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Heavy Metals Concentration in Commercially available *Spirulina* Products

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***Spirulina*, the blue-green algae, has today emerged as a great nutraceutical phenomenon. Though *Spirulina* consumption is growing worldwide, relatively few studies have reported on the quantities of heavy metals they contain and/or their potential effects on the population's health. This study focuses analyses the concentrations of seven typical heavy metals (Pb, As, Cd, Cr, V, Cu and Fe) in 25 *Spirulina* products commercialized in worldwide for direct human consumption. Samples were ground, digested and quantified by Coupled Plasma Mass Spectroscopy (ICP-MS). The concentration ranges found, expressed in mg/kg, dry weight, were: Cr (0.003 - 0.018) followed by Cd (0.003 – 0.069), As (0.006 – 0.578), V (0.005 – 1.199), Pb (0.100 – 1.206), Cu (0.017 – 3.155) and Fe (0.336 – 54.68). The inorganic elements of the present study were significantly lower than recommended daily intake (RDI) level of heavy metal elements (μg/daily) As (100) followed by Pb (300), Zn (13000), Cu (2500), Cd (40), Cr (50) and Ni (400). Based on this study the concentration of inorganic elements were not found to exceed the present regulation levels, they can be considered as safe food.**

Key word: *Spirulina*, Cyanobacteria, Coupled Plasma Mass Spectroscopy, Heavy metals, Recommended daily intake.

Spirulina is a photosynthetic, filamentous non-differentiated, spiral-shaped, multicellular, and blue-green microalgae (cyanobacteria) that grows 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 variety of environments: soil, sand, marshes, brackish water, sea water, and fresh water. In recent year, 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, 1993). On the other hand, about 30% of the current world production of 2000 ton is sols 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, Mexcio and other countries (Fox, 1996). Worldwide medical research has discovered that *Spirulina* with its unique blend of nutrients (good quality proteins, balanced fatty acids profile, antioxidant vitamins, and minerals) has helped to combat many health problems like diabetes, arthritis, anemia, 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,

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Spirulina also is used for health food, feed and for the biochemical products since 1980s (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, at least in the case of *Chlorella* and *Spirulina* (Kallqvist and Meadows, 1978; Gribovskaya *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 aluminum are not such a universal threat, but local conditions must be appraised before they can be completely eliminated as a possible hazard.

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* (Gribovskaya *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). In order to quantify the metal concentration/contamination, the present investigation was carried out in 25 commercially available *Spirulina* products from different countries of origin for quantify the available metals concentration using ICP-MS. The results of these data would be benefit future nutritional and quality studies.

MATERIALS AND METHODS

Spirulina sample collection

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

Elemental determination

The sealed samples of *Spirulina* were purchased by the author from worldwide producers. Triplicate samples of each product were digested for heavy metal analyses. 100 mg of each triplicate was put 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). 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 ^{o}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 instrumental parameters of ICP-MS were as follows: forward power (1400 W), sample cone (Ni) – 1.0 mm and skimmer cone (Ni) – 0.75 mm, spray chamber temperature 10^{o}C and diffusion chamber temperature 18^{o}C, gas controls 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 controls expansion stage (1.8 mbar), intermediate (<104 mbar), analyser (5x106 mbar) and data acquisition for surveying, scanning mode (200 sweep, 4-245 amu), dwell time (160 ìs, 2048 channels), and run time 65 s. 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.

Statistical analysis

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

RESULTS

The results of metals analysis of 25 *Spirulina* products in dry weight are summarized in Table 2. The order of metals concentrations determined in this study for *Spirulina* samples was Cr>Cd>As>V>Pb>Cu>Fe, while the concentrations (mg/kg d.w.) ranging from 0.003 to 0.018 (Cr) followed by 0.003 – 0.069 (Cd), 0.006 – 0.578 (As), 0.005 – 1.199 (V), 0.100 – 1.206 (Pb), 0.017 – 3.155 (Cu) and 0.336 – 54.68 (Fe).

Lead

The table 2 shows that Pb concentrations for different *Spirulina* samples are highly variable. The Pb content ranged between 0.100 to 1.206 mg/kg dw and the highest value of Pb (1.206 mg/kg dw) was detected in sample S1 from Australia. Among the 25 samples tested, the maximum level of Pb content at 1.206 and 0.835 mg/kg d.w. were found in only in two samples namely S1 and S15 respectively. The samples namely S1 and S15 were purchased from Australia and United Kingdom respectively. Whereas the remaining 23 samples, less amount of Pb content was detected ranging from 0.100 to 0.470 mg/kg dw. The order of lead concentration determined from the samples was S1>S15>S6>S7>S8>S16>S25>S5>S21>S25>S22>S18>S17>S4>S3>S14>S10>S20>S13>S11>S9>S23>S12>S9>S2 (Table 2 and Fig. 1a).

Arsenic

The As contents ranged (mg/kg d.w.) between 0.006 detected in sample S1 and 0.578 found in samples S8. Altogether *Spirulina* products tested, only two samples namely S8 and S9 found maximum As contents were 0.578 and 0.515 mg/kg dw respectively. The both S8 and S9 *Spirulina* products procured from USA. Nevertheless, the three samples S7, S4 and S5 had the As contents were 0.111, 0.206 and 0.229 respectively. The rest of 23 samples, the order of As concentration was detected as S1>S10>S13>S12>S11>S23>S6>S25> S24> S3> S17> S22> S2> S21> S15> S14> S18> S19>S20>S16 (Table 2 and Fig. 1b).

Cadmium

The differences in Cd content were not

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 Produts, Inc, Springville, Utah	USA
S4	Tablets	Good ‘N Natural, Yew 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 concentration in mg/kg d.w.						
	Pb	As	Cd	Cr	V	Cu	Fe
S1	0.100	0.006	0.003	0.003	0.0050	0.26	3.44
S2	1.206	0.051	0.069	0.013	0.0470	3.155	12.11
S3	0.835	0.046	0.033	0.008	0.0330	1.375	15.11
S4	0.264	0.206	0.006	0.011	0.510	0.663	41.3
S5	0.269	0.229	0.009	0.009	0.5180	0.017	29.71
S6	0.329	0.036	0.011	0.012	0.3240	0.865	0.329
S7	0.470	0.111	0.011	0.012	0.360	1.276	40.89
S8	0.356	0.578	0.007	0.013	1.1990	0.661	28.93
S9	0.368	0.515	0.015	0.013	1.1990	0.632	69.24
S10	0.218	0.025	0.004	0.009	0.0470	0.629	51.51
S11	0.259	0.033	0.005	0.012	0.2720	1.048	0.334
S12	0.249	0.030	0.008	0.010	0.2380	0.969	0.279
S13	0.277	0.026	0.020	0.011	0.055	1.627	9.502
S14	0.337	0.060	0.016	0.018	0.098	0.848	41.16
S15	0.288	0.053	0.008	0.012	0.06	1.176	16.53
S16	0.253	0.099	0.005	0.014	0.147	0.653	54.68
S17	0.296	0.048	0.004	0.012	0.109	1.028	53.36
S18	0.245	0.060	0.005	0.010	0.067	0.643	37.86
S19	0.316	0.061	0.006	0.015	0.072	1.379	59.92
S20	0.259	0.085	0.004	0.008	0.151	0.801	44.06
S21	0.234	0.052	0.009	0.008	0.112	0.015	40.63
S22	0.311	0.049	0.017	0.014	0.107	0.026	31.09
S23	0.247	0.034	0.005	0.012	0.365	0.966	0.336
S24	0.316	0.038	0.022	0.014	0.096	0.854	48.23
S25	0.340	0.037	0.015	0.010	0.061	0.758	18.21

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

	Pb	As	Cd	Cr	V	Cu	Fe
Pb	1						
As	0.0107	1					
Cd	0.923	-0.081	1				
Cr	0.170	0.173	0.181	1			
V	-0.061	0.947	-0.156	0.175	1		
Cu	0.772	-0.189	0.730	0.265	-0.208	1	
Fe	-0.131	0.349	-0.188	0.319	0.197	-0.230	1

Critical r =0.725 at P < .005, or r =0.900 at P < .001 (n = 24).

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

Elements	Pb	As	Cd	Cr	Cu	Fe
Daily intake (mg/daily)	300	100	40	50	2500	16000

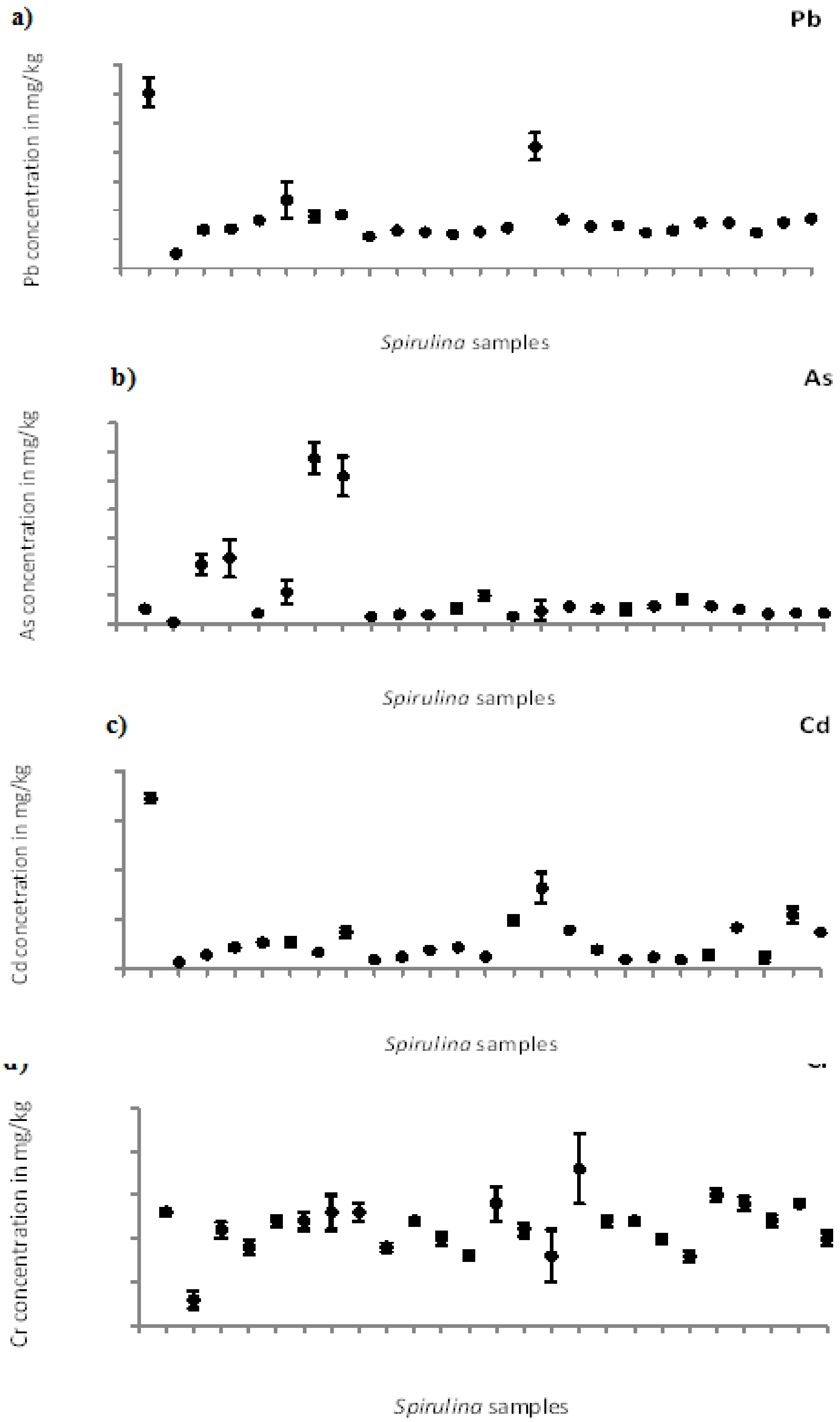

Fig. 1 a-d. Graphical effect of heavy metal concentration (mean ± SD) in commercially available *Spirulina* products.

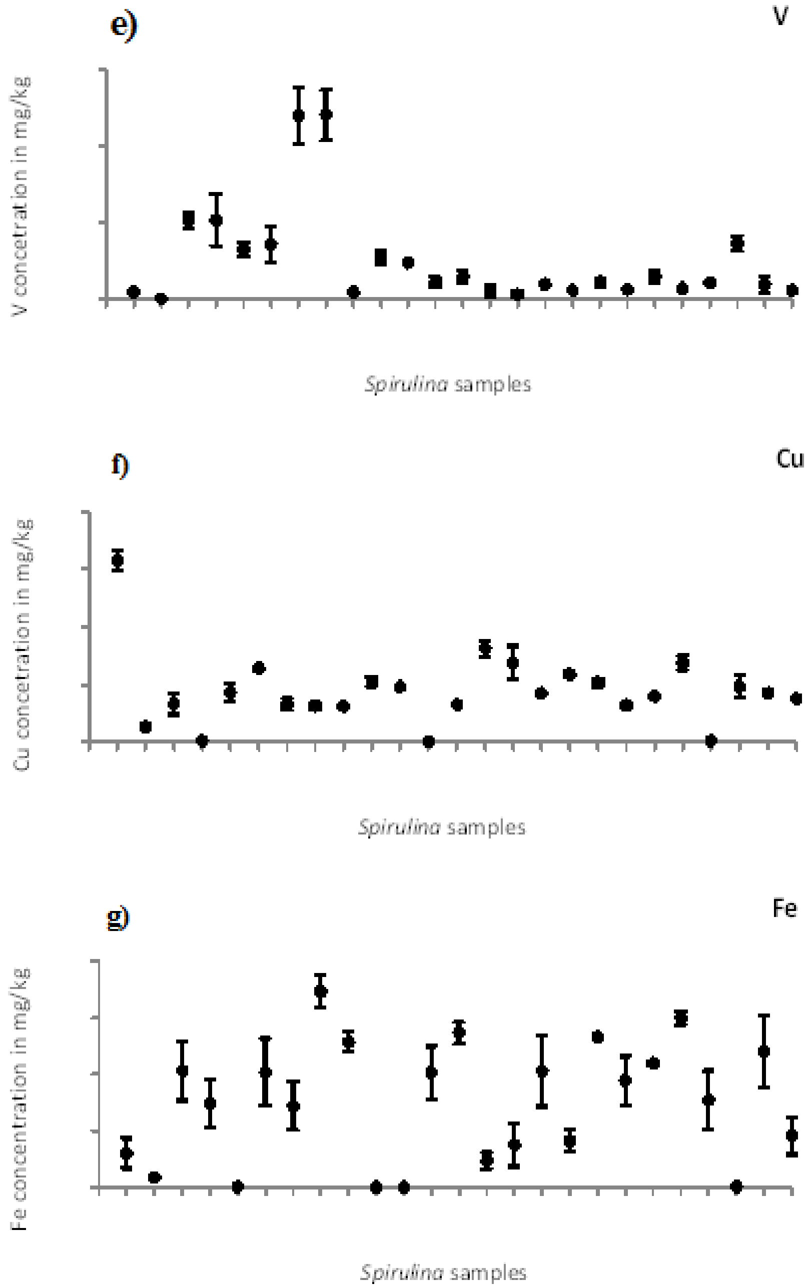

Fig. 1 e-g. Graphical effect of heavy metal concentration (mean ± SD) in commercially available *Spirulina* products. 1-25 products code shown in Table 1

very pronounced in all the *Spirulina* samples tested. The Cd content ranged between 0.003 to 0.069 mg/kg d.w. and the highest value of Cd (0.069 mg/kg d.w.) was detected in sample S2. Among the 25 samples tested, in all the samples had less amount of Cd content was detected (Table 2 and Fig. 1c). A significant positive correlation ($P<0.001$) was observed between Cd and Pb ($r = 0.923$) (Table 3).

Chromium

In all the samples of *Spirulina* (Table 2) had concentrations below the method limit of detection. The Cr contents ranged between 0.003

mg/kg dw detected in sample S1 and 0.018 mg/kg dw in sample S14. The differences in Cd contents were also not very pronounced in all the tested *Spirulina* samples (Table 2 and Fig. 1d).

Vanadium

We tested 25 samples including 16 tablets and nine capsule forms of *Spirulina* products (Table 1). The V contents ranged between 0.005 mg/kg dw, detected in sample S1, and 1.199 mg/kg dw found in samples S9. Among the *Spirulina* products tested, only the two samples S8 (1.199 mg/kg dw) and S9 (1.199 mg/kg dw) were found maximum V contents. On the other hand, another two samples S4 and S5 had the V contents were 0.510 and 0.518 respectively. The remaining of 21 samples, the V concentration was detected between 0.005 and 0.365 (Table 2 and Fig. 1e). A significant positive correlation (P<0.001) was observed between V and As (r = 0.947) (Table 3).

Copper

Table 2 shows that Cu concentrations for different *Spirulina* samples are highly variable. The highest value (3.155 mg/kg dw) corresponded to the sample of S2 from Australia. The samples such as S17, S11, S15, S7, S3, S19 and S13, the Cu concentration was 1.028±0.068, 1.048±0.077, 1.176±0.061, 1.276±0.031, 1.375±0.793, 1.379±0.127 and 1.627±0.141 mg/kg dw, respectively. While the remaining 17 samples, the Cu concentration were detected between 0.015 and 0.969, the order of concentration was S21>S5>S22>S1> S10>S9>S18> S16> S8>S4>S25>S20>S14> S24>S6>S23>S12 (Table 2 and Fig. 1f). A significant positive correlation (P<0.005) was observed between between Cu and Pb (r = 0.772), and also Cu and Cd (r = 0.730) (Table 3).

Iron

The Fe contents ranged between 0.279 mg/kg dw, detected in sample S12, and 69.24 mg/kg dw found in samples S9. In total 25 *Spirulina* products tested, only four samples namely S6, S11, S12 and S23 were found < 1mg/kg dw. Nevertheless, the five samples such as S10 (51.51±3.519 mg/kg dw), S17 (53.36±0.523), S16 (54.68±3.864 mg/kg dw), S19 (59.92±2.346 mg/g dw) and S9 (69.24±5.66 mg/kg dw) were detected highest Fe content, the level was >50 mg/kg dw. While the 16 samples, the Fe concentrations was detected between 3.440 mg/kg dw and 48.230 mg/kg dw (Table 2 and Fig. 1g).

DISCUSSION

The monitoring system of continuous surveillance of contaminant content in food and pharmaceutical products is crucial for consumer protection and facilitates international trade. Risk assessment is a continually evolving process since 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 are the metals which belong to the transition elements with higher specific gravity. However, only seven of them, Pb, As, Cd, Cr, V, Cu and Fe, have received more attention, due to their detrimental effect on health and possibility of food contamination. The recommend daily intake heavy metal elements are summarized in Table 4.

This study reveals that the most abundant metals in the *Spirulina* samples analyzed are Fe, Cu, and the least abundant are usually Cd and As (Table 2). The Pb content of twenty-five *Spirulina* samples was analysed, all the samples did not exceed the recommend daily intake (300mg/daily of Pb). In all the samples, the lead content is <1.3 mg/kg dw. Taiwanese and Japanese manufacturers allow up to 20 mg/ kg in Pb for *Spirulina* products, a value not attained by the samples analysed (Ortega-Calvo *et al.,* 1993; Hsu *et al.,* 2001). Tolerable Daily Intakes (TDI) of lead recommended by the WHO for an adult weighing 70 kg (0.250 mg/day) (WHO, 1993). The Pb contents in *Spirulina* products (Table 2) showed in general good agreement with previous reports (Ortega-Calvo *et al.,* 1993 and Almela *et al.,* 2006).

Accordingly, the total amount of As ingested by humans depends greatly on the amount of *Spirulina* included in their diet. It is well-known that organic and inorganic species of As differ widely in their toxicity (Oygard *et al.,* 1999), inorganic forms being in general more toxic than organic ones (López *et al.,* 1994). The total As contents found varied between 0.100 and 1.206 mg/kg dry weight. Most of the samples had total As concentrations less than 1 mg/kg d.w. In the present experimental results revealed that total As in all the *Spirulina* samples were comparable to other reports, although values of the samples were lower (Netten *et al.,* 2000). Furthermore, our results strongly agreed with those of Almela *et* *al*. (2002) and Besada *et al*. (2009).

Ingestion of the daily dose of *Spirulina* (10 g) recommended for human consumption by the commercial source. The Natural health products directorate of Canada recommended daily tolerable level of Cd < 0.09 mg/kg b.w. /day (Gershwin and Belay, 2008). The previously reported Cd and Cr level in *Spirulina* samples were 0.2 and 7.1 mg/kg d.w. respectively (Ortega-Calvo *et al.*, 1993). It was interesting to note that cadmium and chromium were all below the detection limit in most of *Spirulina* samples. The present investigation the highest Cd content (0.069 mg/kg d.w.) was determined in *Spirulina* samples S2 produced by General Nutrition Corporation, Pittsburgh, USA. In all the *Spirulina* samples analysed the concentrations of Cd not exceed the maximum values permitted by France (0.5 mg/kg dw) (Mabeau and Fleurence, 1993). In general, value for total Cr level (0.003 – 0.018 mg/kg d.w.) was highly similar to Cd value.

Significant differences in V levels between the *Spirulina* samples from different region were found (Table 2). In this study, much higher content (1.199 mg/kg d.w.) of this element were observed from S8 and S9 *Spirulina* samples produced by Jarrow Formulas, Los Angeles, USA and Earthrise Nutritionals LLC, USA respectively. The V content in *Spirulina* samples are yet not studied and reported. Cu concentrations were similar to those reported elsewhere (Netten *et al.*, 2000; Topcluogu *et al.*, 2003, Besada *et al.*, 2009). Cu level ranged between 0.015 mg/g d.w. and 3.155 mg/kg d.w. recorded from *Spirulina* samples S21 and S2 respectively. On the other hand, in 60% of the *Spirulina* samples Cu level is <0.5 mg/kg d.w. The range of Cu content found in present study is very less compare than those previously reported (Ortega-Calvo *et al.*, 1993). Fe content of *Spirulina* samples also differed depending on sample origin; sample S9 had much higher value (69.24 mg/kg d.w.) than that of other samples. The average Fe content in the present study was very less to the levels previously reported by Ortega-Calvo *et al*. (1993) and Hsu *et al*. (2001).

CONCLUSIONS

The heavy metals in 25 marketed *Spirulina* food samples were identified and quantified in this study. Results showed that the contents of Pb, As, Cd, Cr, V, Cu and Fe in the entire test *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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