ELSEVIER

Food Chemistry 71 (2000) 181–188

Food Chemistry

www.elsevier.com/locate/foodchem

Plants as a natural source of concentrated mineral nutritional supplements

Mark P. Elless *, Michael J. Blaylock, Jianwei W. Huang, Christopher D. Gussman

Phytotech, Inc., 1 Deer Park Drive, Suite I, Monmouth Junction, NJ 08852, USA

Abstract

Edible plants enhanced with minerals were tested to determine whether these plants could be used as a new source of mineral dietary supplements that provide essential minerals in a more available form than current, inorganically based mineral supplements. A select cultivar of *Brassica juncea* was identified that can be cultivated under hydroponic conditions to contain high levels of nutritionally important minerals such as Cr, Fe, Mn, Se, and Zn. Sequential extraction, simulated gastric fluid digestion, and simulated intestinal fluid digestion were used to assess the degree of solubility and potential availability of each metal examined. Results from these solubility experiments indicate that the accumulated trace elements achieve greater soluble concentrations than those provided in popular mineral supplements. The consistent high concentration of minerals in the edible plant tissue allows processing small quantities of these enriched plants into capsules or tablets that supply 100% of the recommended daily intake of these elements in soluble form from a natural, vegetative source.

Keywords: Nutraceuticals; *Brassica juncea*; Bioavailability; Mineral supplements

1. Introduction

The industrial revolution has been driven by the need for more efficient ways to provide the necessities of life. In agriculture, the application of biotechnology methods is now being used to generate new and more efficient food, pharmaceutical, and energy products through the manipulation of organisms.

Microbiologists were among the first to develop modern methods for the utilization of organisms in the production of beneficial substances by fermentation. This process consists of two major steps: (1) isolation of an organism with the desired genetic characteristics through screening and selection or molecular techniques followed by (2) use of the appropriate cultivation conditions to optimize productivity. A similar approach is being adopted by the natural products industry and plant scientists to develop plants, which contain many unique biochemical compounds, as a renewable resource for improved medical and pharmaceutical products (Rotman, 1998).

Recently, plant species have been identified that contain nutrients displaying new, beneficial medicinal or therapeutic properties (Fahey, Zhang & Talalay, 1997). The nutritional use of these plants appears to be practical and effective, but the considerable variability in the content of the desired active ingredients in the harvested plant and resulting lack of a predictable, standardized product are perceived barriers to the widespread commercial acceptance of certain plant derived nutritional supplements.

The human body requires a number of minerals in order to maintain good health. Fresh fruits and vegetables, while high in vitamins, are often low in essential minerals (Ashmead, 1982). Even vegetables commonly believed to be high in Fe do not contain adequate dietary concentrations to supply the recommended quantity when consumed in normal-sized portions (Table 1). The rapid growth of the mineral supplement industry is in part due to the need for supplements in diets lacking sufficient mineral content, but supplements may not provide minerals in a soluble and metabolically available form (Fairweather-Tait, 1996).

Plants accumulate minerals essential for their growth from the environment and can also accumulate metals such as Cd, Co, and Ag, which have no known direct benefit to the plant (Baker & Brooks, 1989; Raskin, Kumar, Dushenkov & Salt, 1994). Unusually high concentrations of

* Corresponding author. Tel.: +1-703-390-1100

Current addresses: Drs Elless, Blaylock and Huang now with Edenspace Systems Corporation, 11720 Sunrise Valley Drive, Reston, VA 20191, USA. Mr Gussman now with Lockheed Martin/REAC, 2890 Woodbridge Avenue, Edison, NJ 08837, USA.

0308-8146/00/$ - see front matter
PII: S0308-8146(00)00142-4

Table 1
Micronutrient content of selected plants

Plant	Micronutrient content, mg/g dry biomass				
	Fe	Zn	Mn	Se	Cr
Spinach	0.9	0.07	0.05	< 0.001	< 0.01
Romaine lettuce	0.9	0.07	0.1	< 0.001	< 0.01
Red beet (root)	0.1	0.07	0.01	< 0.001	< 0.01
Collard greens	0.07	0.04	0.01	< 0.001	< 0.01
Carrot (root)	0.02	0.04	0.005	< 0.001	< 0.01
Beet greens	0.3	0.05	0.1	< 0.001	< 0.01
Green beans	0.1	0.03	0.02	< 0.001	< 0.01
Broccoli	0.1	0.04	0.02	< 0.001	< 0.01
Unenriched *B. juncea*	0.13	0.14	0.11	< 0.001	< 0.01
Trace element-enriched *B. juncea*	40	73	25	1.8	4.5
Recommended daily intake[a] (mg/day)	10–15	15	2	0.2	0.2

[a] From National Research Council (1989).

metals are sequestered in a variety of different wild plant species that are not used as food crops to allow their survival in a metal-rich soil environment (Banuelos & Meeks, 1990; Brooks, Morrison, Reeves, Dudley & Akman, 1979; Brooks, Morrison, Reeves & Morrison, 1978; Brooks, Trow, Veillon & Jaffee, 1981; Ernst, Verkliej & Schat, 1992; Reeves & Brooks, 1983).

Large scale laboratory screening studies have identified select cultivars of *Brassica juncea* (Indian mustard), an edible crop species, that accumulate metals in their stems and leaves at concentrations exceeding 2% of their dry weight (Dushenkov, Kumar, Motto & Raskin, 1995; Kumar, Dushenkov, Motto & Raskin, 1995; Salt et al., 1995). Reports of *B. juncea* containing high levels of Se and B after growth in soils rich in these minerals have also been published (Banuelos et al., 1993).

In this paper, these initial screening and selection efforts have been expanded to include several nutritionally important minerals and the development of specific hydroponic cultivation conditions that result in consistent and reliable mineral accumulation in the edible portions of the plants. Recently, Orser et al. (1999) demonstrated that phytoenrichment of *B. juncea* with selenium is possible using hydroponic cultivation, with selenium concentrations averaging 2000 mg/kg, and that this enriched biomass can be used as the source for a nutritional supplement that would provide the recommended daily intake for selenium (50 to 70 μg per day) in a more bioavailable form than commercially available yeast selenium supplements. However, using phytoenriched *B. juncea* as a vehicle to deliver other nutritionally important minerals has yet to be demonstrated.

The objectives of this paper are to demonstrate that (a) phytoenrichment of *B. juncea* with several nutritionally important minerals (i.e., Cr, Fe, Mn, Se, and Zn) is possible, making these plants a natural source of concentrated mineral supplements, (b) these mineral-enriched plants can be routinely cultivated to provide consistent mineral concentrations, (c) as little as 250 mg of dry enriched plant material are able to supply the recommended daily intakes of these minerals, and (d) the minerals incorporated into the enriched *B. juncea* occur in a more soluble, metabolically available form than inorganically-based multivitamins and specific mineral supplements.

2. Material and methods

2.1. Plant culture

Seeds of *B. juncea* (cv. 426308) were sown in potting soil (Pro-Mix^{TM} BX,) in 12 cm diameter pots or in 8×8×6 cm rockwool blocks (Grodan^{TM}) placed in plastic trays to determine the plant growth media most advantageous for routine production practices. *B. juncea* was selected because this plant (a) is a known hyperaccumulator of metals and has been used extensively in the phytoremediation industry and (b) is an edible plant species.

All plants were watered daily with tap water and twice a week with nutrient solutions (pH 6.5) containing (in mM): K, 2.5; Ca, 1.0; Mg, 0.5; S, 0.2; NH_4, 0.1; NO_3, 3.0; P, 0.2; and in μM: Cl, 50; B, 10; Mn, 2.0; Zn, 0.5; Cu, 0.2; Mo, 0.1; Ni, 0.1, and Fe 20. Two weeks after planting, the seedlings were thinned to two plants per pot or block. The plants were grown in a light-and-temperature-controlled greenhouse with a 16 h, 25°C/8 h, 20°C day–night regime. The light intensity at the level of plant shoots was maintained at 650 μmol m^{-2} s^{-1}. Nutrient solutions enriched with trace elements were supplied to 4-week-old plants. For each selected element (Fe as $FeSO_4$ in presence of citric acid, Zn, as $ZnSO_4$, Mn as $MnSO_4$, Se as Na_2SeO_4, and Cr as $CrCl_3$), a stock solution of 0.1 to 0.5 M was prepared and then a

volume of the stock solution was applied to the root growth medium to yield a metal loading capacity ranging from 5 to 500 mg/plant. Therefore, only a single metal was applied to the plant growth medium for a given experiment so that simultaneous uptake of several metals from a mixture of two or more metals was not performed in this study. The plants were harvested 1 week after exposure to the metal enriched nutrient solution by cutting the stem at the root–shoot junction. The shoots were washed with deionized water and the roots were discarded. For comparison purposes, samples of fresh vegetables from a local grocery store and several commercial mineral supplements were analyzed for mineral content and solubility. Although these vegetables were not grown to hyperaccumulate nutritionally important minerals as the enriched *B. juncea* was, this comparison demonstrates that mineral supplements are required to attain 100% RDI of these minerals.

2.2. Plant tissue analysis

All plant samples were dried in an oven at 70°C and ground in a stainless steel Wiley mill to pass through a 0.85 mm screen. Triplicate subsamples of each ground plant material (250 mg) were digested in a mixture of concentrated $HNO_3/HClO_4$ (Jones & Case, 1990). The digested samples were brought to a constant volume with deionized water, and the digests were analyzed for total Fe, Zn, Mn, Se, and Cr by inductively coupled plasma spectroscopy (ICP, Fisons Accuris, Fisons Instruments, Inc., Beverly, MA). No speciation information for any of the five metals examined was determined from this analysis.

2.3. Bioavailability measurements

Two independent experiments were conducted to compare the degree of potential bioavailability (i.e. solubility) between the enriched B. juncea plants and several inorganically-based multivitamins and mineral supplements. The first assay for determining potential bioavailability was conducted using a sequential extraction procedure that used duplicate 1.0 g samples in a 10:1 solution:solid ratio in the following sequence (Ramos, Hernandez & Gonzalez, 1994): 1 M $MgCl_2$ for 2 h (water soluble), 1 M NaOAc at pH 5 for 2 h (acid soluble, pH 5), 0.04M hydroxylamine hydrochloride in 25% (v/v) glacial acetic acid for 2 h (reducible), 0.1 M $HNO_3 + 30\%$ hydrogen peroxide at 70°C for 16 h (organically complexed), and concentrated HNO_3 and 30% hydrogen peroxide for 2 h (residual). Minerals extracted into solution earlier in the sequence are considered more bioavailable because the extractant strength progressively increases in the sequence (Berti, Cunningham & Jacobs, 1995). For this assay, the degree of potential bioavailability of enriched *B. juncea* was compared to a popular multivitamin (Multivitamin I). All samples were centrifuged at 4300 rpm for 10 min, filtered, and the solubilized extracts were analyzed by ICP. Comparison of the means between the enriched *B. juncea* and the multivitamin for this bioavailability assay was performed using the Student *t*-test at an alpha level of 0.10.

Digestions using simulated gastric and intestinal fluid (Glahn, Lai, Hsu, Thompson, Guo & Van Campen, 1998) were also performed to independently confirm the results from the sequential extraction. These digestions were performed in triplicate on both the enriched plant material and the multivitamins/supplements using the recommended daily dose for each (i.e. 250 mg samples for plants and one tablet for the vitamins/supplements). For each sample digested with the simulated gastric fluid, 15 ml of distilled water were added to each sample, mixed to disintegrate the tablets and homogenize the sample, and then adjusted to pH 2 with 5.0 M HCl. Following pH adjustment, 0.75 ml of pepsin solution (1 g pepsin dissolved in 50 ml of 0.1M HCl) was added to each tube. Digestions using simulated intestinal fluid used the extracts from the simulated gastric fluid digestion. The extracts were adjusted to pH 6 with 0.1M $NaHCO_3$, then 3.75 ml of bile extract (0.05 g pancreatin and 0.30 g of bile extract in 35 ml of 0.1 M $NaHCO_3$) were added to each extract, the extracts were again adjusted to pH 7 with 1 N NaOH, and finally 5 ml of 120 mM NaCl and 5 mM KCl were added to each extract. The degree of potential bioavailability between the enriched *B. juncea* and several multivitamins (Multivitamins I, II, and III) and supplements (chromium yeast, chromium picolinate, selenium yeast, zinc picolinate) were compared using this assay. All samples for both the simulated gastric and intestinal fluid digestions were placed in an incubator set at 37°C and shaken at 190–200 rpm. Samples were removed after 15, 30, 45, and 60 min of incubation, centrifuged, filtered, and analyzed by ICP as described previously.

2.4. Statistical evaluation

Comparison of the means between the metals extracted from the enriched *B. juncea* and Multivitamin I during the sequential extraction procedure was performed using the Student's *t*-test at an alpha level of 0.10. Comparison of the means between the metals extracted from the enriched *B. juncea* and multivitamins/supplements during the simulated gastric and intestinal fluid digestions was performed using Duncan's Multiple Range test at an alpha level of 0.05.

2.5. Toxicological evaluation

Toxicological studies were independently conducted to evaluate whether ingestion of Se- or Cr-enriched *B. juncea* causes any adverse reactions in rats. Limit testing

was also performed to measure the LD_{50} for these plant-based nutritional supplements.

3. Results and discussion

3.1. Trace element-enriched B. juncea

A number of minerals essential to human nutrition can be accumulated in select cultivars of *B. juncea* to concentrations much greater than those found in common vegetables (Table 1) and be manipulated by control of the cultivation conditions. The concentrations of Cr, Fe, and Zn in the shoots, for example, are increased 200–500-fold by adding these minerals individually to the growth medium, resulting in plant tissue concentrations exceeding the highest concentrations reported in the literature (Banuelos et al., 1993; Brown, Chaney, Angle & Baker, 1994; Cunningham & Ow, 1996; Kumar et al., 1995; Salt et al., 1995; Welch & LaRue, 1990). Achieving a constant desired metal concentration in the plant tissue is dependent on controlling the metal concentration in the growth medium, as shown for Fe in Fig. 1 but similarly observed for all the metals tested. In three repeated studies, Se concentrations between 1700 and 3000 mg/kg in shoots of *B. juncea* were achieved by adjusting the Se concentration in the growth medium. This process was performed at a commercial greenhouse scale to produce 50-kg dry biomass containing average Se concentrations of 1894 to 2205 mg/kg. Additional operations produced 26 kg dry biomass of Cr-enriched *B. juncea* containing 4870 to 5572 mg Cr/kg, thus demonstrating the ability to produce bulk quantities of plant material enriched with a selected mineral.

3.2. Potential bioavailability of trace elements in B. juncea

The potential bioavailability of minerals in plant tissue was measured in vitro via two independent procedures. One involved a series of sequential extractions (Ramos et al., 1994) that is used to assess bioavailability of trace metals in solid media (Berti et al., 1995). The procedure uses increasingly stringent extractants to characterize the solubility (water soluble, acid soluble, reducible, oxidizable, residual) of trace elements in a given matrix. The second procedure utilizes simulated gastric fluid to predict the solubility of the metal in the stomach (Glahn et al., 1998). These analyses assess the solubilization of the minerals from the plant tissue but not absorption as defined for "bioavailability" (Newman & Jagoe, 1994); however, minerals must be in a soluble form before uptake or metabolism by the body.

Minerals accumulated in *B. juncea* (i.e. Cr, Fe, Mn, Se, Zn) were readily solubilized in the sequential extraction procedure (Table 2). For all minerals examined, more than 80% of the total content in the enriched plant material was solubilized in the water soluble, acid soluble, and reducible fractions. Each of these plant bound minerals was found to be more soluble than those in an over-the-counter multivitamin (Table 2), suggesting that these plant-based minerals are more readily soluble and potentially more available compared to commercially available mineral supplements.

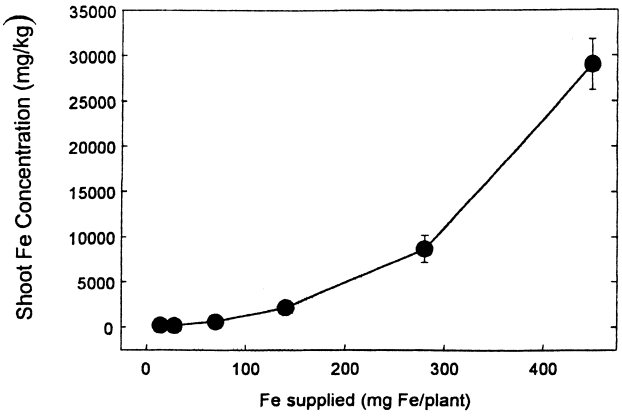

Fig. 1. Iron accumulation in shoots of 5-week old *B. juncea* in response to the level of Fe added to the root growth medium. The plants were grown for 4 weeks before exposure to the Fe-enriched nutrient solution levels indicated in the figure. Error bars are calcu-

Results of the simulated gastric fluid digestion confirm the data obtained from the sequential extraction study. Simulated gastric fluid extracted 31% of the total Fe in the plant sample and 81, 69, 100, and 100% of the total Cr, Mn, Se, and Zn, respectively (Table 3). All of the Se was rapidly solubilized by the simulated gastric fluid and did not increase after 15 min (Fig. 2). Similar results were obtained for Cr, Fe, Mn and Zn (data not shown). Solubility of these metals in the enriched plant material produced soluble-based %RDI values significantly greater than that of commercially available supplements with the exception of the Fe in Multivitamin III, Cr in Cr picolinate, and Zn in Zn picolinate (Table 3).

Results of the simulated intestinal fluid digestion shows that, in most cases, the solubility of the mineral-based supplements decreased in comparison to their behavior in the simulated gastric fluid digestion whereas the metals within the enriched plant matter remained soluble (Table 4). It is believed that the high pH of the intestinal fluid precipitates cationic metals that were soluble in the acidic simulated gastric fluid (i.e. Fe and Mn) as oxyhydroxides, whereas cationic metals associated with the plant matter are apparently chelated with organic complexes which prevents their precipitation. Selenium remained soluble in the simulated intestinal fluid for all supplements and plant materials; however, multivitamins I and II provide less than 30% of the RDI compared to well over 100% for the Se-enriched

Table 2
Solubility of Fe, Zn, Cr, Mn, and Se based on sequential extraction of trace mineral enriched *B. juncea* plants compared with a common trace element/multivitamin supplement[ab]

Supplement	Metal	Water-soluble	Acid-soluble	Reducible	Oxidizable	Residual
		(% of total metal concentration)				
B. juncea	Cr	76.95a	13.30a	3.06a	3.94a	2.74a
Multivitamin I		0.00b	0.00b	0.00b	0.00b	100.00b
B. juncea	Fe	47.85a	19.10a	16.91a	3.41a	12.71a
Multivitamin I		1.82b	0.10b	1.36b	0.21b	96.51b
B. juncea	Mn	87.14a	12.38a	0.28a	0.16a	0.04a
Multivitamin I		67.81a	15.31b	15.55b	0.73b	0.59b
B. juncea	Se	93.91a	3.63a	0.35a	1.84a	0.27a
Multivitamin I		0.00b	0.00b	100.00b	0.00b	0.00b
B. juncea	Zn	92.10a	7.41a	0.37a	0.06a	0.05a
Multivitamin I		3.73b	19.06b	64.96b	5.57b	6.68b

[a] Values are means of two replicates.
[b] Values labeled with the same letter for each metal in each column are not significantly different from each other using Student *t*-test and $\alpha = 0.10$.

Table 3
Solubility (percent of total metal content) and %RDI (recommended daily intake supplied based on the soluble content) of Cr, Fe, Mn, Se, and Zn in capsules/tablets of trace mineral supplements and plant material extracted by simulated gastric fluid[a,b]

Supplement	Cr		Fe		Mn		Se		Zn	
	% Sol	%RDI	% Sol	%RDI	% Sol	%RDI	% Sol	%RDI	% Sol	%RDI
Tablets/capsules										
Multivitamin I[c]	< 1	< 1a	9	9a	36	63a	100	29a	< 1	< 1a
Multivitamin II[d]	< 1	< 1a	1	1b	43	54a	100	14b	8	8a
Multivitamin III[e]	–[h]	–	50	75c	–	–	–	–	76	76b
Cr yeast (200 μg Cr)	< 1	< 1a	–	–	–	–	–	–	–	–
Cr picolinate (400 μg Cr)	96	318[b]	–	–	–	–	–	–	–	–
Se yeast (200 μg Se)	–	–	–	–	–	–	47	135c	–	–
Zn picolinate (30 mg Zn)	–	–	–	–	–	–	–	–	89	179c
Enriched B. juncea										
Cr (200 μg Cr)[f]	81	135c	–	–	–	–	–	–	–	–
Fe (10 mg Fe)[g]	–	–	31	17d	–	–	–	–	–	–
Mn (6.25 mg Mn)[g]	–	–	–	–	69	215b	–	–	–	–
Se (200 μg Se)[f]	–	–	–	–	–	–	100	286d	–	–
Zn (18.2 mg Zn)[g]	–	–	–	–	–	–	–	–	100	122d

[a] The average of three replicates was used to calculate %solubility and %RDI.
[b] Values labeled by the same letter in each column are not significantly different from each other (Duncan's Multiple Range test, $\alpha = 0.05$).
[c] Multivitamin I contains 0.065 mg Cr, 18 mg Fe, 3.5 mg Mn, 0.02 mg Se and 15 mg Zn per tablet.
[d] Multivitamin II contains 0.010 mg Cr, 18 mg Fe, 2.5 mg Mn, 0.01 mg Se, and 15 mg Zn per tablet.
[e] Multivitamin III contains 27 mg Fe and 15 mg Zn per tablet.
[f] Extracted from a 500 mg capsule of plant material.
[g] Extracted from a 250 mg sample of plant material.
[h] Not listed in this material.

plant material. Solubility of these metals in the enriched plant material produced soluble-based %RDI values significantly greater than that of commercially available supplements with the exception of the Fe in Multivitamin III, Cr in Cr picolinate, and Zn in Zn picolinate (Table 4). The high total mineral concentrations in the plant biomass coupled with a high degree of solubility in both the simulated gastric and intestinal fluid supports the use of these plants as mineral supplements.

3.3. Toxicological evaluation

Results from a 3-month evaluation indicate no acute or sub-chronical oral toxicity was found associated with these plant materials. These results were further independently confirmed in limit testing of Se-enriched plant material whereby the LD_{50} for this plant material exceeded the non-lethal dose of 5000 mg/kg body weight. Further testing of these enriched plant materials

are scheduled to be conducted on animals and human subjects.

3.4. Dietary implications of mineral enriched plants

Plants have developed several biochemical processes for the mobilization and uptake of minerals. To chelate and solubilize soil minerals, the roots secrete metal-chelating molecules such as phytosiderophores (Romheld, 1991). Metal-chelating proteins such as metallothioneins (Robinson, Tommey, Kuske & Sachson, 1993) or phytochelatins (Rauser, 1990) also function in plants to bind metals and enhance transport and metabolism. Organic acids have also been implicated in metal transport (Baker & Brooks, 1989). Selenium in *B. juncea* is partially metabolized to selenoamino acids, supporting the proposal that minerals accumulated by plants are subject to metabolic transformation (D.E. Salt, personal communication). In addition, metals are transported when chelated to organic acids, phytochelatins, or metallothioneins. This biochemical complexation maintains the metal in a soluble form that is available for metabolism, and may explain the relatively high bioavailability of minerals found in plants. While further studies involving human nutritional trials and in vitro cell culture are required to confirm the dietary benefits derived from trace elements supplied by plant tissue, the results presented here suggest that they may be more bioavailable than their current inorganic counterparts with the added benefits of other phytochemicals present in the plant tissue.

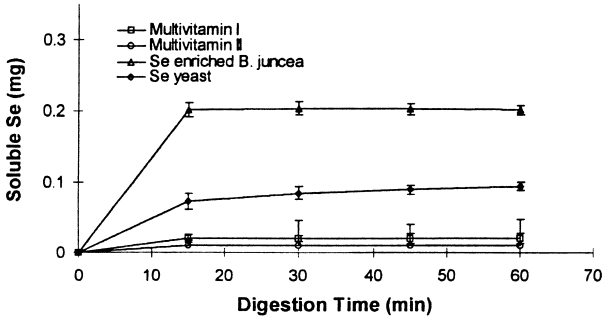

Fig. 2. Solubility of Se in Se enriched *B. juncea* and other Se supplements extracted with simulated gastric fluid with reaction times of 0, 15, 30, 45, or 60 min. Values are means of three replicates. Error bars are calculated as 2 standard errors of the mean.

The low solubility of Cr, Fe, Mn, Se, and Zn in several leading nutritional supplements in simulated gastric fluid clearly shows a need for a supplement that supplies greater available levels of these minerals for absorption to attain the recommended daily intake (RDI), not in total metal form, but in a soluble form (Table 2). For example, multivitamin I and II each contain 100% of the RDI for Fe and Zn; however, only a maximum of 9% and 8% of the RDI for Fe and Zn, respectively, was

Table 4
Solubility (percent of total metal content) and %RDI (recommended daily intake supplied based on the soluble content) of Cr, Fe, Mn, Se, and Zn in capsules/tablets of trace mineral supplements and plant material extracted by simulated intestinal fluid[a,b]

Supplement	Cr		Fe		Mn		Se		Zn	
	% Sol	%RDI	% Sol	%RDI	% Sol	%RDI	% Sol	%RDI	% Sol	%RDI
Tablets/capsules										
Multivitamin I[c]	< 1	< 1[a]	< 1	< 1a	3	5[a]	100	29a	< 1	< 1a
Multivitamin II[d]	< 1	< 1a	< 1	< 1a	1	2a	100	14b	< 1	< 1a
Multivitamin III[e]	–[h]	–	17	25b	–	–	–	–	16	16b
Cr yeast (200 μg Cr)	< 1	< 1a	–	–	–	–	–	–	–	–
Cr picolinate (400 μg Cr)	94	313b	–	–	–	–	–	–	–	–
Se yeast (200 μg Se)	–	–	–	–	–	–	73	208c	–	–
Zn picolinate (30 mg Zn)	–	–	–	–	–	–	–	–	60	121c
Enriched B. juncea										
Cr (200 μg Cr)[f]	97	162c	–	–	–	–	–	–	–	–
Fe (10 mg Fe)[g]			26	14c						
Mn (6.25 mg Mn)[g]					28	85b				
Se (200 μg Se)[f]	–	–	–	–	–	–	100	286[d]	–	–
Zn (18.2 mg Zn)[g]									48	58d

[a] The average of three replicates was used to calculate percent solubility and percent RDI.
[b] Values labeled with the same letter in each column are not significantly different from each other (Duncan's Multiple Range Test, $\alpha = 0.05$).
[c] Multivitamin I contains 0.065 mg Cr, 18 mg Fe, 3.5 mg Mn, 0.02 mg Se and 15 mg Zn per tablet.
[d] Multivitamin II contains 0.010 mg Cr, 18 mg Fe, 2.5 mg Mn, 0.01 mg Se, and 15 mg Zn per tablet.
[e] Multivitamin III contains 27 mg Fe and 15 mg Zn per tablet.
[f] Extracted from a 500 mg capsule of plant material.
[g] Extracted from a 250 mg sample of plant material.
[h] Not listed in this material.

solubilized by the simulated gastric fluid. Because eventual absorption of the metals by the body requires the metals to be soluble, achieving the 100% RDI criterion by total metal content alone is insufficient to meet daily nutritional requirements.

Bioavailability requires solubility, absorption, and eventual metabolism by the body (Newman & Jagoe, 1994). Even though solubility in stomach acid is important for digestion of food intake, metals must remain soluble in the intestinal fluid before absorption can occur. Soluble ions, not solid precipitates, are required for absorption. The poor performance of the mineral-based supplements, particularly the multivitamins, in providing the metals (except Se) in soluble form in the intestinal fluid simulant clearly shows that these supplements do not provide their metals in bioavailable form. The enriched plant matter can provide intestinal fluid soluble Cr, Mn, Se, and Zn at levels that exceed at least 50% of each metal's respective RDI in as little as 0.25 g of plant material, thereby providing a bioavailable source of these metals. Bioavailability will be directly measured in later pharmokinetic studies.

The most challenging aspect of providing trace elements in a plant-based material is to obtain a sufficient concentration for the supplement to be ingested without consuming large quantities of plant tissue. The use of forage crops enriched in Se from Se enriched soils to supplement the diets of animals has been proposed (Banuelos, 2000; Banuelos, Ajwa, Terry & Zayed, 1997) and attempts to increase the Se levels of garlic (Ip, Lisk & Scimeca, 1994) and Brussels sprouts (Stoeswand, Anderson, Muson & Lisk, 1989) have been performed. However, to achieve sufficient selenium levels to reduce mammary carcinogenesis in rats, dietary fractions of 2% for garlic and 20% for Brussels sprouts were required — prohibitively large quantities for a normal human diet.

The plants used to produce nutritional supplements in this study are able to supply a greater %RDI in soluble form than popular multivitamins and supplements and provide these nutrients in a much smaller quantity ($<$ 0.5 g) for human consumption (Tables 3 and 4). The %RDI of Cr, Mn, Se, and Zn supplied by these plants, calculated from the soluble metal concentration, exceeds the %RDI of most of the mineral-based supplements and allows small quantities of these plants to meet the RDI of these minerals in soluble form.

The consumption of dietary supplements that have higher micronutrient concentrations with greater bioavailability is important in meeting the varying nutritional requirements of all individuals. The results reported here indicate that certain species of plants, when cultivated under appropriate conditions, can accumulate sufficient quantities of important micronutrients that meet the recommended dietary intake of each micronutrient in a bioavailable form. The ability to achieve percent level concentrations of the trace elements in the dry plant matter allows the material to be supplied in a traditional capsule or tablet form. The hydroponic cultivation process can be used in this instance to produce a consistent, predictable concentration of the essential elements while avoiding undesirable heavy metals and other elements found in soil-grown plants. We propose that these plants can be processed into a natural mineral dietary supplement with improved performance.

The economics of cultivating mineral accumulating crops compares quite favorably with current sources of quality mineral supplements. The plants can be cultivated, harvested, and processed at a cost of less than $100 per kg, which compares very favorably, up to 40 times less, to the costs associated with bulk purchases for chromium picolinate and selenium yeast supplements.

References

Ashmead, H. (1982). Chelated mineral nutrition in plants. In D. W. Ashmead, *Animals and man* (pp. xi–xv). Springfield, IL: C.C. Thomas.

Baker, A. J. M., & Brooks, R. R. (1989). Terrestrial higher plants which hyperaccumulate metallic elements — A review of their distribution, ecology and phytochemistry. *Biorecovery, 1*, 81–126.

Banuelos, G. S. (2000) Factors influencing field phytoremediation of selenium-laden soils. In N. E. Terry, and G. S. Banuelos, *Phytoremediation of contaminated soil and water*. Ann Arbor Press, Ann Arbor, MI.

Banuelos, G. S., & Meeks, D. W. (1990). Accumulation of selenium in plants grown on selenium-treated soil. *Journal of Environmental Quality, 19*, 772–777.

Banuelos, G. S., Ajwa, H. A., Terry, N. E., & Zayed, A. (1997). Phytoremediation of Se laden soils: A new technology. *Journal of Soil and Water Conservation, 52*, 426–430.

Banuelos, G. S., Cardon, G., Mackey, B., Ben-Asher, J., Wu, L., Beuselinck, P., Akohoue, S., & Zambrzuski, S. (1993). Plant and Environment Interactions. Boron and selenium removal in boron-laden soils by four sprinkler irrigated plant species.. *Journal of Environmental Quality, 22*, 786–792.

Berti, W., Cunningham, S. D., and Jacobs, L. W. (1995) Sequential chemical extraction of trace elements: Development and use in remediating contaminated soils. In *Proc. 3rd International Conference on biogeochemistry of trace elements*.

Brooks, R. R., Morrison, R. S., Reeves, R. D., Dudley, T. R., & Akman, Y. (1979). Hyperaccumulation of nickel by *Alyssum Linnaeus* (Cruciferae). *Proceedings of the Royal Society of London, Serial B, 203*, 387–403.

Brooks, R. R., Morrison, R. S., Reeves, R. D., & Malaisse, F. (1978). Copper and cobalt in African species of Aeolanthus Mart (Plectranthinae, Labiatae). *Plant and Soil, 50*, 503–507.

Brooks, R. R., Trow, J. M., Veillon, J. M., & Jaffre, J. M. (1981). Studies on manganese accumulating Alyxia from New Caledonia. *Taxonomy, 30*, 420–423.

Brown, S. L., Chaney, R. L., Angle, J. S., & Baker, A. J. M. (1994). Phytoremediation potential of *Thlaspi caerulescens* and Bladder Campion for zinc- and cadmium-contaminated soil. *Journal of Environmental Quality, 23*, 1151–1157.

Cunningham, S. D., & Ow, D. W. (1996). Promises and prospects of phytoremediation. *Plant Physiology, 110*, 715–719.

Dushenkov, V., Kumar, P. B. A. N., Motto, H., & Raskin, I. (1995). Rhizofiltration: The use of plants to remove heavy metals from aqueous streams. *Environmental Science and Technology, 29*, 1239–1245.

Ernst, W. H. O., Verkleij, J. A. C., & Schat, H. (1992). Metal tolerance in plants. *Acta Botonica Neerlanlica, 41*, 229–248.

Fahey, J. W., Zhang, Y., & Talalay, P. (1997). Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. *Proceedings of the National Academy of Science, USA, 94*, 10367–10372.

Fairweather-Tait, S. J. (1996). Bioavailability of dietary minerals. *Biochemical Society Transactions, 24*, 775–780.

Glahn, R. P., Lai, C., Hsu, J., Thompson, J. F., Guo, M., & Van Campen, D. R. (1998). Decreased citrate improves iron availability from infant formula: application of an in-vitro digestion/Caca-2 cell culture model. *Journal of Nutrition, 128*, 257–264.

Ip, C., Lisk, D. J., & Scimeca, J. A. (1994). Potential of food modification in cancer prevention. *Cancer Research (Supplement), 54*, 1957s–1959s.

Jones, J. B., & Case, V. W. (1990). Sampling, handling and analyzing plant tissue samples. In R. L. Westerman, *Soil Testing and Plant Analysis, 3rd ed. SSSA Book Series No. 3* (pp. 389–427). Madison, WI: Soil Science Society of America Inc.

Kumar, P. B. A. N., Dushenkov, V., Motto, H., & Raskin, I. (1995). Phytoextraction: The use of plants to remove heavy metals from soils. *Environmental Science and Technology, 29*, 1232–1238.

National Research Council (1989). *Recommended dietary allowances*. (10th). Washington DC: National Academy Press.

Newman, M. C., & Jagoe, C. H. (1994). Ligands and the bioavailability of metals in aquatic environments. In J. L. Hamelick, P. F. Bergman, H. L. Bergman, & W. H. Benson, *Bioavailability: Physical, chemical, and biological interactions, CRC Press, Lewis Publishers* (pp. 39–61). FL: Boca Raton.

Orser, C. S., Salt, D. E., Pickering, I. J., Prince, R., Epstein, A., & Ensley, B. D. (1999). Brassica plants to provide enhanced mineral nutrition: selenium phytoenrichment and metabolic transformation. *Journal of Medicinal Food, 1*, 253–261.

Ramos, L., Hernandez, L. M., & Gonzalez, M. J. (1994). Sequential extraction of copper, lead, cadmium, and zinc in soils from or near Donana National Park. *Journal of Environmental Quality, 23*, 50–57.

Raskin, I., Kumar, P. B. A. N., Dushenkov, S., & Salt, D. E. (1994). Bioconcentration of heavy metals by plants. *Current Opinion in Biotechnology, 5*, 285–290.

Rauser, W. E. (1990). Phytochelatins. *Annual Reviews of Biochemistry, 59*, 61–86.

Reeves, R. D., & Brooks, R. R. (1983). Hyperaccumulation of lead and zinc by two metallophytes from mining areas in Central Europe. *Environmental Pollution, Serial A, 31*, 277–285.

Robinson, N. J., Tommey, A. M., Kuske, C., & Jackson, P. J. (1993). Plant Metallothioneins. *Biochemistry Journal, 295*, 1–10.

Romheld, V. (1991). The role of phytosiderophores in acquisition of iron and other micronutrients in graminaceous species: An ecological approach. *Plant and Soil, 130*, 127–134.

Rotman, D. (1998, September/October) The next biotech harvest. *Technology Review* (pp. 34–41).

Salt, D. E., Blaylock, M., Kumar, N., Dushenkov, S., Ensley, B., Chet, I., & Raskin, I. (1995). Phytoremediation: A novel strategy for the removal of toxic metals from the environment using plants. *Bio/Technology, 13*, 468–474.

Stoeswand, G. S., Anderson, J. L., Munson, L., & Lisk, D. J. (1989). Effect of dietary Brussels sprouts with increased selenium content on mammary carcinogenesis in the rat. *Cancer Letters, 45*, 43–48.

Welch, R. M., & LaRue, T. A. (1990). Physiological characteristics of Fe accumulation in the 'Bronze' Mutant of *Pisum sativum* L, cv 'Sparkle' E107 (brz brz). *Plant Physiology, 93*, 723–729.