

Determination of macrominerals and trace elements in the alga *Spirulina platensis*

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Abstract. The alga *Spirulina*, in particular *Spirulina platensis*, an ubiquitous component of sea and ocean with high nutritional content, is considered a potential alimentary source for third world countries affected by heavy famine problems. On other side, *Spirulina*-based products are nowadays commonly commercialized and recommended by suppliers as health food, diet pills, vitamins supplement etc.. However, a careful nutritional and toxicological evaluation of these products is necessary, since algae show bioaccumulation properties towards heavy metals. In this frame, samples of commercial and natural *Spirulina platensis* of Cuban origin, have been characterized for their macromineral and trace elements content. Measurements have been carried out by INAA and ICP-AES employing standard reference materials. The use of two techniques allowed the determination of a wider spectrum of elements and in some cases, the cross-checking of the data obtained. The toxic heavy metals levels found in both products are such that they do not constitute, on the basis of the recommended daily doses a health hazard for consumers.

Key words. Macrominerals – trace elements – INAA – ICP-AES.

Introduction

Spirulina is an ubiquitous marine alga present in ocean and sea waters, which has been recognized to be a more than potential alimentary source [1] to be used as substitute and/or supplement in conventional diets. Among the various species of *Spirulina*, the blue-green alga *Spirulina platensis* has drawn more attention because shows a high nutritional content, characterized by a 70% protein content and by the presence of minerals, vitamins, amino acids, essential fatty acids, etc. [2], is easily digestible and because size and dimensions facilitate harvesting from the natural or artificial growing sites [3]. All this makes *Spirulina* attractive as an alternative (and inexpensive) food source for third world countries still affected by heavy famine problems. Nowadays, *Spirulina* is commercialized (as pills, tablets and powder) and recommended as health food, diet pills, vitamin supplement and protein source.

The evaluation of macrominerals and trace elements in *Spirulina* as in any other edible, is important from both the nutritional and the toxicological point of view. It is well known, in fact, the need of establishing heavy metals concentration in foodstuffs, because of the long-term effect on human health caused by accumulation in target organs [4]. Most of the trace elements present in the algal biomass are heavy metals and algae have been reported to be strongly active in heavy metals concentration [5-7]. Further, while some trace elements are considered toxic (i.e. As, Br, Cd, Hg, Pb, Sb), others are considered essential (Cu, Zn) or necessary to human body (Cr, Se) but become health hazardous when certain intake values are exceeded [8-10].

In this paper, both macrominerals and trace elements have been determined in natural and commercial *Spirulina platensis* of Cuban origin by using Instrumental Neutron Activation Analysis (INAA) and Inductively Coupled

Plasma-Atomic Emission Spectroscopy (ICP-AES). The use of two techniques allows on one side the cross-checking of the data, and on other side the determination of a wider spectrum of elements since either INAA or ICP-AES do not or hardly can measure some of them.

INAA allows the determination of a large number of trace elements, including lanthanides, per sample and it has been widely applied to different fields of science [11-17] (geology, biology, metallurgy, archaeometry, environmental analysis, forensic survey, etc...). It is a non-destructive technique, which does not require any complex sample treatment so that possible sample contamination is minimized [10]. Therefore, it is characterized by a high degree of precision and reproducibility [18]. On the other hand, some important trace elements (Cu, Mg, Mn, Pb) can not or are hardly measurable by INAA, while they can be easily measured by ICP-AES. Thus, the simultaneous use of the two techniques allows the determination of a wider spectrum of elements (19, 20) and the cross-checking of the data for those elements which can be measured by both techniques.

Experimental

Two types of samples have been analyzed: a natural *Spirulina platensis* product, harvested in the Caribbean Sea around Cuba received as dried seaweed (scales), and a commercial product (pills) prepared from the natural one. On the commercial product container (plastic bottle) net weight (20 g), recommended dose (0.8 ÷ 1.6 g/day) and expiration date (3 years) are reported. No information were available on the exact geographical position and the environmental conditions of the natural algae growing site as well as on the industrial processing phases which led to the preparation of the final commercial product.

For INAA analysis, the TRIGA MARK II nuclear reactor of the ENEA-Casaccia Laboratories (Rome) has been used. Samples were sealed in polyethylene vials and irradiated, together with standard reference materials, at a neutron flux of $2 \times 10^{12} \text{ n cm}^{-2} \text{ s}^{-1}$ for 28 hrs, on the rotatory track of the reactor. Determination of elements which yield radioisotopes with half-lives of few minutes or hours has been carried out by irradiation of the samples for 1 min at a neutron flux $2 \times 10^{13} \text{ n cm}^{-2} \text{ s}^{-1}$, in the pneumatic device of the nuclear reactor. About 5 g of each product, crushed in an agate mortar to avoid metallic contaminations, were sampled randomly and placed in an oven at $105 \text{ }^\circ\text{C}$ for 8 hrs. Five 0.5 g and five 0.025 g aliquots were used for the longer and shorter irradiation, respectively. The activity per sample, which was about 100 mR immediately after irradiation, became nearly zero after two days. Two Reference Materials (RMs) of the European Inter-Institutes Committee (CII) were used: "Oeillet" and "Pomme Fruit". These RMs were previously standardized using the NIST-SRM 1573 standard ("Tomato Leaves"). For Al, Cu, V which were absent in the RMs and in the NIST-SRM 1573, "Orchard Leaves" (NIST-1571) was used. After irradiation, γ -spectrometry measurements at proper times (Fig. 1) were carried out using a computerized multichannel analyzer, connected to a Ge (HP) Canberra detector (resolution 1.9 keV) equipped with a suitable software program. In table I, a list of the measured nuclides and their nuclear characteristic are reported together with the limits of detection (LOD).

For ICP-AES analysis, 1 g of each sample (no particular cleaning procedure [21] has been adopted), sampled randomly, was digested in a teflon capsule with 10 mL of 65% nitric acid and 0.5 mL of hydrogen peroxide, using a microwave digestion system (CEM, Matthews, NC), for 40 min. Then, the clear solution was filtered on a $0.45 \mu\text{m}$ Millipore (Millipore Co, Bedford, MA) filter. Filter and container were washed three times with doubly-distilled water which was collected together with the sample solution. The solution was then brought to a final volume of 40 mL with doubly-distilled water. For the determination of Ca, Na, K, Fe a further dilution to 100 mL was needed. Measurements were carried out using a Jobin-Yovin TYPE III PLUS (France) spectrometer. The instrument was calibrated using

Spectrosol standards (BDH Chemicals Ltd, Poole, England). Instrumental parameters, elements determined by ICP-AES, relative analytical lines and LOD are listed in table II.

Table I. Nuclear data and Limit of Detection (LOD) of the elements determined by INAA. (m: minutes; h: hours; d: days; y: years).

Element	Product nuclide	Cross Section [19] (barn)	Half life	γ -Ray used (keV)	LOD ^a (ppm)
Ag	^{110m} Ag	3.2	253 d	658.0	
Br	⁸² Br	2.69	1.47 d	776.5	
Ca	⁴⁷ Sc	0.7	3.42 d	159.4	240
Ce	¹⁴¹ Ce	0.57	32.38 d	145.4	58 ^b
Cl	³⁸ Cl	0.428	37.3 m	1642.4	36
Co	⁶⁰ Co	37.2	5.272 y	1332.5	0.86 ^b
Cr	⁵¹ Cr	15.9	27.7 d	320.0	88 ^b
Cs	¹³⁴ Cs	29.0	2.062 y	795.7	1.2 ^b
Cu	⁶⁵ Cu	2.17	5.1 m	1039.2	6.1
Eu	¹⁵² Eu	5900	12.7 y	1408.0	0.3 ^b
Fe	⁵⁹ Fe	1.15	45.1 d	1099.2	6.3
Hg	²⁰³ Hg	3.8	46.9 d	279.0	5.2 ^b
K	⁴² K	1.46	12.36 h	1524.7	220
La	¹⁴⁰ La	9.0	40.27 h	1596.2	3.5 ^b
Mg	²⁷ Mg	0.0382	9.462 m	1014.4	152
Mn	⁵⁶ Mn	13.3	2.576 h	846.6	54 ^b
Mo	⁹⁹ Mo	0.45	2.75 d	141.0	
Na	²⁴ Na	0.53	15.03 h	1368.5	3
Ni	⁵⁸ Co	0.113	70.78 d	810.7	
Sb	¹²² Sb	6.25	2.70 d	564.0	6 ^b
Sc	⁴⁶ Sc	26.5	83.85 d	889.2	0.9 ^b
Se	⁷⁵ Se	51.8	120.4 d	264.6	9 ^b
Sm	¹⁵³ Sm	206	1.948 d	103.1	0.41 ^b
V	⁵² V	4.88	3.75 m	1434.2	
Zn	⁶⁵ Zn	0.78	243.8 d	1115.5	12 ^b

^a Calculating according to [20].

^b The values are expressed in ppb (ng/g).

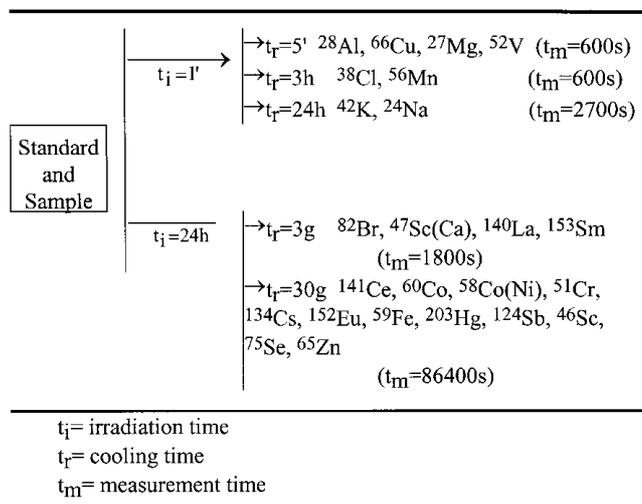


Fig 1. Scheme for INAA of the algal samples and standards.

Table II. Instrumental parameters, analytical lines and limit of detection (LOD) for the elements determined by ICP-AES.

Element	Line used (nm)	LOD (ppm)
Cd	214.532	0.01
Cr	205.644	0.02
Fe	234.437	0.01
Ni	217.553	0.02
Pb	224.688	0.05
V	311.174	0.01
Zn	213.949	0.01

Results and discussion

Precision and accuracy of the methods can be evaluated from tables III and IV where the measurements on the RMS “Oeillet” and “Pomme Fruit” by INAA are reported. Each value is the mean of five determinations, precision is evaluated by the estimated standard deviation, s , and relative standard deviation, RSD, while accuracy (i.e. inaccuracy) is evaluated by $D = \frac{\bar{x} - X}{X} \times 100$, whereas \bar{x} and X are the mean and certified values, respectively. RSD values are within the range 0.6–17.4% depending upon the elements and the con-

Table III. Precision and accuracy in the INAA of the “Oeillet” Reference Material. Concentrations are expressed $\mu\text{g/g}$. \bar{x} = our value; s = estimated standard deviation; RSD = relative standard deviation; X = certified value; $D(\%) = \frac{\bar{x} - X}{X} \times 100$.

Element	\bar{x}	S	RSD (%)	X	(%)
Br	8.30	0.72	8.7	8.10 ± 0.28	2.5
Ca%	1.58	0.13	8.2	1.91 ± 0.08	17.3
Co	0.469	0.024	5.2	0.427 ± 0.093	9.8
Cr	4.40	0.48	10.8	4.26 ± 0.49	3.3
Fe	1024	55	5.4	1095 ± 64	6.5
K%	4.50	0.08	1.8	4.71 ± 0.13	4.5
La	3.60	0.30	8.3	–	–
Mo	1.43	0.09	12.9	1.60 ± 0.22	9.5
Na%	0.092	0.001	1.1	0.101 ± 0.009	8.9
Rb	11.4	0.29	2.5	–	–
Sb	0.180	0.030	16.7	–	–
Sc	0.783	0.005	0.64	–	–
Sm	1.00	0.08	8.0	–	–
Zn	74.7	2.61	3.5	74.5 ± 5.2	0.3

Table IV. Precision and accuracy in the INAA of the “Pomme Fruit” Reference Material. Concentrations are expressed $\mu\text{g/g}$. \bar{x} = our value; s = estimated standard deviation; RSD = relative standard deviation; X = certified value; $D(\%) = \frac{\bar{x} - X}{X} \times 100$.

Element	\bar{x}	S	RSD (%)	X	(%)
Br	0.380	0.010	2.6	–	–
Ca%	0.186	0.018	9.7	0.158 ± 0.006	17.7
Cl%	0.013	0.002	15.3	0.012 ± 0.003	8.3
Co	0.187	0.012	6.2	0.173 ± 0.024	8.1
Cr	1.20	0.15	12.5	1.15 ± 0.12	4.3
Cs	0.023	0.002	8.7	–	–
Fe	58.1	4.0	6.9	55 ± 5.0	5.6
K%	1.61	0.02	1.2	1.52 ± 0.04	6.1
La	0.322	0.035	10.9	–	–
Mg%	0.104	0.007	6.7	0.098 ± 0.003	6.1
Mn	11.8	0.3	2.5	11.1 ± 0.7	6.3
Na	60.0	4.0	6.7	70 ± 10	14.3
Rb	6.40	0.28	4.4	–	–
Sb	0.474	0.084	17.8	–	–
Sc	0.070	0.002	2.9	–	–
Se	0.16	0.02	12.5	0.16 ± 0.00	0
Zn	13.1	0.2	1.5	12.5 ± 0.7	4.8

centrations range. Accuracy is quite good, considering that all values, with few exceptions, are below 10%. Further, the measured values (\bar{x}) fall in most cases within or very close to the standard deviation range of the certified values, being Ca the only exception.

Tables V and VI report the measurements of the trace elements by INAA and ICP-AES in both the natural and commercial *Spirulina* products. The low standard deviation values obtained for lanthanides, Cs and Sc are a proof of the good homogeneity of the sample [22]. The percent difference (Δ) between the concentration values of those elements determined by both techniques are reported in the two Tables together with literature data [23] for natural *Spirulina platensis* of US origin (Tab. V) and for commercial products of different origin (Taiwan, Israel, Mexico, Japan, USA) but available on the US market (Tab. VI).

The data obtained utilizing INAA and ICP-AES are in good agreement though values are higher in the natural sample (6.8–23%) than in the commercial ones (0.4–9.3%). In the latter, an exception is Mn ($\Delta = 31\%$) and the reason could be ascribed to the scarce accuracy of the INAA measurement. In fact, since the ^{56}Mn half-life (2.58 hrs) is short and there is, at close photopeak energy (1014 keV), the interference of the ^{27}Mg nuclide, the error may be high. If the data regarding the natural *Spirulina platensis* are compared with literature data (Tab. V), Mo and Ni and V are absent in our case while Hg and Se levels are significantly lower. Higher concentration values are instead obtained for Ca, Cr, Cu, Fe, Na, Mn, Pb and Zn. While some

Table V. Concentration values of metals in the natural sample ($\mu\text{g/g} \pm s$).

Element	Literature* [23]	INAA	ICP-AES
Ag	n.m.	0.076 ± 0.009	n.d.
Br	n.m.	17.9 ± 0.3	n.d.
Ca%	0.044	0.432 ± 0.005	0.336 ± 0.032
Cd	–	n.m.	–
Ce	n.m.	0.637 ± 0.015	n.d.
Cl%	n.m.	0.489 ± 0.050	n.d.
Co	n.m.	0.720 ± 0.010	n.m.
Cr	1.6	14.2 ± 0.2	11.3 ± 0.4
Cs	n.m.	0.048 ± 0.001	n.d.
Cu	2.7	n.m.	12.0 ± 0.7
Eu	n.m.	0.041 ± 0.001	n.d.
Fe	300	1176 ± 25	900 ± 32
Hg	9.30	0.120 ± 0.012	n.d.
K%	1.50	1.82 ± 0.14	n.m.
La	n.m.	0.514 ± 0.006	n.d.
Mg%	0.194	0.067 ± 0.021	0.132 ± 0.003
Mn	16.9	554 ± 4	592 ± 1
Mo	1.1	–	n.d.
Na%	0.749	23.5 ± 0.6	n.m.
Ni	1.9	–	n.m.
Pb	1.3	n.d.	12.9 ± 0.2
Sb	n.m.	0.064 ± 0.008	n.m.
Sc	n.m.	0.075 ± 0.001	n.d.
Se	2.5	0.198 ± 0.004	n.d.
Sm	n.m.	0.060 ± 0.004	n.d.
V	0.2	–	–
Zn	7.4	375 ± 8	336 ± 14

n.d.: not detectable; n.m.: not measured; -: absent.

* Values determined by ICP-AES.

Table VI. Concentration values of metals in the commercial sample ($\mu\text{g/g} \pm s$).

Element	Literature* [23]	INAA	ICP-AES
Ag	n.m.	0.067 ± 0.008	n.d.
Br	n.m.	19.2 ± 0.4	n.d.
Ca%	0.1÷0.9	0.722 ± 0.010	0.694 ± 0.020
Cd	n.m.	n.m.	–
Ce	n.m.	2.26 ± 0.08	n.d.
Cl%	n.m.	0.630 ± 0.032	n.d.
Co	n.m.	0.670 ± 0.053	n.m.
Cr	1÷6	9.02 ± 0.30	8.18 ± 0.15
Cs	n.m.	0.055 ± 0.002	n.d.
Cu	5÷11	49.6 ± 3.2	37.5 ± 0.6
Eu	n.m.	0.063 ± 0.001	n.d.
Fe	300÷1000	1116 ± 25	1030 ± 40
Hg	9÷18	0.096 ± 0.005	n.d.
K%	0.7÷1.2	0.890 ± 0.041	n.m.
La	n.m.	1.83 ± 0.02	n.d.
Mg%	0.2÷0.3	0.132 ± 0.045	0.116 ± 0.003
Mn	33÷35	54.5 ± 0.4	37.5 ± 1.1
Mo	0.5÷1.5	–	n.d.
Na%	0.2÷0.8	12.3 ± 0.4	n.m.
Ni	1÷3	–	n.m.
Pb	2÷5	n.d.	12.1 ± 0.9
Sb	n.m.	0.140 ± 0.024	n.m.
Sc	n.m.	0.248 ± 0.002	n.d.
Se	1÷5	0.124 ± 0.006	n.d.
Sm	n.m.	0.243 ± 0.005	n.d.
V	n.m.	3.16 ± 0.42	–
Zn	20÷35	240 ± 11	239 ± 9

n.d.: not detectable; n.m.: not measured; -: absent.

* Values determined by ICP-AES.

of these trace elements are important from the nutritional point of view, other ones are a clear indication of pollution of the harvesting site. This is feasible considering that, as already said, algae can accumulate heavy metals so that their use as bioaccumulator and/or bioindicator has been suggested [24,25]. If the same comparison is made for the commercial product, again Mo and Ni are absent, Hg and Se are present at consistently lower levels while V is now present (3.16 ppm), with respect to the data reported in the literature [23]. Similar concentration values are now obtained for Ca, Cr, Cu, Fe and Mn while still higher levels of K, Na, Pb and Zn are still in our commercial product. Thus, some qualitative and quantitative substantial variations are observed in the trace elements profile between the natural and the commercial product. This fact could be imputed to changes caused by the commercialization of the algae (biomass treatment and processing, package, distribution). Essentially K, Na, Cr, Mn, Zn levels decrease in the commercial product indicating that washing and/or removal processes have been probably used. The concentration of Ca, Cu, V increases while that one of Pb does not change. While the increase of Ca can be due to the use of excipients, it is not easy to explain the increase of Cu and V. Tentative explanations may be based on the presence, as impurities, of these elements in the additives used or in their unwilling introduction during the commercialization process.

It should be pointed out that the levels of Pb and of the other toxic heavy metals does not make the use of the commercial product hazardous for human health. The maximum

allowed daily intakes established by international organization [9,26] are such that the quantity of product needed to exceed such limits is above those ones corresponding to a “normal” use. For example, since the maximum allowed daily intake of Pb is 500 μg , about 42 g of this product should be assumed by a single individual to exceed it and this is well above the recommended dose ($0.8 \div 1.6$ g/day).

Conclusions

In conclusion, there seems opportune set still in consequence the utility of the INAA in the determination of heavy metals in edible vegetables, noting that in the microelements analysis we could determinate very small quantity and so not well appreciable with also techniques highly qualified like the ICP-AES which, on the contrary, becomes fundamental for the determination of some toxicological elements, like Cd, Cu, Mn Ni and Pb.

The proposal for *Spirulina* as food resource needs its characterisation for the metal content; really on one side some metals are toxic while other ones are necessary for human diet and on the other side algae can act as food bioaccumulator. Some qualitative and quantitative differences are observed in the trace elements profile between the natural and commercial products, probably due to the commercialisation process. The toxic heavy metal levels fall in the range of normality with no hazard for users.

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