ORIGINAL ARTICLE

Heavy metal analysis in commercial *Spirulina* products for human consumption

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Abstract For consumption of health foods of *Spirulina,* by the general public, health food stores are increasingly offering more exotic products. Though *Spirulina* consumption is growing worldwide, relatively few studies have reported on the quantities of heavy metals/minerals they contain and/or their potential effects on the population's health. This study reveals the concentrations of six typical heavy metals/minerals (Ni, Zn, Hg, Pt, Mg, and Mn) in 25 *Spirulina* products commercialized worldwide for direct human consumption. Samples were ground, digested and quantified by Coupled Plasma Mass Spectroscopy (ICP–MS). The concentrations (mg/kg d.w.) were range from 0.001 to 0.012 (Pt) followed by 0.002–0.028 (Hg), 0.002–0.042 (Mg), 0.005–2.248 (Mn), 0.211–4.672 (Ni) and 0.533–6.225 (Zn). The inorganic elements of the present study were significantly lower than the recommended daily intake (RDI) level of heavy metal elements (mg/daily) Ni (0.4), Zn (13), Hg (0.01), Pt (0.002), Mg (400) and Mn (4). Based on this study the concentration of inorganic elements was not found to exceed the present regulation levels, and they can be considered as safe food.

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1. Introduction

Spirulina is a photosynthetic, filamentous non-differentiated, spiral-shaped, multicellular cyanobacteria that grow naturally in warm climates (Sánchez et al., 2003). It is a ubiquitous organism that was used as food in Mexico 400 years ago during the Aztec civilization. They are found in a variety of environments: soil, sand, marshes, brackish water, sea water, and fresh water. In recent years, the most commonly used *Spirulina* are *S. platensis* and *S. maxima* (Khan et al., 2005). Today, there are several companies producing *Spirulina* as a food supplement, which is sold in many health food stores around the world (Belay et al., 1993). On the other hand, about 30% of the current world production of 2000 tons is sold for animal food applications (Belay et al., 1996). *Spirulina* is being grown in the United States, Hawaii, Thailand, Taiwan, Chile, Vietnam, India, Japan, Cuba, Spain, Argentina, Mexico and other countries (Fox, 1996). Worldwide medical research has discovered that *Spirulina* with its unique blend of nutrients (good quality proteins, balanced fatty acid profile, antioxidant vitamins, and minerals) has helped to combat many health

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problems like diabetes, arthritis, anaemia, cancer and so forth. *Spirulina* capsules have also proved effective in lowering blood lipid level, and in decreasing white blood corpuscles (Ruan et al., 1988, 1990), as well as improving immunological function. In addition, *Spirulina* also is used for health food, feed and for the biochemical products since 1980s. *Spirulina* seems to be one of the best solutions and safe food for children, teenagers and adults as a high-quality food supplement (Becker, 1988; Borowitzka, 1988; Richmond, 1988).

Metals like lead, mercury, cadmium, and arsenic are the most likely to adulterate *Spirulina* products. Each is found as a trace contaminant in certain pesticides and fertilizers, so they are common in agricultural areas. Nickel, copper, and zinc are common fertilizer components or contaminants, but they are substantially less toxic and have a narrow range of optimum concentrations for algae and cynaobacteria, at least in the case of *Chlorella* and *Spirulina* (Kallqvist and Meadows, 1978; Gribovskay et al., 1980; Pande et al., 1981; Kotangale et al., 1984). They are likely to terminate algae growth before being accumulated at levels toxic to humans. Tin, chromium, selenium, and aluminium are not such a universal threat, but local conditions must be appraised before they can be completely eliminated as a possible hazard. It is well known that an overdose of trace elements is harmful to health. It was reported in Japan that consuming cadmium contaminated rice could cause Itai-Itai disease (Kaneta et al., 1986). Overdoses of lead can cause lead poisoning, resulting in intelligence retardation and slow reactions and arsenic could cause black-foot-disease (Foulke, 1993).

Cyanobacteria may be especially effective accumulators: certain types excrete hydroxamate chelating agents (Murphy et al., 1976) that can act as carrier molecules or increase the trace metal pool available near the cell surface. Experiments demonstrate that *Spirulina platensis* accumulates trace metals more effectively than *Chlorella vulgaris* (Gribovskay et al., 1980), an advantage with regard to trace elements essential to humans but a liability if toxic metals are present. Inductively Coupled Plasma Mass Spectrometry (ICP–MS) has been validated for analysis of trace metals in plants (Leiterer et al., 1997). However, ICP–MS is currently being used to detect metals in seaweeds (Netten et al., 2000) and algae food products (Dawczynski et al., 2007). The aim of the study was to determine the heavy metal concentration/contamination in 25 commercially available *Spirulina* products from different countries of origin, in order to assess their contamination sources during their treatment processes as well as the harvesting conditions.

2. Methods and materials

2.1. Spirulina sample collection

Totally 25 *Spirulina* samples in the form of tablets and capsules were obtained from specialist shops from seven different countries of origin. The product code and country of origin are summarized in Table 1.

2.2. Sample preparation

From 25 *Spirulina* samples, 16 samples were in tablet form and 9 were in capsule form. The tablet form of samples was ground well aseptically using a mortar and pestle and capsules were removed from the capsular form of samples and the powders were directly used for analysis.

Table 1 List of *Spirulina* products and their country of origin.

Code Number	Product type	Manufacturing company	Country of Origin
S1	Tablets	TAAU Australia Pvt Ltd, NT	Australia
S2	Capsules	General Nutrition Corp, Pittsburgh	USA
S3	Capsules	Nature's Way Products, Inc, Springville, Utah	USA
S4	Tablets	Good 'N Natural, New York	USA
S5	Tablets	Now Foods, Bloomingdale	USA
S6	Tablets	Nature Pure, Inc., Larkspur, California	USA
S7	Tablets	Source Naturals, Inc, Santa Cruz, California	USA
S8	Tablets	Jarrow Formulas, Los Angeles, CA	USA
S9	Tablets	Earthrise Nutritionals LLC, Irvine, CA	USA
S10	Tablets	Nutrex Hawaii Inc, Kailua-Kona, Hawaii	USA
S11	Capsules	Pure Planet Products, Inc., Long Beach, CA	USA
S12	Tablets	Puritan's Pride, Inc., Oakdale, New York	USA
S13	Capsules	21st Century HealthCare, Inc., Arizona	USA
S14	Tablets	Japan Algae Co., Ltd., Tokyo	Japan
S15	Tablets	All Seasons Health, Hampshire	United Kingdom
S16	Capsules	Fushi Wellbeing Ltd., London	United Kingdom
S17	Tablets	Biovea, London	United Kingdom
S18	Capsules	Parry Nutraceuticals, Chennai	India
S19	Tablets	Lifestream International Ltd, Northcote, Auckland	New Zealand
S20	Tablets	Green Health, Auckland	New Zealand
S21	Tablets	RBC Life Sciences, Inc., Burnaby, British Columbia (BC)	Canada
S22	Tablets	Swiss Herbal Remedies Ltd., Richmond Hill, Ontario	Canada
S23	Capsules	Herbal Select, Guelph, Ontario	Canada
S24	Capsules	Gourmet Nutrition F.B. Inc., STE-Julie (Quebec)	Canada
S25	Capsules	Terra Vita Fine Whole Herbs, Brampton, Ontario	Canada

Table 2 Heavy metal concentration in *Spirulina* samples.

Spirulina samples	Heavy metal mg/kg d.w.					
	Ni	Zn	Hg	Pt	Mg	Mn
S1	0.211	0.533	0.002	0.001	0.002	0.076
S2	4.672	5.627	0.028	0.01	0.03	0.587
S3	2.016	2.397	0.017	0.008	0.018	0.603
S4	2.147	1.628	0.02	0.011	0.026	0.436
S5	2.199	1.235	0.017	0.008	0.014	1.17
S6	3.726	6.225	0.022	0.009	0.028	0.007
S7	2.601	2.817	0.018	0.011	0.028	0.007
S8	3.577	1.871	0.023	0.012	0.031	0.309
S9	3.519	3.267	0.021	0.011	0.03	0.008
S10	2.442	2.041	0.017	0.008	0.021	0.137
S11	3.785	3.007	0.019	0.008	0.028	0.008
S12	3.597	2.859	0.019	0.008	0.024	0.006
S13	2.857	2.901	0.019	0.008	0.026	1.643
S14	2.852	2.114	0.026	0.008	0.042	0.011
S15	2.437	1.568	0.02	0.008	0.028	0.008
S16	2.712	2.434	0.019	0.009	0.033	1.777
S17	2.948	2.513	0.021	0.009	0.028	1.8
S18	2.633	1.876	0.016	0.009	0.023	1.328
S19	3.731	3.184	0.017	0.009	0.036	2.248
S20	2.225	1.548	0.008	0.009	0.019	1.132
S21	1.618	1.478	0.014	0.006	0.02	0.005
S22	1.589	4.626	0.016	0.007	0.023	0.009
S23	3.272	4.428	0.02	0.008	0.018	0.008
S24	3.558	3.733	0.017	0.009	0.034	1.433
S25	2.319	2.586	0.017	0.008	0.024	0.006

2.3. Elemental determination

Triplicate powder samples of each product were digested for heavy metal analyses. 100 mg of each triplicate was placed into a Teflon PFA type digestion vessel and dissolved in 65% HNO$_3$ (Merck, Germany; Suprapur grade) using a microwave sample preparation system (CEM Co. Model MDS-200) (Sahan et al., 2007). The temperature in the interior of the vessels can be monitored with the 300 Automatic Temperature Control Probe. Maximum operating temperature and pressure were 300 °C and 100 bar, respectively. Repeated concentration–dilution procedures were performed and the final volume was 10–20 ml which was used for heavy metal determination.

Heavy metal content was determined by Inductively Coupled Plasma Mass Spectroscopy (ICP–MS) using a Perkin Elmer apparatus model Elan-6000 (Waltham, Massachusetts, USA) following the manufacturer's recommended standard operating procedure. The certified reference materials were purchased from Merck Co. and used for the preparation of standard solutions. The instrumental parameters of ICP–MS such as forward power (1400 W), sample cone (Ni) – 1.0 mm and skimmer cone (Ni) – 0.75 mm, spray chamber temperature of 10 °C and diffusion chamber temperature of 18 °C, gas control nebuliser (0.7 ml/min), coolant (13 ml/min, auxiliary 0.5 ml/min), sample flow rate (1.0 ml/min) and sampling distance (10 mm) from load coil and vacuum control expansion stage (1.8 mbar), intermediate (<104 mbar), analyser (5 × 106 mbar) and data acquisition for surveying, scanning mode (200 sweep, 4–245 amu), dwell time (160 µs, 2048 channels), and run time of 65 s were employed for this analysis. Standard concentrations for each element were prepared and

used according to the recommendations by the manufacturer to calibrate the instrument before using it on real samples.

2.4. Statistical analysis

The statistical analysis of mean ± SD and correlation co-efficient was carried out using PASW statistics 18 software package.

3. Results

The results of heavy metal analysis of 25 *Spirulina* products in dry weight are summarized in Table 2. The order of metal concentrations determined in this study for *Spirulina* samples was Pt > Hg > Mg > Mn > Ni > Zn, while the concentrations (mg/kg d.w.) range from 0.001 to 0.012 (Pt) followed by 0.002–0.028 (Hg), 0.002–0.042 (Mg), 0.005–2.248 (Mn), 0.211–4.672 (Ni) and 0.533–6.225 (Zn).

3.1. Nickel

Table 2 shows that Ni concentrations for different *Spirulina* samples are highly variable. The Ni content ranged between 0.211 and 4.672 mg/kg d.w. and the highest value of Ni (4.672 mg/kg d.w.) was detected in sample S2. Among the 25 samples tested, only three samples namely S1, S21 and S22 had minimum level of Ni content as 0.211, 1.618 and 1.589 mg/kg d.w. respectively. Whereas in the remaining 22 samples, high amount of Ni content was detected ranging from 2.016 to 4.672 mg/kg d.w. The order of Nickel concentration determined from the samples was S3 > S4 > S5 > S20 > S25 > S15 > S10 > S7
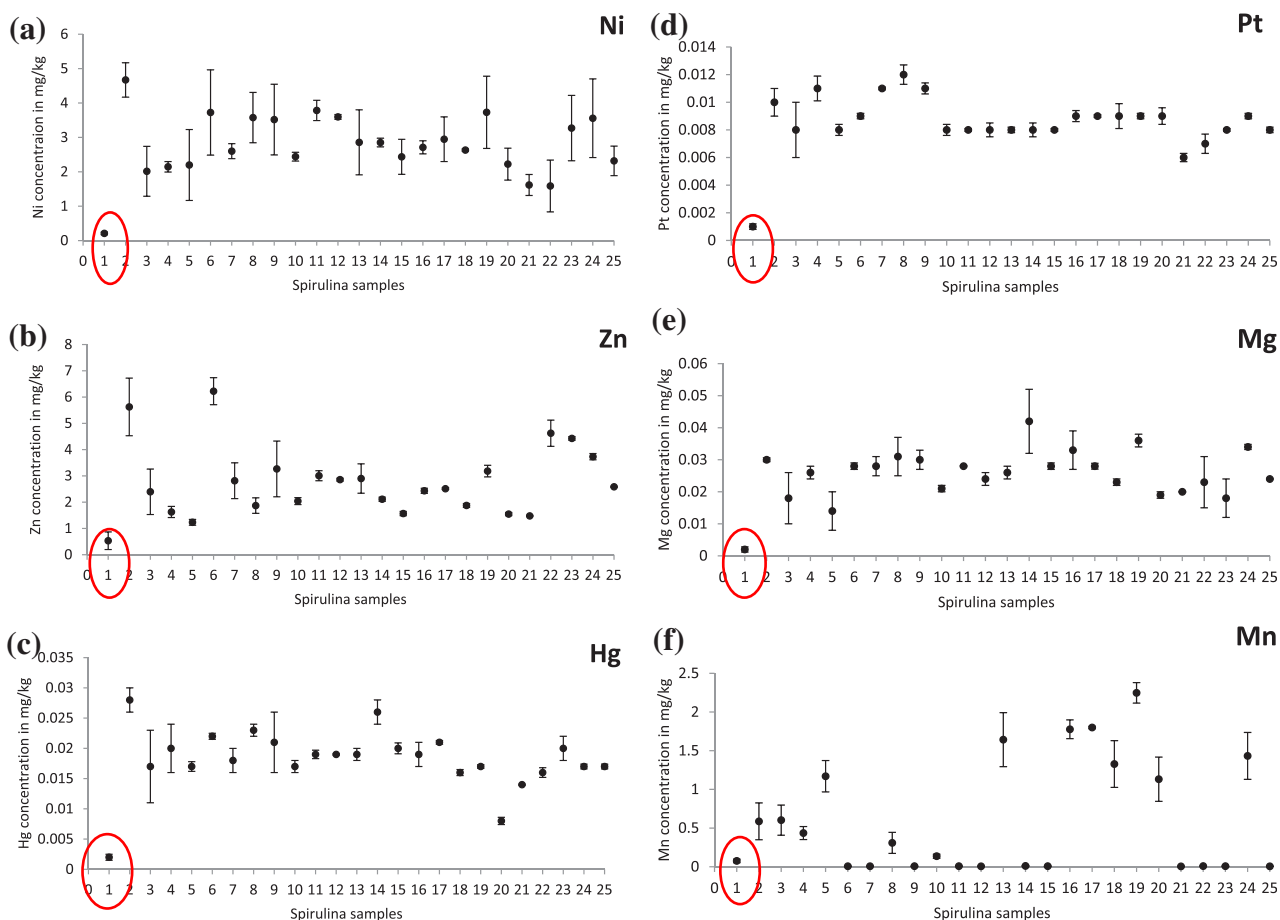
Figure 1   (a–f) Graphical effect of heavy metal concentration in commercially available *Spirulina* products. 1–25 – product code shown in Table 1.

> S18 > S16 > S14 > S13 > S17 > S23 > S9 > S24 > S8 > S12 > S6 > S19 > S11 > S2 (Table 2 and Fig. 1a).

3.2. Zinc

The Zn content ranged between 0.533 and 6.225 mg/kg d.w. detected in all *Spirulina* products. Altogether *Spirulina* products tested, only one sample S1 was found to have minimum Zn content of 0.533 mg/kg d.w. Nevertheless, the remaining 24 samples had the Zn content of > 1.200 mg/kg d.w. On the other hand, the samples S6, S2, S22 and S23 had the highest content of Zn such as 6.225, 5.627, 4.626 and 4.428 respectively (Table 2 and Fig. 1b).

3.3. Mercury

The differences in Hg content were not very pronounced in all the *Spirulina* samples tested. The Hg content ranged between 0.002 and 0.028 mg/kg d.w. and the highest value of Hg (0.028 mg/kg d.w.) was detected in sample S2. Among the 25 samples tested, in all the samples < 0.03 mg/kg d.w. of Hg content was detected (Table 2 and Fig. 1c).

3.4. Platinum

All the tested *Spirulina* samples had Pt concentrations below the method's limit of detection (Table 2). The Pt contents ranged

0.001 mg/kg dw as detected in sample S1 and 0.012 mg/kg dw in sample S8. The differences in Pt contents were also not very pronounced in all the tested *Spirulina* samples (Table 2 and Fig. 1d).

3.5. Magnesium

Totally 25 samples were tested including 16 tablets and nine capsule forms of *Spirulina* products (Table 1). The Mg contents ranged between 0.002 mg/kg dw, detected in sample S1 and 0.042 mg/kg dw found in sample S14. Among the *Spirulina* products tested, all the samples had < 0.05 mg/kg d.w. of Mg content (Table 2 and Fig. 1e).

3.6. Manganese

Table 2 shows that Mn concentrations for different *Spirulina* samples are highly variable. The highest value (2.248 mg/kg dw) corresponded to the sample of S19. The samples S20, S5, S18, S24, S13, S16 and S17 had maximum (> 1.10 mg/kg d.w.) level of Mn, the concentrations were 1.132, 1.170, 1.328, 1.433, 1.643, 1.777 and 1.800 mg/kg dw, respectively. While in the remaining 17 samples, the Mn concentration was detected between 0.005 and 0.603 mg/kg d.w., the order of concentration was S21 > S25 > S12 > S6 > S7 > S23 > S11 > S15 > S9 > S22 > S15 > S1 > S10 > S8 > S4 > S2 > S3 (Table 2 and Fig. 1f).

4. Discussion

Sprirulina products have been used in many countries, as a dietary supplement. Recently these products have become popular in the food industry because of high protein and number of interesting essential fatty acids and vitamins. Very little control exists over the composition of these products, which could be contaminated with a number of agents including heavy metals and certain radioactive isotopes. The monitoring system of continuous surveillance of contaminant content in food and pharmaceutical products is crucial for consumer protection and facilitates international trade (Kuhnlein and Chan, 2000). Risk assessment is a continually evolving process as information on contaminants, the health effects involved and their occurrence in food are all factors that should be continuously studied and monitored (Kuhnlein and Chan, 2000). The heavy metals such as lead, mercury, cadmium and arsenic are mostly found to adulterate in *Spirulina* products. Each is found as a trace contaminant in certain pesticides and fertilizers, so they are common in agricultural areas (Pande et al., 1981; Kotangale et al., 1984).

In this study, twenty-five products were analysed, the most abundant heavy metals in the *Spirulina* samples are Zn and Ni, and the least abundant are usually Hg and Pt (Table 2). There are limited studies concerning inorganic contaminants in products of this kind. The Ni content of twenty-five *Spirulina* samples was analysed, significant differences in Ni levels between the *Spriulina* samples were found. The maximum Ni level reported previously in an unknown *Spirulina* product A is 7.7 mg kg^{-1} dry weight, and slightly higher than those of *Spirulina* B and *Spirulina* C (Ortega-Calvo et al., 1993). In the current study the highest Ni level was recorded in *Spirulina* sample S2 (4.672 mg/kg d.w.) and there were slight differences in all the samples.

Concentration of Zn content is remarkably higher than that of other metals in all *Spirulina* samples. Zinc content was tested in all *Spirulina* products ranging from 0.533 to 6.225 mg/kg (Table 2), this level below the maximum amount (1.5–10 mg/100 g) allowed in macroalgae for human consumption in Japan and France (Indegaard and Minsaas, 1991) and also below the daily intake level of 13 mg (Iyengar, 1985). The recommended daily intake of heavy metal elements is summarized in Table 4. The experimental values for Zn were similar to those from other studies (Hou and Yan, 1998; Netten et al., 2000; Besada et al., 2009), and very disperse. It has been reported by Ito and Miyosshi (1990) for instance, that certain elements such as zinc appear to be taken up at different rates at different stages of growth. Local variation in salinity has also

Table 3 Correlation co-efficient matrix for heavy metals in *Spirulina* samples.

	Ni	Zn	Hg	Pt	Mg	Mn
Ni	1					
Zn	0.631	1				
Hg	0.751	0.512	1			
Pt	0.663	0.286	0.670	1		
Mg	0.681	0.352	0.733	0.648	1	
Mn	0.158	−0.084	−0.059	0.160	0.215	1

Critical $r = 0.725$ at $P < 005$ ($n = 24$).

Table 4 Recommended daily intake of heavy metal elements (Iyengar, 1985).

Elements	Ni	Zn	Hg	Pt	Mg	Mn
Daily intake (mg/daily)	0.4	13	0.01	0.002	400	4

been shown to be an important factor in the biological uptake of a number of elements (Cho et al., 1995; Struck et al., 1997).

It was interesting to note that Hg and Pt were all below the detection limit in most of the globally collected *Spirulina* samples. The lowest and highest levels of mercury were also found in samples S1 and S2 respectively. All of the twenty-five samples were < 0.03 mg/kg d.w. indicating that mercury is not a ubiquitous contaminant. For all the products analysed, mercury level in all *Spirulina* samples does not exceed the legislation of UN World Health Organization/FAO i.e. the tolerable amount of 50 µg/day (Henrikson, 1989). Hg concentrations were similar to those reported elsewhere (Companella et al., 1998; Hsu et al., 2001; Al-Homaidan, 2006; Besada et al., 2009).

Table 2 shows that Mg concentrations for twenty-five *Spirulina* products are not very pronounced. On the other hand, the highest Mn concentration (2.248 mg/kg d.w.) was documented in *Spirulina* samples S19 and lowest (0.005 mg/kg d.w.) recorded in sample S21. Correlation co-efficient between the nutrients was analysed. A significant positive correlation ($P < 0.005$) exists between Hg and Ni ($r = 0.751$), and Mg and Hg ($r = 0.733$) (Table 3). The range of Mn content found in the present study is very less compared to those previously reported by Hsu et al. (2001).

5. Conclusions

The heavy metals in 25 marketed *Spirulina* food samples were identified and quantified in this study. Results showed that the contents of Ni, Zn, Hg, Pt, Mg and Mn in the entire tested *Spirulina* food samples were all within the daily intake levels. Therefore, all the tested *Spirulina* food samples were considered to be safe food.

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