Journal of Applied Phycology (2019) 31:3567–3579
https://doi.org/10.1007/s10811-018-1681-1

Essential and toxic elements in commercial microalgal food supplements

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Received: 23 July 2018 / Revised and accepted: 29 October 2018 / Published online: 13 November 2018

Abstract

Arthrospira spp. (known commercially as *Spirulina*) and *Chlorella* spp., valued for their evidence-based nutritional and bioactive properties, are cultivated for the purpose of production of food supplements for worldwide distribution. However, the quality and safety of the final product depends on culturing and manufacturing conditions. The present study investigated the content of toxic elements (As species, Al, Cd, Hg, Ni, Pt, Pb, Cr (VI), rare earth elements) and minerals (Ca, Co, Cr, Cu, Fe, K, Na, Mg, Mn, P, Zn) in *Chlorella* ($n = 10$) and *Spirulina* ($n = 13$) food supplements registered in the European Union. Considering the most common recommended daily dosage 3.0, supplementation with any of the studied product would contribute significantly to mineral intake, with the exception of Fe which was found at high but acceptable levels in both *Spirulina* and *Chlorella* formulas. The majority of products revealed agreement between factual mineral content and that declared on the label, the only exception being Cu content in *Chlorella* products found to be significantly higher (> 130%). All studied supplements were found to have Cd, Hg, and Ni levels much below safety limits, although selected ones were characterized by increased content of Al, Pb, and inorganic As. No hexavalent Cr was detected in the studied products. The study highlights that microalgal supplements can be safe for consumers if appropriate measures are taken to ensure consumer safety, although it underlines the continuous need to monitor these products in order to fully eliminate those of low quality.

Keywords Microalgal supplements · *Spirulina* · *Chlorella* · Mineral content · Contamination · Metals · Food safety · Food quality

Introduction

The market of food supplements is on the rise, with increasing numbers of individuals interested in using these formulas for various purposes in Europe, the USA, and Asia. There is particular interest in targeting those products that are based on ingredients of natural origin (Kennedy 2005; Hirayama et al. 2008; Bailey et al. 2013). Within this group, supplements that are based on microalgal biomass are not only gaining economic attention, but at the same time, their bioactive properties are being extensively explored using complementary research models*:* *in vitro* and in *in vivo* experiments, and randomized, placebo-controlled clinical trials involving different groups of patients (Panahi et al. 2016; de la Jara et al. 2018). These products use biomass of cyanobacteria belonging to the genera of *Arthrospira* (known commercially as *Spirulina*), *Nostoc*, and the former "*Aphanizomenon*," and green algae representing the genera *Haematococcus*, *Dunaliella*, and *Chlorella* (Pulz and Gross 2004). The greatest market success has been achieved by *Spirulina* and *Chlorella* formulas, with the main cultivation plants located in the USA and Asia, particularly in China, the current leader in global microalgal biomass production (García et al. 2017).

Spirulina- and *Chlorella*-based products are known to be a rich source of proteins, fatty acids, pigments, minerals (e.g., calcium, magnesium, phosphorus, zinc, copper, and iron), digestive and restriction enzymes, and selected vitamins such as bioavailable forms of B_{12}, C, and E (Buono et al. 2014; Wells

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Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10811-018-1681-1) contains supplementary material, which is available to authorized users.

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et al. 2016). As evidenced using clinical trials, both supplements reveal promising bioactive properties encompassing immunomodulatory, antihypertensive, antilipidemic, and hypoglycemic effects (Nielsen et al. 2010; Kim et al. 2016; Juszkiewicz et al. 2018). This not only advocates the role of these products as functional foods but also supports their potential therapeutic use. For example, supplementation with *Spirulina* has been shown to decrease the viral load and increase CD4 cells in HIV-infected patients, modulate lipid metabolism, control appetite and promote weight loss in obese subjects (Zeinalian et al. 2017), and increase ability to resist mental and physical fatigue in men (Johnson et al. 2016), while *Chlorella* supplementation reveals a protective antioxidant effect in smokers (Panahi et al. 2013), may be efficiently used in adjunctive therapy for depressive disorders (Panahi et al. 2015), and to improve glycemic status in patients with non-alcoholic fatty liver disease (Ebrahimi-Mameghani et al. 2017).

There are no doubts that a biomedical exploration of the properties of *Spirulina* and *Chlorella* imparts a reliability to these products and helps to strengthen their market. It is, however, of high importance to ensure the safety of the final commercial product. *Arthrospira* spp. and *Chlorella* spp. are not known to produce any toxic compounds and their safety is well established (Buono et al. 2014; Wells et al. 2016; García et al. 2017). Nevertheless, one should note that cultivation of microalgae for scientific purposes may vary from that conducted on a large, commercial scale. Over recent years, the quality of selected microalgal supplements available in trade has been put in doubt due to the detection of cyanotoxins, e.g., neurotoxic anatoxin-a and β-methylamino-l-alanine, hepatotoxic microcystins resulting from alterations to culture purity and the co-occurrence of toxigenic species such as *Microcystis aeruginosa* (Vichi et al. 2012; Manali et al. 2017; Roy-Lachapelle et al. 2017). There have also been reports of increased levels of aluminium, lead, and inorganic arsenic, possibly as a consequence of the unsuitable location of cultivation ponds and the use of chemical methods to harvest biomass (Papazi et al. 2010; Hedegaard et al. 2013; Rzymski et al. 2015). However, some individuals, particularly those suffering from autoimmune disorders, can be specifically susceptible to microalgal supplements (Lee and Werth 2004), and it is likely that the occurrence of adverse effects such as diarrhea, nausea, abdominal pain, and skin rash reported after consumption of these products (Rzymski et al. 2015; Rzymski and Jaśkiewicz 2017) may have origins in their altered and imperfect quality. Therefore, it is imperative that the content of (potentially) toxic elements in these formulas be investigated, yet updated information in this regard is limited (Al-Dhabi 2013; Hedegaard et al. 2013; Rzymski et al. 2015).

Recent studies have shown that various food supplements such as multi-ingredient formulas can reveal high discrepancies between the factual nutritional value and that which is declared on the product label (Brandon et al. 2014; Niedzielski et al. 2016; Poniedziałek et al. 2018). Large-scale cultivation of microalgae for food production that most often employs raceway pond systems is prone to various environmental conditions, mitigation of which may be challenging. The nutritional value of produced biomass can be influenced by the presence and availability of certain compounds in cultivation medium. It is therefore of interest to examine whether the content of minerals declared on the labels of commercial microalgal supplements matches their factual levels within the accepted margin.

The aim of the present study was to assess the content of toxic metals (Al, Cd, Hg, Ni, Pb), hexavalent chromium, arsenic species, rare earth elements (REEs), and essential macro- and trace elements (Ca, Co, Cr, Cu, K, Mg, Mn, Mo, Na, Se, Fe, Zn) in *Spirulina* and *Chlorella* food supplements originating from different world regions and registered for distribution in the European Union. The determined levels of minerals were compared to those declared on the product labels.

Materials and methods

Food supplements

A total of 13 *Spirulina*-based and 10 *Chlorella*-based food supplements were randomly purchased from online stores. The inclusion criteria were official registration as a food supplement, country of origin declared on the label, powder or tablet form. The general characteristics of the studied products are given in Table 1. The following number of *Spirulina* and *Chlorella* products declared the content of the studied minerals on the label: Ca, 9 and 10; Cu, 9 and 8; K, 9 and 10; Mg, 10 each; Mn, 11 and 9; Fe, 11 and 10; Se, 3 and 1; Na, P, and Zn, 1 each 1; Cr and Mo, 1 and 0; Co, none.

Total element content analysis

The whole batch of each supplement was ground, thoroughly mixed, and weighed. Afterwards, 0.50 g of each formula was digested in 8 mL of suprapure HNO_3 in closed Teflon vessels using the microwave sample digestion system Mars 6 (CEM, USA). The digestion procedure consisted of two steps: ramp to temperature 180 °C for 20 min and hold at 180 °C for 30 min. After digestion, the solution was diluted to a final volume of 15 mL with ultrapure water obtained in the Milli-Q system (Millipore, USA). Each supplement was prepared in triplicate.

The content of essential macroelements (Ca, Mg, K, Na, and P) and trace elements (Co, Cr, Cu, Mn, Mo, Se, Fe, and Zn), toxic metals (Al, Cd, Ni, Hg and Pb), light REEs

Table 1 General characteristics of the studied group of microalgal food supplements (S, *Spirulina*; Ch, *Chlorella*)

Supplement	Declared species	Country of origin	Form	Daily dose recommendations [g]
S-1	*Spirulina* sp.	China	Powder	4.0
S-2	*Spirulina* sp.	China	Powder	4.0
S-3	*Spirulina* sp.	China	Powder	1.5
S-4	*Spirulina platensis*	China	Powder	Not given
S-5	*Spirulina* sp.	Taiwan	Powder	3.0
S-6	*Spirulina Pacifica*	USA	Tablets	3.0
S-7	*Arthrospira platensis*	China	Powder	3.0
S-8	*Spirulina platensis*	China	Tablets	3.0
S-9	*Spirulina* sp.	China	Tablets	2.0–3.0
S-10	*Spirulina maxima*	China	Tablets	1.5
S-11	*Spirulina* sp.	China	Tablets	2.0
S-12	*Spirulina* sp.	China	Tablets	2.0
S-13	*Spirulina* sp.	India	Powder	5.0
Ch-1	*Chlorella sp.*	China	Tablets	3.0
Ch-2	*Chlorella vulgaris*	Taiwan	Tablets	3.0
Ch-3	*Chlorella pyrenoidosa*	Japan	Tablets	3.0
Ch-4	*Chlorella* sp.	India	Tablets	Not given
Ch-5	*Chlorella* sp.	China	Powder	2.0–3.0
Ch-6	*Chlorella* sp.	China	Powder	Not given
Ch-7	*Chlorella pyrenoidosa*	China	Powder	3.0
Ch-8	*Chlorella vulgaris*	China	Powder	3.0
Ch-9	*Chlorella vulgaris*	Portugal	Powder	0.6
Ch-10	*Chlorella vulgaris*	China	Tablets	3.0

(LREEs: Ce, Eu, Gd, La, Nd, Pr, Sc, Sm), and heavy REEs (HREEs: Dy, Er, Ho, Lu, Tb, Tm, Y, and Yb) was determined using the inductively coupled plasma optical emission spectrometer Agilent 5110 ICP-OES (Agilent, USA). ICP commercial analytical standards (Romil, England) were applied for the calibration. The following common instrumental parameters were used for determination of all elements: RF power 1.2 kW, plasma gas (argon) flow 12 L min^{-1}, nebulizer gas (argon) flow 0.7 L min^{-1}, axial plasma observation. The applied wavelengths (nm), limits of detection (mg kg^{-1}), and levels of instrumental precision (%) for each determined element are summarized in Table S1. Traceability was checked using the standard reference materials: CRM S-1, CRM NCSDC (73349), CRM 2709, CRM 405, and CRM 667. The recovery (80–120%) was acceptable for all the elements determined.

Arsenic speciation analyses

To determine the inorganic and organic As content, 1.00 g of each supplement (homogenized by rubbing and sieving through a 0.02-mm sieve) was placed in a glass flask containing 10 mL 1 M phosphoric acid and several drops of Triton-100 and extracted in an ultrasonic bath (30 min at ambient temperature). Next, the solution was filtered using a paper filter (the filter was washed by 200 mL of water and 20 mL of phosphoric buffer). The pH of the solution was adjusted to 6–6.5 by the addition of 10 mol L^{-1} solution of NaOH, and finally, the solution was diluted to 20 mL with phosphate buffer. Each supplement was prepared in triplicate. The inorganic arsenic species, arsenite As (III) and arsenate As(V) in the acid extracts, were determined by HPLC–HG-ICP-OES immediately following the extraction procedure. The HPLC instrument was a liquid chromatograph (Shimadzu, Japan) with an anion-exchange column (Supelco, USA) LC-SAX1 (250 mm, 4.6 mm i.d.). The chromatographic run was isocratic at 3 mL min^{-1} of phosphate buffer (1 mmol L^{-1} Na_2HPO_4 and 10 mmol L^{-1} $KH_2PO_4.2H_2O$) with an injection volume of 200 µL. PEEK (polyetheretherketone) tubing was inserted into a Tygon sleeve for transfer of the eluent from the LC column to the hydride generation unit. A spectrometer, model Agilent 5110 ICP-OES (Agilent, USA), with in-spray chamber generation of arsenic hydrides was used. For quantification, calibration curves based on the peak area for As (III) and As(V) were used. The final results were given as the mean concentration of As (III) and As(V); the remaining fraction (determined after ICP-OES analysis) was identified as organic As (Niedzielski et al. 2013).

Hexavalent chromium determination

To determine Cr (VI), the colorimetric method with 1,5-diphenylcarbazide was applied (Poniedziałek et al. 2018). For 2 mL of the sample extracted by 1 M phosphoric acid, several drops of 0.5% (*m*/*m*) solution of diphenylcarbazide in acetone were added. Each supplement was prepared in triplicate. The eventual presence of a red-colored complex of Cr (VI) was determined spectrophotometrically at 540 nm.

Statistical analyses and calculations

The results were analyzed using STATISTICA 10.0 software (StatSoft, USA). Because most of the data were not normally distributed (Shapiro-Wilk test, $p < 0.05$), the non-parametric Mann-Whitney *U* test was used for comparison in element content between *Spirulina* and *Chlorella* products. In all analyses, $p < 0.05$ was considered as statistically significant.

The content of Cd, Hg, and Pb was with maximum allowance levels set for FS by the European Commission, 1.0, 0.1, and 3.0 mg kg^{-1}, respectively (Commission Regulation (EC) No 629/2008: amending Regulation (EC) No 1881/2006). The total content of REEs was confronted with a maximum allowance threshold of 7.0 mg kg^{-1} dry weight (equivalent to 0.7 mg kg^{-1} fresh weight) set by China, the only country yet to regulate REEs in foodstuffs (SAC 2012). The contents of Al and Ni were related to their Tolerable Weekly Intake (TWI) set by the European Food Safety Authority (EFSA) at 1.0 and 0.0195 mg kg^{-1}body weight, respectively (EFSA 2008, 2015), assuming a daily consumption of 3.0 g of a supplement (the most common recommended dose, see Table 1) for a week by a 70-kg adult. Similarly, determined levels of essential elements were compared to Adequate Intake (AI) established by the EFSA (2017a). The following AI (per day) were assumed: Ca, 950 mg; P, 550 mg; Mg, 350 mg; Na, not set; K, 3500 mg; Cu, 1.6 mg; Cr, not set; Co, not set; Mn, 3.0 mg; Mo, 0.065 mg; Fe, 11 mg; Se, 0.07 mg; Zn, 11.7 mg. The determined content of Ca, Cu, K, Fe, Mg, and Mn was compared with that declared on the label. These minerals were selected because unlike Se, Na, P, Zn, Ce, Mo, and Co, these minerals were listed on the labels of the majority of the studied products. Seventy to 130% of declared value was considered as an acceptable margin (Niedzielski et al. 2016; Poniedziałek et al. 2018). In the case of As, the provisional Tolerable Weekly Intake (PTWI) established at a level of 15 μg kg^{-1} body weight (bw) of total As previously by the Joint FAO/WHO Expert Committee on Food Additives (JECFA 2012) has been disapproved by EFSA as inappropriate as adverse effects have been reported for exposures at lower levels of As. Instead, a benchmark dose lower confidence limit (BMDL01) was identified for cancers of the lung, skin, and bladder, as well as skin lesions, at a level of 0.3 μg kg^{-1} bw day^{-1} for an increased cancer risk at 1% (EFSA 2009), and the content of As in the studied supplements was related to this value, assuming a daily consumption of 3.0 g of a product by a 70-kg adult.

Results

Toxic elements

Aluminium

The mean ± SD (median) content of Al amounted to 2155.6 ± 1774.7 (1299.8) mg kg^{-1} in *Spirulina* supplements and did not differ significantly from that found in *Chlorella* preparations—1732.8 ± 1991.5 (1095.3) mg kg^{-1} ($p > 0.05$, Mann-Whitney *U* test). Three *Spirulina* and two *Chlorella* products whose weekly consumption at a daily dose of 3.0 g by a 70-kg adult would exceed TWI for Al were identified (Fig. 1).

Arsenic

The As content was below detection limits (0.1 mg kg^{-1}) in six *Spirulina* (46.1%) and 5 *Chlorella* (50.0%) products. In other supplements, the following order of As species was found: As organic > As(V) > As (III), with sum of inorganic As ranging from 1.7–2.2 mg kg^{-1} in *Spirulina* and 2.3–2.7 mg kg^{-1} in *Chlorella* (Fig. 1). Considering the usual recommended daily dose of 3 g of supplement consumption, use of these products would lead to daily exposure to inorganic As at a level of 5.1–6.7 for *Spirulina* and 6.2–8.1 μg in the case of *Chlorella*. Such exposure would constitute respectively 24.3–31.9% and 29.5–38.6% of BMDL01 assuming consumption by a 70-kg adult.

Nickel

The Ni content in *Spirulina* and *Chlorella* supplements was comparable and amounted to a mean ± SD (median) of 1.52 ± 0.72 (1.33) and 1.38 ± 0.63 (1.29) mg kg^{-1}, respectively. The highest content in the former group of products was 3.26 mg kg^{-1}, and in the latter, 2.81 mg kg^{-1}. Weekly supplementation with any studied supplement at a daily dose of 3.0 g by a 70-kg adult would not exceed 40% of the established TWI for Ni, and in most cases, it was lower than 20% of the TWI (Fig. 1).

Hexavalent chromium

The Cr (VI) content was below the limit of detection (0.01 mg kg^{-1}) in all studied samples.

Cadmium

The Cd content in all tested products fell much below the maximum allowance level (1.0 mg kg^{-1}) set by the

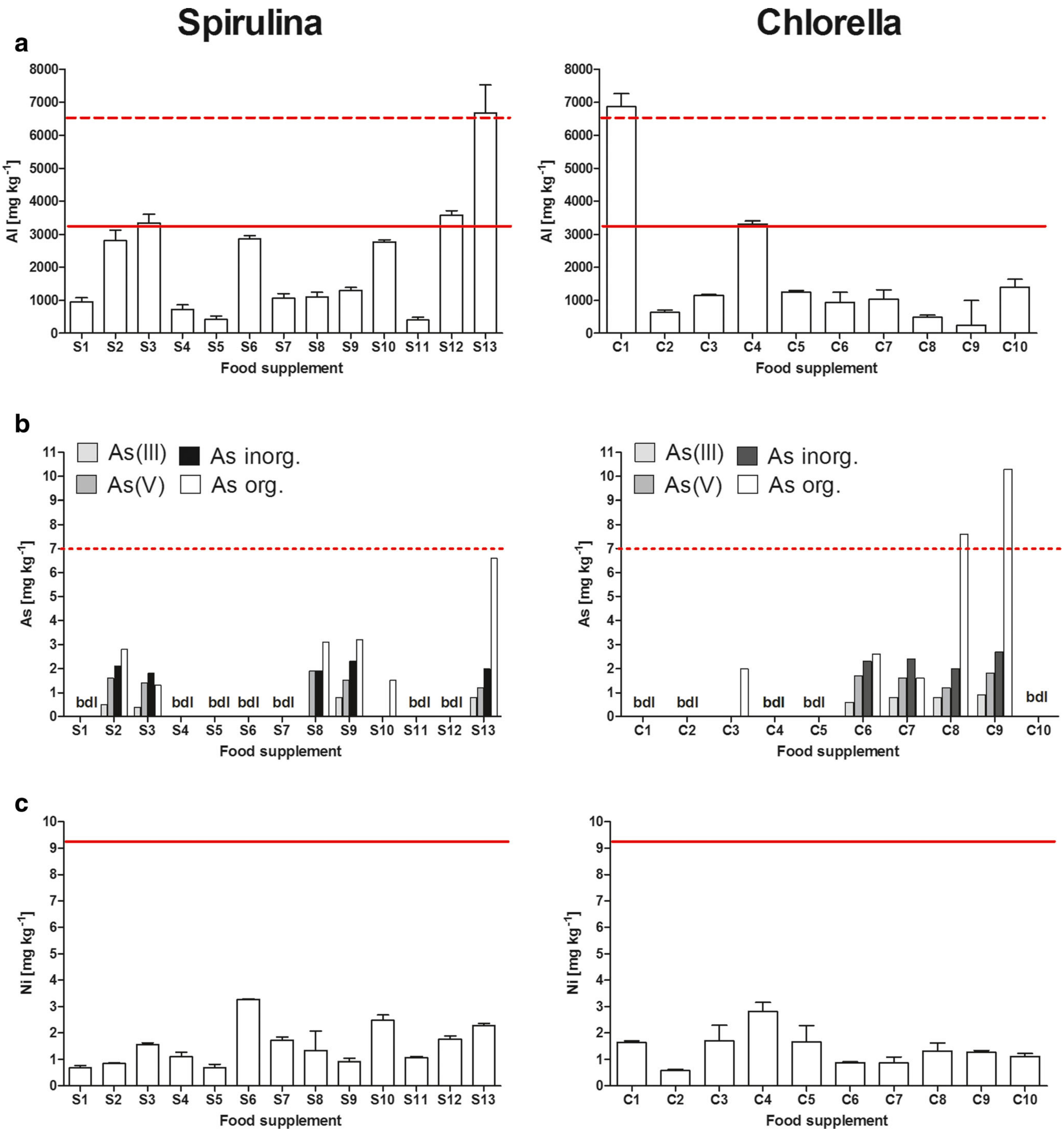

Fig. 1 The content (mean ± SD) of Al (**a**), As species (**b**), and Ni (**c**) in *Spirulina* (left column; $n = 13$) and *Chlorella* (right column; $n = 10$) in food supplements. The red line represents Tolerable Weekly Intake (TWI) set by the EC considering a weekly consumption at a daily dose of 3.0 g by a 70-kg adult; the dashed red line represents Provisional Tolerable Weekly Intake (PTWI) set by the World Health Organization considering the same consumption as for TWI calculation. The dotted red line represents the Benchmark Dose Lower Confidence Limit (BMDL01) set for inorganic As at a consumption of 3.0 g of supplement by a 70-kg adult. bdl, below detection limits

European Commission (Fig. 2) and did not differ significantly between *Spirulina*- and *Chlorella*-based supplements ($p > 0.05$, Mann-Whitney U test) with mean ± SD (median) content amounting to 0.125 ± 0.055 (0.128) and 0.142 ± 0.071 (0.134) mg kg^{-1}, respectively.

Mercury

Hg was detected in seven *Spirulina* (53.8%) and eight *Chlorella* (80.0%) food supplements at mean ± SD (median) levels of 0.027 ± 0.031 (0.022) and 0.41 ± 0.017 (0.042),

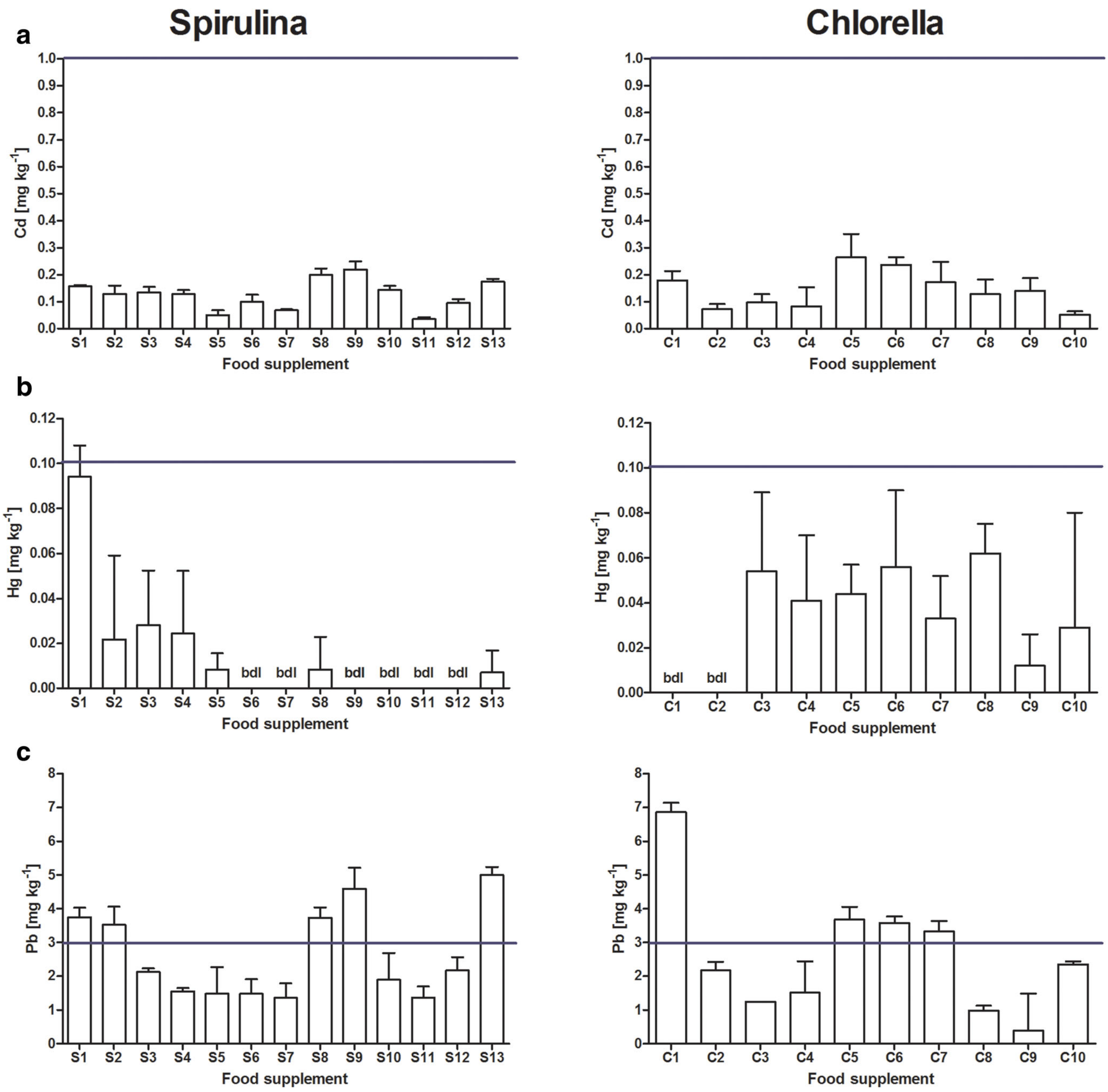

Fig. 2 The content (mean ± SD) of Cd (**a**), Hg (**b**), and Pb (**c**) in *Spirulina* (left column; $n = 13$) and *Chlorella* (right column; $n = 10$) food supplements. The blue line represents maximum allowance limit for food supplements established by the European Commission. bdl, below detection limits

respectively, not differing significantly from each other ($p > 0.05$, Mann-Whitney *U* test). Most of the tested products revealed Hg content much below the maximum allowance level set for food supplements by the European Commission (Fig. 2).

Lead

The mean ± SD (median) content of Pb in *Spirulina* and *Chlorella* supplements was 2.6 ± 1.9 (2.3) and 2.6 ± 1.3 (2.1) and did not differ significantly ($p > 0.05$, Mann-Whitney *U* test). Four *Chlorella* (40%) and five *Spirulina* (30.8%) products exceeded the maximum allowance level of Pb (3.0 mg kg^{-1}) set by the European Commission, with one *Chlorella* supplement exceeding it over 2-fold (Fig. 2).

Rare earth elements

The total mean ± SD (median) content of REEs in *Spirulina* and *Chlorella* was 2.14 ± 1.89 (1.25) and 2.03 ± 11.28 (1.63) mg kg^{-1}, respectively. A significantly higher content of LREEs was noted in both product groups ($p < 0.05$, Mann-Whitney *U* test) with a mean LREEs:HREEs ratio of 4:1. The maximum allowed REE content (7.0 mg kg^{-1} dw) was exceeded by only one *Spirulina* product (Table 2).

Essential elements

Macroelements

The content of macroelements in *Spirulina* and *Chlorella* food supplements is summarized in Table 3. Their mean content decreased in the order K > Ca > P > Na > Mg for *Spirulina* products and K > P > Ca > Na > Mg for *Chlorella* supplements. A significantly higher content of Ca was observed for *Spirulina* products while *Chlorella* displayed a greater P level. In the case of most products, consumption of the most commonly recommended daily dose of 3.0 g would not lead to their significant intake (Table 3). Most of the studied supplements revealed an agreement between factual and declared content of Ca, K, and Mg (Fig. 3).

Trace elements

The mean content of trace elements decreased in the following order: Fe > Mn > Zn > Cu > Cr > Co > Mo = Se in *Spirulina* supplements and Fe > Mn > Zn > Cu > Cr > Mo > Se > Co in *Chlorella* products. Their contents, which are summarized in Table 3, did not differ between *Spirulina* and *Chlorella* products, except for Cu levels being significantly higher in the latter. They were also mostly low when compared with AI values by assuming a daily consumption of a 3.0-g dose. The only exception was the level of Fe which in a similar scenario would be provided at over 25% of AI on average, with some *Spirulina* and *Chlorella* supplements providing even substantially more (Table 3). For the majority of *Spirulina* products, the factual and declared content of Cu, Mn, and Fe was found to correspond very closely. In the case of *Chlorella* supplements, larger discrepancies were noted, particularly with respect to Cu content, which was found to significantly exceed the value declared on the label (Fig. 3).

Discussion

Considering the biological activities of *Spirulina* and *Chlorella* food supplements, now evidenced also on a clinical level, it is expected that the market for these products will continue to develop. Thus, it is imperative to ensure that the quality of the final product is high and safe for consumers through, e.g., implementation of a food safety management system according to ISO 22000, efficient enforcement of various food regulations, systematic monitoring of contaminant levels, and credibility of information declared on the product label (Fernández-Segovia et al. 2014; Poniedziałek et al. 2018). The present study is a comprehensive assessment of *Spirulina* and *Chlorella* food supplements registered for trade in the EU as regards levels of essential and (potentially) toxic elements.

A variable content of toxic elements was found in the investigated products. This particularly concerned Al whose levels notably differed between various products, reaching over 6000 mg kg^{-1} in selected ones. It has been previously reported that some microalgal food supplements can contain a high content of this element, most likely as a result of the use of aluminium chloride as a flocculant to harvest biomass (Papazi et al. 2010; Rzymski et al. 2015). Although inexpensive and efficient, such a method may contaminate the final product with Al at unsafe levels, exceeding maximum dietary tolerance thresholds (Rzymski et al. 2015). Despite the poor bioavailability of Al ingested orally, and that once absorbed most of its load is excreted readily with urine (Taylor et al. 1998), exposure to this element has greatly increased over the decades and is now estimated to reach annually as much as 11 kg per capita (Exley 2013). Therefore, an effort must be made to reduce its content in food, and setting a (still not established) maximum threshold level of Al in different foodstuffs (including food supplements) would be a beneficial step forward in this regard. Moreover, one should note that some individuals, particularly those with renal failure diseases, may be more prone to the toxic effects of Al due to its increased retention and consequently, bioaccumulation (Yokel and McNamara 2001). There are number of alternative methods, such as physical or biological flocculation, whose efficiency is reliable enough to be applied to the harvest of microalgal biomass (Salim et al. 2011; Vandamme et al. 2013; Choy et al. 2018), and because they do not lead to biomass contamination, their use is highly advised in the production of *Spirulina* and *Chlorella* food supplements.

Previous studies have already reported that some food supplements containing biomass (e.g., plant material) may reveal increased levels of toxic elements such as Cd or Pb. This is most probably due to contamination of the ambient environment from which the biomass was originally derived, and the bioaccumulation process. Some microalgae are also known for their tolerance and uptake of toxic metals so the chemical quality of the culture medium is likely to have a profound effect on the presence of contaminants in the produced supplement. Importantly, the present study found that Cd and Hg content in studied *Spirulina* and *Chlorella* formulas was much below the maximum allowance limits set for food supplements by the EFSA. However, the Pb levels in selected products, exceeding the allowance threshold of 3.0 mg kg^{-1}, are rising concerns. Microalgae sequester Pb preferentially over other toxic metals, and additionally, Pb is more environmentally abundant than Cd or Hg—contamination with this

Table 2 Mean content (mg kg^{-1}) of rare earth elements (REEs) in *Spirulina* ($n = 13$) and Chlorella ($n = 10$) supplements

	Light REEs								Heavy REEs								
Spirulina supplements ($n = 13$)																	
	La	Ce	Eu	Gd	Nd	Pr	Sm	Sc	Dy	Er	Ho	Lu	Tb	Tm	Y	Yb	ΣREEs
S1	0.254	0.013	0.004	0.111	0.153	0.514	n.d.	0.059	n.d.	0.005	n.d.	n.d.	n.d.	0.014	0.101	0.025	1.253
S2	0.096	0.011	0.003	0.109	0.155	0.512	n.d.	0.049	n.d.	n.d.	0.004	n.d.	n.d.	0.027	0.112	0.029	1.107
S3	0.335	0.058	0.014	0.177	0.338	0.428	0.007	0.109	0.034	0.036	0.019	n.d.	n.d.	0.007	0.362	0.056	1.980
S4	0.094	0.015	0.003	0.080	0.147	0.363	n.d.	0.142	0.043	n.d.	0.012	n.d.	n.d.	n.d.	0.157	0.033	1.089
S5	0.061	0.005	n.d.	0.092	0.104	0.380	n.d.	0.025	n.d.	n.d.	0.014	n.d.	n.d.	n.d.	0.026	0.012	0.719
S6	0.190	0.100	0.036	0.279	0.246	0.742	n.d.	0.146	0.029	0.035	0.021	0.008	0.014	0.047	0.938	0.099	2.930
S7	0.097	0.017	n.d.	0.086	0.166	0.437	n.d.	0.041	n.d.	n.d.	0.015	n.d.	n.d.	n.d.	0.051	0.013	0.923
S8	0.201	0.035	n.d.	0.076	0.286	6.793	n.d.	0.051	n.d.	0.004	n.d.	n.d.	0.116	0.005	0.307	0.019	7.893
S9	0.084	0.014	0.007	0.119	0.169	0.604	n.d.	0.053	n.d.	0.003	0.009	n.d.	n.d.	0.033	0.098	0.025	1.218
S10	0.189	0.030	0.009	0.135	0.217	0.406	n.d.	0.058	n.d.	0.004	0.028	n.d.	0.011	n.d.	0.122	0.024	1.233
S11	0.532	0.005	0.005	0.095	0.131	0.639	n.d.	0.065	0.028	n.d.	0.037	n.d.	0.006	0.007	0.026	0.005	1.581
S12	0.367	0.068	0.014	0.219	0.404	0.550	n.d.	0.119	0.021	0.004	n.d.	n.d.	0.008	0.058	0.183	0.034	2.049
S13	0.502	0.095	0.029	0.184	0.571	0.812	n.d.	0.172	0.087	0.033	n.d.	n.d.	0.007	0.030	0.537	0.053	3.112
Mean (SD)	0.231 (0.159)	0.036 (0.034)	0.012 (0.011)	0.136 (0.062)	0.237 (0.133)	1.01 (1.74)	–	0.084 (0.047)	0.040 (0.024)	0.016 (0.016)	0.018 (0.010)	–	0.027 (0.044)	0.025 (0.019)	0.232 (0.258)	0.033 (0.025)	2.14 (1.895)
Chlorella supplements ($n = 10$)																	
Ch1	0.743	1.174	0.026	0.312	0.817	0.836	n.d.	0.284	0.120	0.100	n.d.	0.052	0.087	0.072	0.736	0.116	5.475
Ch2	0.085	0.024	0.004	0.147	0.266	0.471	n.d.	0.011	n.d.	0.011	n.d.	0.036	0.049	n.d.	0.045	0.018	1.167
Ch3	0.150	0.121	0.018	0.238	0.240	0.514	n.d.	0.007	n.d.	n.d.	0.016	0.034	0.037	n.d.	0.036	0.028	1.439
Ch 4	0.353	0.557	0.019	0.148	0.382	0.903	n.d.	0.154	0.022	0.022	n.d.	0.021	0.017	n.d.	0.112	0.022	2.732
Ch 5	0.109	0.153	0.014	0.184	0.235	0.717	n.d.	0.036	n.d.	0.013	0.027	0.033	0.026	n.d.	0.066	0.028	1.641
Ch 6	0.082	0.104	0.021	0.173	0.203	0.508	n.d.	0.019	n.d.	n.d.	0.022	0.028	0.027	n.d.	0.042	0.022	1.251
Ch 7	0.090	0.134	0.021	0.171	0.224	0.831	n.d.	0.020	n.d.	0.010	0.007	0.028	0.019	n.d.	0.044	0.021	1.62
Ch 8	0.084	0.110	0.017	0.138	0.232	0.960	n.d.	0.046	n.d.	0.005	n.d.	0.024	0.036	n.d.	0.071	0.021	1.744
Ch 9	0.042	0.138	n.d.	0.083	0.219	1.108	n.d.	n.d.	n.d.	0.022	n.d.	0.050	0.035	n.d.	0.008	0.012	1.717
Ch 10	0.160	0.237	0.007	0.072	0.261	0.448	n.d.	0.092	0.024	0.008	n.d.	0.033	0.021	n.d.	0.110	0.022	1.495
Mean (SD)	0.190 (0.213)	0.275 (0.347)	0.016 (0.007)	0.167 (0.070)	0.308 (0.186)	0.730 (0.233)	–	0.074 (0.092)	0.055 (0.056)	0.024 (0.031)	0.018 (0.009)	0.034 (0.021)	0.035 (0.021)	–	0.127 (0.216)	0.031 (0.030)	2.028 (1.284)

n.d. not detected; *SD* standard deviation

Table 3 The content of macroelements and trace elements in *Spirulina* ($n = 13$) and *Chlorella* ($n = 10$) food supplements. The reported p value is for Mann-Whitney U test. Percentage of Adequate Intake (%AI) established by the European Food Safety Authority was calculated assuming a daily consumption of 3.0 g dose of a supplement

		Mean ± SD (median)	Min–max	p	%AI
Macroelements		[g kg^{-1}]			Mean (min–max)
Ca	*Spirulina*	12.5 ± 32.1 (3.3)	2.2–119.3	*	3.9 (0.7–37.6)
	Chlorella	5.9 ± 2.6 (5.4)	2.9–12.3		1.9 (0.9–3.9)
P	*Spirulina*	11.9 ± 14.1 (8.3)	6.6–58.7	***	6.5 (3.6–32.0)
	Chlorella	12.4 ± 3.8 (11.8)	5.0–18.6		6.7 (2.7–10.1)
Mg	*Spirulina*	3.4 ± 1.3 (2.9)	1.9–6.6	n.s.	2.9 (1.6–5.6)
	Chlorella	3.5 ± 1.8 (3.3)	1.3–6.3		3.0 (1.1–5.4)
Na	*Spirulina*	11.0 ± 6.6 (10.4)	4.2–28.1	*	–
	Chlorella	5.0 ± 4.8 (3.3)	0.5–13.2		–
K	*Spirulina*	15.7 ± 2.8 (16.0)	8.5–20.9	n.s.	1.3 (0.7–1.8)
	Chlorella	14.5 ± 4.5 (12.4)	9.8–23.0		1.2 (0.8–2.0)
Trace elements		[mg kg^{-1}]			Mean (min–max)
Co	*Spirulina*	1.0 ± 2.5 (0.3)	0.08–9.4	n.s.	–
	Chlorella	0.2 ± 0.09 (0.21)	0.07–0.39		–
Cr	*Spirulina*	3.6 ± 6.3 (1.6)	0.6–24.4	n.s.	–
	Chlorella	2.5 ± 1.3 (2.3)	1.1–4.8		–
Cu	*Spirulina*	4.4 ± 4.5 (2.4)	0.9–15.1	**	0.8 (0.2–2.8)
	Chlorella	10.1 ± 8.8 (6.3)	2.6–29.4		1.9 (0.5–5.5)
Fe	*Spirulina*	994.7 ± 475.8 (873.6)	368.5–2286.6	n.s.	27.1 (10.0–62.3)
	Chlorella	1045.9 ± 382.3 (1121.0)	438.4–1661.5		28.5 (11.9–45.3)
Mn	*Spirulina*	45.8 ± 25.7 (36.3)	26.1–109.8	n.s.	4.6 (2.6–11.0)
	Chlorella	53.8 ± 14.1 (51.1)	24.2–74.6		5.4 (2.4–7.5)
Mo	*Spirulina*	0.3 ± 0.2 (0.2)	0.09–0.90	n.s.	1.4 (0.4–4.1)
	Chlorella	0.8 ± 1.8 (0.2)	0.14–5.8		3.7 (0.6–26.8)
Se	*Spirulina*	0.3 ± 0.5 (0.1)	0.0–1.6	n.s.	1.3 (0.0–7.0)
	Chlorella	0.7 ± 0.3 (0.8)	0.4–1.1		3.0 (1.7–4.7)
Zn	*Spirulina*	23.2 ± 13.3 (19.7)	9.6–61.4	n.s.	0.6 (0.2–1.6)
	Chlorella	28.4 ± 16.9 (23.3)	11.2–73.5		0.7 (0.3–1.9)

n.s. not significant ($p > 0.05$)

*$p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

element of microalgal biomass is therefore more plausible (Rzymski et al. 2014). The increased contents of this metal have previously been reported in *Spirulina* and *Chlorella* supplements implicated in cases of human intoxication; extracts of which revealed a cytotoxicity *in vitro* (although the causative agent was not unambiguously elucidated) (Rzymski et al. 2015). An effort is required to eliminate microalgal biomass contaminated significantly with Pb to decrease exposure and ensure consumer safety. One should, however, note that selected multi-ingredient food supplements that contain a mixture of vitamins and minerals have been reported to contain even greater Pb content (Poniedziałek et al. 2018). The potential monitoring enforcement should therefore encompass not only microalgal-based formulations but all food supplements.

Previous studies have shown that some food supplements may be contaminated with the hexavalent form of Cr (Martone et al. 2013) which is recognized as a human carcinogen (Sun et al. 2015). Thus, it remains important to screen microalgal products to exclude such contamination and, as shown in the present study, none of the *Spirulina* and *Chlorella* products contained detectable levels of the Cr (VI) form. The As speciation analyses revealed, in turn, that content of this metalloid (if detectable) was mostly constituted of an organic form that reveals low or even no toxicity for humans (Hughes 2002). This is in line with previous observations (Hsu et al. 2001; Hedegaard et al. 2013; Rzymski et al. 2015). However, one should note that selected *Spirulina* and *Chlorella* products had detectable levels of inorganic As species, particularly the As(V) form. According to the EFSA (2014), the mean daily exposure to inorganic As in Europe varies from 0.24 to 0.38 µg kg^{-1} bw per day for adults, equivalent to 16.8–26.6 µg of As per day for a 70-kg adult. This considered, use of a usual 3.0 g dose of supplements with detected inorganic As would contribute significantly to its exposure, although one should note that in any case, the BMDL01 value would be exceeded. There are currently no

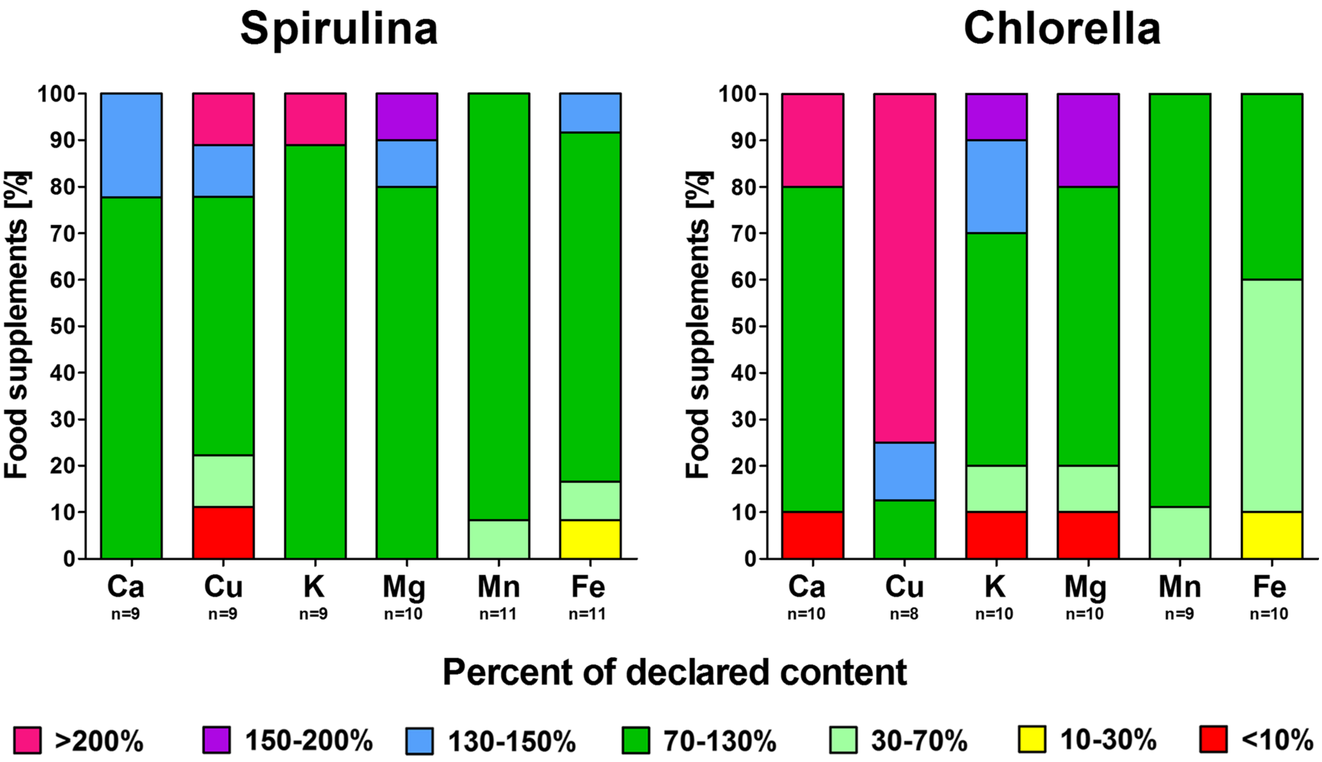

Fig. 3 The content of Ca, Cu, K, Mg, Mn, and Fe determined in *Spirulina* (left) and *Chlorella* (right) food supplements presented as a percentage of the content declared on the label

maximum limits for As in food supplements set in European Union—this study would suggest that the establishment of such limits would lead to the prevention of low-quality products from contaminating the market.

To the best of our knowledge, the present study is the first to analyze the level of REEs in microalgal food supplements. They represent an emerging group of pollutants whose emissions have risen over recent decades due to certain human activities (Pagano et al. 2015) resulting in increased human exposure via different routes (Poniedziałek et al. 2017). The outcomes of these exposures are yet to be fully explored, although selected REEs reveal cytotoxicity and have been potentially linked to anti-testicular effects, male sterility, hypertension in females, reduced IQ, and neurological alterations in children (Zhu et al. 1996; Marzec-Wróblewska et al. 2015; Wang et al. 2017; Gwenzi et al. 2018). Diet may constitute an important route of human exposure to REEs, and various foodstuffs such as mushrooms or vegetables already have been reported to exceed the maximum 0.7 mg kg^{-1} fresh weight (7.0 mg kg^{-1} dry weight) threshold limit, set so far only in China (SAC 2012; Li et al. 2013; Rzymski et al. 2017; Siwulski et al. 2017; Zhuang et al. 2017). Interestingly, some REEs, namely Ce, Gd, La, and Sc, have been reported to increase the growth of some microalgae and their content of lutein, violaxanthin, β-carotene, and chlorophylls (Goecke et al. 2017). Microalgae from the *Arthrospira* and *Chlorella* genera were also experimentally shown to bioconcentrate some REEs such as Ce or Nd (Sadovsky et al. 2016; Kücüker et al. 2017). In the present study, all studied microalgal food supplements, except for one product based on *Spirulina* (exceeding maximum allowance limit) and one based on *Chlorella* (reaching 78% of the limit), were characterized by a relatively low content of REEs. The highest share was represented by light REEs (with Pr reaching the highest mean content in *Spirulina* and *Chlorella* products) which are known to accumulate to a greater extent in the human body (Wei et al. 2013; Hao et al. 2015). All in all, the findings of the present study indicate that microalgal food supplements, with certain exceptions, do not represent a significant dietary source of REEs.

While *Spirulina* and *Chlorella* food products are known to contain a high content of proteins, polyunsaturated fatty acids, and various pigments, the nutritional value of their mineral content, both macro- and trace elements, is relatively low, particularly if one considers the most commonly recommended dose of 3.0 g daily. In this regard, they cannot compete with a number of other commonly eaten food products such as vegetables or fruits. On the other hand, the Tolerable Upper Intake Level (UL) and Safety Upper Level (SUL)/Guidance Level (GL) as set respectively by the EFSA (2017b) and the Expert Group on Vitamins and Minerals (2003) were not exceeded for any essential element considered by this study. This is an important finding as food supplements should only serve as an accessory source of nutrients in a balanced diet and not as their substitution. An excessive intake of minerals can lead to various adverse effects including neurotoxic responses (Mn), cardiotoxicity (Co), gastrointestinal symptoms (Cu, Fe, K, Mg), altered immune function (Zn), or unwanted cardiovascular events (Ca) (Verkaik-Kloosterman et al. 2012). One should note, however, that the studied *Spirulina* and *Chlorella* food supplements served as a rich source of Fe. On average, daily supplementation with the most commonly recommended dose of 3.0 g would constitute 25% of AI with some *Spirulina* and *Chlorella* products reaching over 60 and 40% of AI, respectively. As found in previous studies, bioavailability of Fe from *Spirulina* biomass (in which it is mostly present as ferrihydrite) is lower than that of ferrous sulfate, often used in mineral food supplements, but higher than that in whole wheat (Kapoor and Mehta 1992; Perfiliev et al. 2018). However, in the case of Fe-fortified *Spirulina*, mineral bioavailability can be even higher than that from meat products (Puyfoulhoux et al. 2001). Considering the Fe content in microalgal food supplement and the fact that they can contain bioavailable forms of vitamin B_{12} (Nakano et al. 2010; De et al. 2011; Merchant et al. 2015), these products are potentially of high interest to individuals on vegetarian diets which are becoming increasingly popular in different populations (Dinu et al. 2017). It would be therefore interesting to conduct a clinical trial investigating whether *Spirulina* supplementation may have a beneficial effect on Fe burden. Although one recent study has shown that such intervention unexpectedly decreased the concentration of serum Fe (Suliburska et al. 2016), one should note that this parameter alone, without assessing a number of other biochemical markers (e.g., transferrin, ferritin, hemoglobin, hepcidin, etc.), is not sufficient to evaluate Fe status in humans (Rzymski and Ganz 2018).

The nutritional value of the food supplement declared on the container label is an important factor in assisting the consumers' decision-making processes when purchasing a product. A worrisome finding of some recent investigations is that actual declared levels of ingredients in various food supplements does not agree with their actual content, with the majority of investigated products being far from meeting the acceptable margin (Brandon et al. 2014; Niedzielski et al. 2016; Verkaik-Kloosterman et al. 2017; Poniedziałek et al. 2018). Contrary to this, most of the studied products revealed good agreement in this respect. This particularly concerned the content of Ca, K, Mg, Mn, and Fe in *Spirulina* supplements. Some discrepancies were found for some *Chlorella* products with most of those studied having a significantly higher content of Cu than that declared on the label. Although the origins of such an increased content of Cu levels can only be hypothesized, one should note that Cu compounds can be used in *Chlorella* cultures as selective inhibitors of some grazing rotifers which can occur in open pond cultivation systems and cause a significant economic loss (Pradeep et al. 2015; Day et al. 2017); such an application may significantly alter Cu content in the final food product.

Conclusion

In summary, the study screened the content of minerals and toxic elements in selected *Spirulina* and *Chlorella* food supplements registered on the European market and originating from Asia, North America, and Europe. Considering the most commonly recommended daily dose of 3 g, the use of such products would rather not contribute significantly to mineral intake, except for Fe whose levels were found to be high in both *Spirulina* and *Chlorella* formulas. An important finding is that most of the studied products revealed a good agreement between declared and factual content of elements. The microalgal food supplements appear not to contribute significantly to dietary intake of REEs. Even though some of the products screened by this study revealed increased levels of toxic metals such as Al or Pb, this should by no means be a reason to disregard the very promising bioactive properties of *Arthrospira* or *Chlorella*. It is rather an indication that the quality control of such products should be strengthened so that only those microalgal food supplements which ensure consumer safety can reach the market.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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