1875.0

[a] RDA, Recommended Dietary Allowance value; ADDI, Adequate Daily Dietary Intake; UL, Tolerable Upper Intake Level; ML, Maximum Level of Daily Intake without Detriment to Health.

safe dosage of *L. japonica* for adults should be below 33.3 g/day. Metal risk assessment can be examined through the comparison of the level of metal AMLMs in the intestine and the metal UL or ML values. According to a similar method as for the RDA values, the UL value of *L. japonica* used as powder for adults was 230.8 g/day.

ABBREVIATIONS USED

AMLMs, monolayer liposome-bond metals; WSMs, water-soluble metals; NIST, National Institute of Standards and Technology; RDA, Recommended Dietary Allowance; ADDI, Adequate Daily Dietary Intake; UL, tolerable upper intake level; ML, Maximum Level of Daily Intake with Detriment to Health.

LITERATURE CITED

(1) Galland-Irmouli, A. V.; Fleurence, J.; Lamghari, R.; Lucon, M.; Rouxel, C.; Barbaroux, O.; Bronowicki, J. P.; Villaume, C.; Guéant, J. L. Nutritional value of proteins from edible seaweed *Palmaria palmata* (Dulse). *J. Nutr. Biochem.* **1999**, *10*, 353−359.

(2) Skibola, C. The effect of Fucus vesiculosus, an edible brown seaweed, upon menstrual cycle length and hormonal status in three pre-menopausal women: a case report. *BMC Compl. Altern. Med.* **2004**, *4*, 10−17.

(3) McDermid, K. J.; Stuercke, B. Nutritional composition of edible Hawaiian seaweeds. *J. Appl. Phys.* **2003**, *5*, 513−524.

(4) Dawczynski, C.; Schubert, R.; Jahreis, G. Amino acids, fatty acids, and dietary fiber in edible seaweed products. *Food Chem.* **2007**, *103*, 891−899.

(5) Gómez-Ordóñez, E.; Jiménez-Escrig, A.; Rupérez, P. Dietary fibre and physicochemical properties of several edible seaweeds from the northwestern Spanish coast. *Food Res. Int.* **2010**, *43* (9), 2289−2294.

(6) Wondimu, T.; Ueno, A.; Kanamaru, I.; Yamaguchi, Y. K.; McCrindle, R.; Hanaoka, K. I. Temperature-dependent extraction of trace metals in edible brown alga *hijiki*, *Hizikia fusiforme*. *Food Chem.* **2007**, *104*, 542−555.

(7) Wang, J.; Zhang, Q.; Zhang, Z. S.; Li, Z. E. Antioxidant activity of sulfated polysaccharide fractions extracted from *Laminaria japonica*. *Int. J. Biol. Macromol.* **2008**, *42*, 127−132.

(8) Yuan, Y. V.; Walsh, N. A. Antioxidant and antiproliferative activities of extracts from a variety of edible seaweeds. *Food Chem. Toxicol.* **2006**, *44*, 1144−1150.

(9) Moreda-Piñeiro, J.; Alonso-Rodríguez, E.; López-Mahía, P.; Muniategui-Lorenzo, S.; Prada-Rodríguez, D.; Moreda-Piñeiro, A.; Bermejo-Barrera, P. Development of a new sample pre-treatment procedure based on pressurized liquid extraction for the determination of metals in edible seaweed. *Anal. Chim. Acta* **2007**, *598*, 95−102.

(10) Ródenas de la Rocha, S.; Sánchez-Muniz, F. J.; Gómez-Juaristi, M.; Larrea Marín, M. T. Trace metals determination in edible seaweeds by an optimized and validated ICP-MS method. *J. Food Compos. Anal.* **2009**, *22*, 330−336.

(11) Subba Rao, P. V.; Mantri, V. A.; Ganesan, K. Mineral composition of edible seaweed *Porphyra vietnamensis*. *Food Chem.* **2007**, *102*, 215−218.

(12) Dawczynski, C.; Schäfer, U.; Leiterer, M.; Jahreis, G. Nutritional and toxicological importance of macro, trace, and ultra-trace elements in algae food products. *J. Agric. Food Chem.* **2007**, *55* (25), 10470−10475.

(13) Sloth, J. J.; Julshamn, K. Survey of total and inorganic arsenic content in blue mussels (*Mytilus edulis*) from Norwegian fiords: revelation of unusual high levels of inorganic arsenic. *J. Agric. Food Chem.* **2008**, *56* (4), 1269−1273.

(14) Li, L.; Xie, M.; Sun, Z.; Wu, X.; Sun, D.; Wang, X. The study on the element transference characteristics and element speciation in the extract of *Cyclocarya paliurus* (Batal.) Iljinskaja leaves. *Chem. J. Chin. U.* **2000**, *21*, 707−709.

(15) Shen, L. H.; Luten, J.; Robberecht, H.; Bindels, J.; Deelstra, H. Modification of an in-vitro method for estimating the bioavailability of zinc and calcium from foods. *Z. Lebensm.-Unters. -Forsch.* **1994**, *199*, 442−445.

(16) Hemalatha, S.; Platel, K.; Srinivasan, K. Influence of food acidulants on bioaccessibility of zinc and iron from selected food grains. *Mol. Nutr. Food Res.* **2005**, *49*, 960−965.

(17) Peña-Farfal, C.; Moreda-Piñeiro, A.; Bermejo-Barrera, A.; Bermejo-Barrera, P.; Pinochet-Cancino, H.; Gregori-Henríquez, I. Speeding up enzymatic hydrolysis procedures for the multi-element determination in edible seaweed. *Anal. Chim. Acta* **2005**, *548*, 183−191.

(18) Cámara, F.; Barberá, R.; Amaro, M. A.; Farré, R. Calcium, iron, zinc and copper transport and uptake by Caco-2 cells in school meals: influence of protein and mineral interactions. *Food Chem.* **2007**, *100*, 1085−1092.

(19) Vitali, D.; Dragojević, I. V.; Šebečić, B. Bioaccessibility of Ca, Mg, Mn and Cu from whole grain tea-biscuits: impact of proteins, phytic acid and polyphenols. *Food Chem.* **2008**, *110*, 62−68.

(20) García-Nebot, M. J.; Alegría, A.; Barberá, R.; Clemente, G.; Romero, F. Addition of milk or caseinophosphopeptides to fruit beverages to improve iron bioavailability? *Food Chem.* **2010**, *119*, 141−148.

(21) Hemalatha, S.; Gautam, S.; Platel, K.; Srinivasan, K. Influence of exogenous iron, calcium, protein and common salt on the bioaccessibility of zinc from cereals and legumes. *J. Trace Elem. Med. Biol.* **2009**, *23*, 75−83.

(22) Oomen, A. G.; Rompelberg, C. J. M.; Bruil, M. A.; Dobbe, C. J. G.; Pereboom, D. P. K. H.; Sips, A. J. A. M. Development of an in vitro digestion model for estimating the bioaccessibility of soil contaminants. *Arch. Environ. Contam. Toxicol.* **2003**, *44*, 281−287.

(23) Versantvoort, C. H. M.; Oomen, E.; Van de Kamp, E.; Rompelberg, C. J. M.; Sips, A. J. Applicability of an in vitro digestion model in assessing the bioaccessibility of mycotoxins from food. *Food Chem. Toxicol.* **2005**, *43*, 31−40.

(24) Jiang, X. J.; Gan, W. E.; Han, S. P.; Zi, H. J.; He, Y. Z. Design and application of a novel integrated electrochemical hydride generation cell for the determination of arsenic in seaweeds by atomic fluorescence spectrometry. *Talanta* **2009**, *79*, 314−318.
(25) García Salgado, S.; Q, J.; Nieto, M. A.; Bonilla Simón, M. M. Assessment of total arsenic and arsenic species stability in alga samples and their aqueous extracts. *Talanta* **2008**, *75*, 897−903.
(26) Farías, S.; Smichowski, P.; Vélez, D.; Montoro, R.; Curtosi, A.; Valdopívez, C. Total and inorganic arsenic in Antarctic macroalgae. *Chemosphere* **2007**, *69*, 1017−1024.
(27) Li, S. X.; Deng, N. S.; Zheng, F. Y. Speciation analysis of iron in traditional Chinese medicine by flame atomic absorption spectrometry. *J. Pharm. Biomed. Anal.* **2003**, *32* (1), 51−57.
(28) Li, S. X.; Deng, N. S.; Zheng, F. Y. Effect of digestive site acidity and compatibility on the species, lipophily and bioavailability of coordinated complex of iron, manganese and zinc in *Prunus persica* Batsch and *Carthamus tinctorus*. *Bioorg. Med. Chem. Let.* **2004**, *14* (2), 505−510.
(29) Li, S. X.; Zheng, F. Y.; Liu, X. L.; Cai, W. L. Speciation analysis and bioavailability assessment of manganese in phytomedicine by extraction with octanol and determination by flame atomic absorption spectrometry. *Phytochem. Anal.* **2005**, *16* (6), 405−410.
(30) Li, S. X.; Zheng, F. Y. Speciation analysis, bioavailability and risk assessment of copper complexes in phytomedicines using flame atomic absorption spectrometry. *Planta Med.* **2008**, *74*, 1302−1307.
(31) Alberti-Fidanza, A.; Burini, G.; Perriello, G.; Fidanza, F. Trace element intake and status of Italian subjects living in the Gubbio area. *Environ. Res.* **2003**, *91*, 71−77.
(32) Biego, G. H.; Joyeux, M.; Hartemann, P.; Debry, G. Daily intake of essential minerals and metallic micropolutants from foods in France. *Sci. Total Environ.* **1998**, *217*, 27−36.
(33) Jorhem, L.; Becker, W.; Slorach, S. Intake of 17 metals by Swedish women, determined by a 24-h duplicate portion study. *J. Food Compos. Anal.* **1998**, *11*, 32−46.
(34) WHO. *Trace Metals in Human Nutrition and Health*; World Health Organisation: Geneva, Switzerland, 1996.
(35) Institute of Medicine. *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*; a report of the Panel of Micronutrients, Subcommittees on Upper Reference Levels of Nutrients and of Interpretation and Uses of Dietary Reference Intakes and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes; National Academy Press: Washington, DC, 2001.
(36) Leśniewicz, A.; Jaworska, K.; Żyrnicki, W. Macro- and micronutrients and their bioavailability in polish herbal medicaments. *Food Chem.* **2006**, *99*, 670−679.

Received for review September 10, 2010. Revised manuscript received December 8, 2010. Accepted December 13, 2010. This work was supported by the National Natural Science Foundation of China (No. 20775067, 20805041, and 20977074), Outstanding Youth Science Foundation of Fujian Province, China (No. 2010J06005), the Science and Technology Committee of Fujian Province, China (No. 2008F5063), and the Program for New Century Excellent Talents in Fujian Province University (No. 200720).