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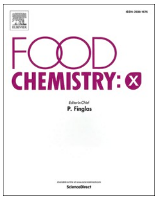

Occurrence and health risk assessment of toxic metals and rare earth elements in microalgae: Insight into potential risk factors in new sustainable food resources

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ABSTRACT

Microalgae are a promising sustainable food source with high nutritional value and environmental benefits. This study investigated the presence of toxic metals and rare earth elements (REEs) in 68 microalgal-based food products and conducted a probabilistic risk assessment to evaluate potential health risks. The findings revealed high detection rates of REEs (80.96% to 100%) and heavy metals (83.82% to 100%), with REE concentrations ranging from 0.0055 to 0.5207 mg/kg. Heavy metals were detected at the following average concentrations: As (2.80 mg/kg) > Cr (1.27 mg/kg) > Pb (0.30 mg/kg) > Cd (0.20 mg/kg) > Hg (0.01 mg/kg). Carcinogenic risk analysis for Cd (3.004×10^{-3}), Cr (1.484×10^{-3}), and As (1.1283×10^{-2}) indicated that 95th percentile values exceeded established safety thresholds (10^{-4}). These findings highlight the critical need for stringent monitoring and the establishment of comprehensive regulatory frameworks for the safety of novel microalgae foods.

1. Introduction

Microalgae, diminutive yet prolific in biomass production, have garnered global attention not only as a rich source of essential nutrients but also as key players in sustainable nutrition and potential combatants against malnutrition (Islam et al., 2023; Torres-Tiji et al., 2020). These single-celled organisms are renowned for their rapid growth and capacity to transform inorganic substances into a biomass rich in proteins, polyunsaturated fatty acids, and bioactive compounds (Islam et al., 2023; Torres-Tiji et al., 2020). The United Nations World Food Conference has notably recognized Spirulina platensis as a “food of the future”, underscoring its nutritional value and minimal resource requirements for cultivation (Liu, Wu, et al., 2024). A number of microalgal species, including *Chlorella spp.*, *Arthrospira spp.* (*Spirulina*), *Odontella*, *Tetraselmis spp.*, *Nannochloropsis spp.*, and *Euglena spp.* have been approved as novel food sources in various regions, such as China, the USA, and Europe (Janssen et al., 2022; Liu, Yu, et al., 2024).

In addition to their role in nutrient supplementation, microalgae are of great importance in the development of sustainable food systems. They are used as functional ingredients in diverse products, such as pasta and beverages (Kumar et al., 2022; Mathys & Caporgno, 2018). Commercially, microalgae are predominantly employed as additives in various food products, including dietary supplements and bakery items, biscuits and breads (Barkia et al., 2019; Krishna Koyande et al., 2019). Moreover, microalgae represent a sustainable alternative protein source, demanding significantly fewer resources including land, water, nitrogen, and phosphorus, than traditional protein sources. This makes them as an environmentally friendly solution for global protein demands

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Abbreviations: REEs, Rare Earth Elements; HMs, Heavy Metals; EDI, Estimated Daily Intake; FIR, Food Intake Rate; THQ, Target Hazard Quotient; RfD, Reference Dose; HI, Hazard Index; CR, Target Carcinogenic Risk; CSF, Carcinogenic Slope Factor; P95, 95th percentile values; PTDI, Provisional Tolerable Daily Intake.

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(Amorim et al., 2021). The efficiency and nutritional density of microalgae as a promising solution to meet the global challenge of sustainable food security, while also minimizing environmental impacts. The potential for microalgae to revolutionize the food industry is becoming increasingly apparent as technology and biotechnological advances enhance their commercial viability and consumer acceptance (Williamson et al., 2024).

The utilization of microalgae as a novel food source highlights the necessity for a heightened focus on food safety risk factors. The cell walls of microalgae are equipped with specific functional groups such as carboxyl, hydroxyl, and sulfhydryl, which enable these photosynthetic organisms to effectively bind with various harmful substances present in their growth environments (Abdelfattah et al., 2023). This remarkable adsorption capacity enables microalgae to remove pollutants from their environments, which is advantageous for bioremediation (Nagarajan et al., 2019). However, this same characteristic also gives rise to significant safety concerns when microalgae are used in food applications. This is due to their high affinity for heavy metals, organic pollutants, and other environmental contaminants, which can accumulate to levels that pose health risks to consumers (Sharma et al., 2022; Tibon et al., 2023).

In recent decades, the escalation of industrialization, urbanization, and intensive agriculture has resulted in the release of trace metals and metalloids, such as thallium, tellurium, and other rare earth elements (REEs) into the environment (Doulgeridou et al., 2020). These elements are used extensively in the production of alloys, agricultural enhancements, medicinal applications, and high-tech manufacturing. As a result they accumulate in environmental water bodies, thereby integrating into the food chain through various pathways including mining discharges, industrial waste, and urban runoff (Kowalczyk et al., 2022). The environmental presence of these contaminants poses significant risks, as chronic exposure to even at low levels can induce a spectrum of adverse health outcomes (Gwenzi et al., 2018), including neurotoxicity (Xu et al., 2017), developmental disruptions (Cabrera-Rodríguez et al., 2018), and hepatic changes from early life stages (Nakamura et al., 1997). The pervasive use of these elements in modern technology, coupled with their anthropogenic release, necessitates comprehensive safety assessments of aquatic food resources to ensure the protection of public health against toxic elements contamination (Hao et al., 2015; Taroncher, Rodriguez-Carrasco, et al., 2023).

As microalgae are increasingly cultivated for their nutritional benefits, the potential for contamination highlights the necessity for rigorous monitoring and regulation of cultivation practices to ensure the safety of microalgae products for human consumption. This aspect of microalgal cultivation presents a significant challenge in the utilization of these organism as sustainable food sources without compromising consumer health. This study aims to investigate the concentrations of REEs, heavy metals, and other toxic elements in commercially available food-grade microalgal powders in China. The study focuses on specific varieties of microalgae, including spirulina, chlorella, and microalgae protein or peptides. The analysis and risk assessment of contaminants in microalgal samples sourced from 16 provinces are expected to yield essential insights into novel food risk factors.

2. Materials and methods

2.1. Chemicals and reagents

Concentrated nitric acid (HNO_3) and hydrofluoric acid (HF) of spectroscopic grade were procured from SCR in Shanghai, China, and employed in the preparation of samples. The elemental standards utilized in this study were obtained from the National Center for Analysis and Testing of Steel Materials in Beijing, China. The concentrations of standards were set at either 1000 mg/L or 100 mg/L. The standards encompassed a comprehensive array of elements, including lead, cadmium, arsenic, mercury, selenium, chromium, tin, copper, iron, manganese, zinc, nickel, aluminum, antimony, potassium, sodium, calcium, magnesium, boron, barium, strontium, molybdenum, thallium, titanium, vanadium, and cobalt. The elemental standards used in this study, available in both single-element and multi-element formulations, have been officially certified by the relevant state authority and have received standard substance certification. These certifications guarantee the precision and dependability of the standards for analytical applications. The internal standard solution, prepared at a concentration of 1000 mg/L, comprises elements including scandium, germanium, indium, rhodium, rhenium, and bismuth. Similarly, the internal standards, whether single or multi-element, have also been certified by the relevant state authority and have been awarded a certificate of standard substance. In practical applications, these internal standards are employed at a concentration of 10 μg/mL for elements such as rhodium (Rh), indium (In), and rhenium (Re) in order to maintain precision in instrumental analysis. The experiments were conducted using ultrapure water with a resistivity of 18.2 MΩ·cm. The water used in this study was obtained from a Milli-Q water purification system (Millipore Corporation, USA).

2.2. Sample collection

A total of 68 microalgae samples were procured from online sources and retail outlets between March and December 2023. The collection comprised 45 samples of spirulina powder, 10 of chlorella powder, and 13 of spirulina peptide samples. The samples were procured from 16 different provinces and obtained from 68 distinct retailers, encompassing both food-grade and export-grade algal powders. Each sample was assigned a unique identification number, homogenised, and stored at 4 °C prior to undergoing further analysis.

2.3. Sample preparation and instrument analysis

The preparation of the algae powder samples was conducted in accordance with the China National Food Safety Standards, spcifically GB 5009.94–2012 and GB 5009.268–2016. Initially, approximately 0.3 g of each sample was transferred into a microwave digestion vessel, to which 7 mL of nitric acid was added. To ensure the reliability and accuracy of the data, each sample was analysed in triplicate. The vessel was then covered and left to stand for 1 h to facilitate pre-digestion interactions. Subsequently, the vessel was sealed and subjected to microwave-assisted digestion according to the standard operating procedures of the instrument. Following digestion, the vessel was opened carefully to release gas and rinsed three times in a temperature-controlled wash tank. The rinse solutions were combined in a centrifuge tube and diluted to a final volume of 25 mL, thus ensuring uniformity and preparing the analysis-ready sample along with a reagent blank. The analysis of the samples was conducted using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS, NexION 350×; PerkinElmer, Waltham, MA, USA), with the specific parameters provided in supplementary Table S1.

2.4. Quality control and assurance

For each set of fifteen samples, a laboratory blank was analysed concurrently in order to monitor potential contamination and interferences. The control solution was ultrapure water (18 MΩ × cm) from the Milli-Q Integral system (Millipore, Bedford, MA, USA). The levels of the analytes in the samples were deemed to be accurate and reportable if they exceeded the concentrations found in the blanks by a factor of two. Otherwise, the analytes were classified as not detected (ND). The inclusion of surrogate standards in the samples prior to extraction enabled the evaluation of recovery rates. The accuracy and precision of the method were additionally evaluated by reanalysing the standard reference material GBW10014a, obtained from the Institute of Geochemistry, Chinese Academy of Geological Sciences, China. The results of the ICP-MS analysis demonstrated that the concentrations of

REEs, heavy metals, and toxic elements in the standard materials were within the reference values (see Tables S2), thereby confirming the accuracy and precision of the method.

The results of ICP-MS detection of standard cabbage material indicate that rare earth elements, heavy metals, and toxic elements are all within the detection range, with the exception of Mn, which exhibits a minor deviation. This is evidenced by Tables S3 and S4, which demonstrate satisfactory accuracy and precision in metal analysis.

2.5. Probabilistic exposure and risk assessment

The Estimated Daily Intake (EDI) was calcualted using the most recent data on the consumption of aquatic plant in China, as reported by the Food and Agriculture Organization. In order to assess the potential health risks, a number of indicators were employed, including the Target Hazard Quotient (THQ), Hazard Index (HI), and Target Carcinogenic Risk (CR) (Xiong et al., 2020). A Monte Carlo simulation, a widely used statistical mathematical method for predicting the likelihood of adverse outcomes in a population, was employed to account for data variability and uncertainty, even in cases of limited sample sizes. The probabilistic assessment model permits the evaluation of a broad range of risk levels, thereby accommodating populations with disparate risk tolerances. The simulation was conducted 10,000 times, with 100 iterations, in order to ensure the robustness and reliability of the risk assessment results (Karami et al., 2019).

The calculation of EDI for contaminants was conducted in accordance with the following formula:

$$\mathrm{EDI_iCFIR/BW}$$

In this equation, C (mg/kg, wet weight) represents the concentration of the heavy metal, FIR is the average daily food intake rate of microalgae (per person), and BW is the average body weight (adult: 66 kg). The most recent data on the consumption of aquatic plants in China, as reported by the Food and Agriculture Organization, indicates an annual consumption rate of 12.26 kg of microalgae per person (Taroncher, Rodríguez-Carrasco, et al., 2023). This figure translates to a daily FIR of microalgae of 33.59 g per person.

The Target Hazard Quotient (THQ) is a key risk index for evaluating non-carcinogenic human health risks associated with exposure to hazardous substances. The premise is that a THQ value exceeding 1 indicates a potential risk to human health, suggesting that the intake of the substance may result in adverse health effects. The calculation formula for THQ is provided by Adebiyi et al. (Adebiyi et al., 2020).

$$\mathrm{THQEDI/RfD}$$

The Reference Dose (RfD) represents the chronic oral reference dose for heavy metals.

In this study, the reference dose (RfD) for an adult weighing approximately 66 kg is set at 0.07 mg/kg/day (70 μg/kg/day), in accordance with the referenced guidelines. The United States Environmental Protection Agency (USEPA) provides reference dose (RfD) values for heavy metals and toxic elements in micrograms per kilogram of body weight per day, as follows: The following values were obtained: Pb (3.5), Cd (1), Cr (1500), Mn (140), Co (0.3), Hg (0.1), Al (1000), V (9000), Ni (20), Cu (40), Zn (20), Sb (0.4), Tl (0.01), Se (5), and As (0.3). These values are fundamental to the evaluation of potential non-carcinogenic health risks associated with these substances (Bonsignore et al., 2018).

The Hazard Index (HI) is a method of assessing the cumulative effects of a mixture in aquatic products. The HI is defined as follows (Tan, 2021):

$$\mathrm{HI} = \sum_{i=1}^{n} THQ_{(\mathrm{i})}$$

Similarly, an HI value exceeding 1 indicates a potential risk to human health from heavy metals. In the absence of such evidence, the risk can be considered negligible.

The calculation of carcinogenic risk (CR) is based on the guidelines set forth by the United States Environmental Protection Agency (EPA). The defined risk categories are as follows: negligible carcinogenic risk ($\mathrm{CR} < 1 \times 10^{-6}$), acceptable carcinogenic risk ($1 \times 10^{-6} < \mathrm{CR} < 1 \times 10^{-4}$), and unacceptable carcinogenic risk ($\mathrm{CR} > 1 \times 10^{-4}$). Previous research has identified arsenic, cadmium, lead and chromium as carcinogenic elements. The calculation of the CR is performed using the following formula, as proposed by Bonsignore et al. (Bonsignore et al., 2018).

$$\mathrm{CR} = \mathrm{EDI} \times \mathrm{CSF} \times 10^{-3}$$

The Carcinogenic Slope Factor (CSF) values are 1.5 (As), 6.3 (Cd), 0.0085 (Pb), and 0.5 (Cr) mg/kg/d, respectively.(Wang et al., 2020).

2.6. Statistical analyses

The data analysis was conducted using Excel 2016 (Microsoft, USA) and IBM SPSS 25.0 software (IBM Corporation, Armonk, NY, USA). Spearman's correlation analysis was performed with Origin 2022 (OriginLab Corporation, Northampton, MA, USA). Monte Carlo simulations were conducted using the Excel@risk 8.2 software (Palisade Corporation, Newfield, NY, USA). In instances where the detection results fell below the limit of detection, a surrogate value equal to half of the detection limit was employed in the statistical analyses.

3. Results

3.1. Detection rate and contamination levels of toxic metals and REEs in edible microalgae

The detection rates of rare earth elements (REEs) in food-grade microalgal powder ranged from 80.96% to 100% (Table S2). Elements such as Eu, Gd, and Dy were found in all samples, while Lu and Sc showed slightly lower detection rates of 84.13% and 80.96%, respectively. The detection rates for the remaining REEs exceeded 90%, underscoring their pervasive presence in microalgal products. These high detection rates align with findings from previous study which reported similarly high detection rates of REEs in Italian seaweed (Squadrone et al., 2019). In addition to REEs, heavy metals such as Pb, As, Hg, Cadmium Cd, and Cr were detected at significant levels in the analysed samples, with detection rates ranging from 52.94% to 100%. Other elements, including V, Ni, Tl, and Se, exhibited detection rates exceeding 90%.

Fig. 1A illustrates the concentrations of 16 REEs in microalgal powder, which varied widely from 0.0055 mg/kg to 0.5207 mg/kg. Among the REEs, Ce had the highest average concentration at 0.5207 mg/kg, while Tm and Lu had the lowest average concentrations at 0.0055 mg/kg and 0.0063 mg/kg, respectively. Fig. 1B further examines the concentrations of 16 heavy metals and toxic elements in the microalgal powder, revealing a broad range from 0.01 mg/kg to 273.97 mg/kg. Notably, Al was the most abundant toxic element at 273.97 mg/kg, while Hg exhibited the lowest concentration among the tested heavy metals and toxic elements at 0.01 mg/kg. The concentrations of the heavy metals followed the order: As (2.80 mg/kg) > Cr (1.27 mg/kg) > Pb (0.30 mg/kg) > Cd (0.20 mg/kg) > Hg (0.01 mg/kg).

These findings indicate significant contamination levels of both REEs and heavy metals in food-grade microalgal powder. The high detection rates suggest widespread contamination, while the variability in concentration levels highlights the extent of contamination. The extensive presence of these contaminants underscores the need for comprehensive monitoring and regulation to mitigate potential health risks associated with the consumption of microalgal products.

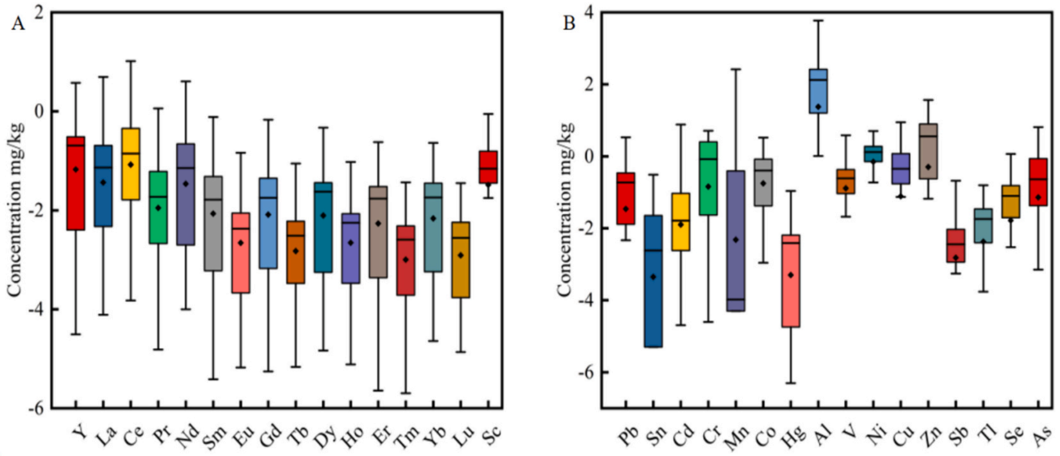

Fig. 1. Concentration distribution of REEs and Heavy metals and toxic elements in microalgae powder (Data were log 10 transformed. A: REEs; B: Heavy metals and toxic elements).

3.2. *Correlation analysis of heavy metals and rare earth elements*

Fig. 2 presents a correlation heatmap illustrating the interrelationships among major rare earth elements (REEs), heavy metals, and toxic elements in microalgae. This analysis reveals significant positive correlations across the 16 REEs, underscoring the pervasive co-occurrence of these elements within the samples. Particularly noteworthy are the correlation coefficients between Sc and various REEs, ranging from 0.58 to 0.69, which indicate moderate to strong associations. Furthermore, correlation coefficients among other REEs are even more pronounced, often surpassing 0.75, suggesting a robust interdependence among these elements in microalgae. Notably, exceptionally high correlations, with coefficients of 0.99 and above, were observed among several pairs of REEs, implying nearly identical distribution patterns within the samples.

The analysis also identified divergent behaviors among heavy metals and toxic elements. Mn exhibited a positive correlation with As but displayed negative correlations with other elements, suggesting complex and varied interactions within the microalgal matrix. Pb demonstrated very strong positive correlations with Cd, Cr, and Hg, with coefficients exceeding 0.8, indicating a closely linked presence and possibly shared sources or pathways of contamination in microalgae. Additionally, a strong correlation was noted between Cd and Hg, further emphasizing the interconnectedness of these toxic elements.

Al exhibited a strong positive correlation with V, while V also showed a robust correlation with Ni. Se demonstrated a notable positive correlation with As, highlighting potential co-contamination patterns. These interconnected contamination patterns of heavy metals and toxic elements in microalgal samples reflect a complex network of interactions and affinities among the elements, suggesting their varying degrees of co-occurrence.

These findings reveal critical insights into the potential implications for microalgal contamination and bioaccumulation. The strong correlations among certain heavy metals and REEs may indicate common sources of pollution or similar bioaccumulation mechanisms in the aquatic environment where these microalgae are cultivated. Understanding these interrelationships is essential for developing effective monitoring and mitigation strategies to ensure the safety and sustainability of microalgal food products.

3.3. *Health risk assessment of exposure to REEs and toxic metals from microalgae consumption*

The dietary intake and associated health risks of REEs, heavy metals, and toxic elements in microalgal powder were rigorously evaluated using Monte Carlo simulation, providing a probabilistic risk assessment framework (Table 1 and Figs. 3–4). This approach allowed for the comprehensive estimation of the Target Hazard Quotients (THQ) and cumulative Hazard Index (HI) values.

The THQ values for heavy metals and toxic elements were found to be substantially higher than those for REEs. Specifically, the mean, median, and 95th percentile THQ values for REEs were all significantly below 1, indicating a relatively low non-carcinogenic risk from REE exposure through microalgal consumption. However, the systematic simulation of the hazard index (HI) for REEs revealed mean, median, and 95th percentile values that exceeded 1, suggesting potential cumulative non-carcinogenic risks when considering the combined exposure to multiple REEs. Conversely, the assessment of heavy metals and toxic elements painted a more concerning picture. The mean THQ value for these elements exceeded the safety threshold of 1, indicating potential health risks. Notably, the 95th percentile values for Cobalt (Co), Thallium (Tl), and Arsenic (As) were particularly alarming, at 3.186, 5.705, and 4.16, respectively. These values substantially surpass the safety threshold, underscoring significant non-carcinogenic risks associated with the ingestion of these contaminants from microalgal products.

The cumulative non-carcinogenic risk, as reflected by the HI values for heavy metals and toxic elements, consistently exceeded 1 across mean, median, and 95th percentile estimates. This indicates that the combined exposure to these toxic elements through microalgal consumption poses a considerable health risk. The elevated HI values were primarily driven by high THQ values for Co, Tl, and As, highlighting these elements as major contributors to the overall health risk. Furthermore, the probabilistic risk assessment using Monte Carlo simulation, which included 10,000 iterations to account for variability and uncertainty, reinforced the robustness of these findings. The high THQ and HI values necessitate the implementation of stringent monitoring and regulatory measures to mitigate the health risks associated with the consumption of microalgal products.

Fig. 4 provides a detailed illustration of the carcinogenic risk (CR) values for Pb, Cd, Cr, and As in microalgal powder. The 95th percentile CR values were calculated as 5.784×10^{-6} for Pb, 3.004×10^{-3} for Cd, 1.484×10^{-3} for Cr, and 1.1283×10^{-2} for As. For Pb, a cumulative probability of 32.3% was observed for CR values ranging between 10^{-6} and 10^{-4}, indicating an acceptable carcinogenic risk level with no probability of exceeding the 10^{-4} threshold. For Cd, the analysis indicated a 33.3% probability that CR values would fall within the same range; however, a significant 51.9% probability was identified for values surpassing the 10^{-4} threshold, highlighting a substantial carcinogenic risk. Similarly, Cr showed a cumulative probability of 39.5% for CR values within the 10^{-6} to 10^{-4} range, with a 48.1% likelihood of

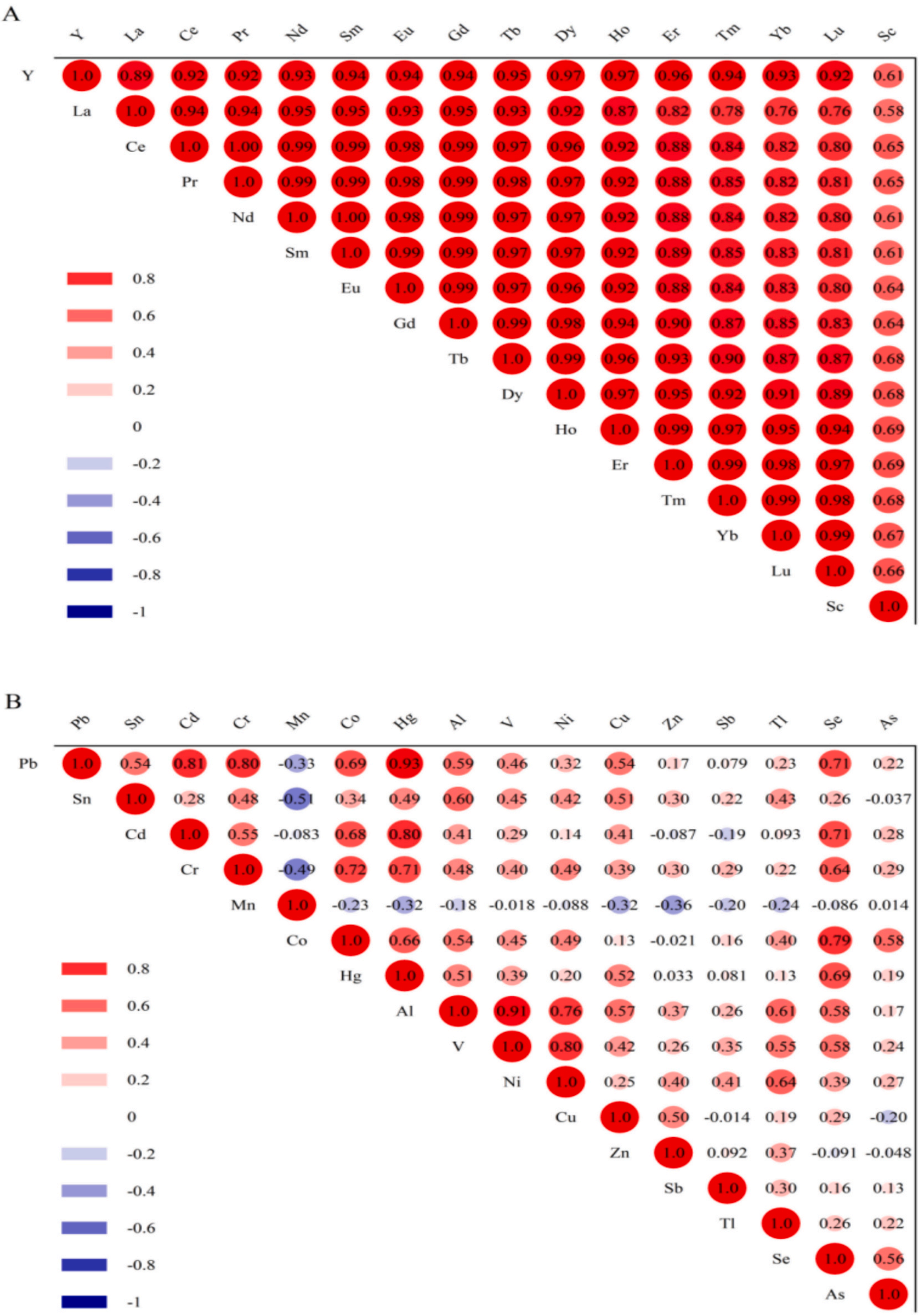

Fig. 2. Thermogram of correlation between major REEs and Heavy metals and toxic elements in microalgae (A: REEs; B: Heavy metals and toxic elements).

exceeding the 10^{-4} threshold, indicating potential carcinogenic concerns. As presented the most concerning findings, with a 25.5% cumulative probability for CR values within the acceptable range, but a notably higher 59.5% probability of exceeding the 10^{-4} threshold. This underscores a significant carcinogenic risk associated with arsenic in microalgal powder. These detailed probabilistic assessments underscore the critical need for robust risk management strategies to mitigate the potential health risks posed by heavy metals and REEs in microalgal food products. The findings highlight the necessity for stringent regulatory frameworks and continuous monitoring to ensure consumer safety.

4. Discussion

The diverse composition of microalgae makes them ideal candidates for innovative functional foods, as they offer a sustainable source of

Table 1
EDI values of rare earth elements and Heavy metals and toxic elements in algae powder.

REEs	EDI (μg/kg bw/day)			Toxic metals	EDI (μg/kg bw/day)		
	Mean	Median	P95		Mean	Median	P95
Y	0.224	0.07	0.966	Pb	0.15	0.04	0.675
La	0.142	0.041	0.625	Sn	0.016	0.001	0.087
Ce	0.265	0.086	1.135	Cd	0.1	0.019	0.477
Pr	0.035	0.011	0.151	Cr	0.658	0.178	2.942
Nd	0.128	0.038	0.562	Mn	4.58	0.04	26.47
Sm	0.028	0.009	0.122	Co	0.263	0.135	0.956
Eu	0.005	0.002	0.022	Hg	0.003	0.001	0.014
Gd	0.005	0.002	0.022	Al	140.12	31.25	651.59
Tb	0.004	0.001	0.017	V	0.364	0.127	1.531
Dy	0.026	0.008	0.111	Ni	0.726	0.464	2.321
Ho	0.006	0.002	0.024	Cu	0.502	0.106	2.356
Er	0.018	0.006	0.076	Zn	2.99	0.662	13.918
Tm	0.003	0.001	0.012	Sb	0.008	0.002	0.039
Yb	0.02	0.007	0.083	Tl	0.013	0.004	0.057
Lu	0.003	0.001	0.013	Se	0.069	0.019	0.306
Sc	0.055	0.026	0.205	As	0.282	0.081	1.248

Note: REEs, Rare earth elements; EDI, estimated daily intake; P95, estimated 95th percentile values.

proteins and micronutrients. However, their capacity to absorb pollutants gives rise to considerable safety concerns with regard to their use as a foodstuff. This study presents the initial investigation into the presence of rare earth elements (REEs), heavy metals, and toxic elements in microalgae, with a view to evaluating potential health risks. The findings emphasize the need for comprehensive monitoring and rigorous safety assessments to ensure the safe use of microalgae in the sustainable food industry, protecting consumer health.

4.1. Comparison with previous studies on toxic metals and REEs in microalgae

The results of our study indicate the presence of considerable contamination of both REEs and toxic metals in food-grade microalgal powders. The high detection rates observed suggest that contamination is a widespread phenomenon. This finding is consistent with previous research that has identified similar concerns in different contexts and geographical regions. For example, Rzymski et al. (Rzymski et al., 2019) investigated commercial microalgal supplements and found significant levels of toxic elements, including arsenic, lead, and cadmium, although these concentrations were generally below safety limits. However, certain products exhibited elevated levels of Al and inorganic arsenic, which highlights the variability in contamination levels depending on the cultivation and processing conditions. Similarly, a study by Fabre et al. (Fabre et al., 2020) examined the removal efficiency of Hg and other toxic elements by various macroalgae species, revealing high contamination levels and the ability of macroalgae to bioaccumulate these elements. The results of this study corroborate our findings regarding the considerable prevalence of Hg and other heavy metals in microalgal products, underscoring the necessity for meticulous monitoring and regulatory oversight. REE concentrations were found to range from 0.0063 to 0.5208 mg/kg in the course of our analysis. This range is approximately three times higher than the REE concentration in seaweed (162.35 ng/g) reported by Li (2012), yet significantly lower than the levels in Italian seaweed documented by Squadrone et al. (2019) at 3.12 mg/kg. While specific standards for REE levels in microalgal food products have yet to be established, prior research (Gu et al., 2015) has suggested a safe daily intake threshold of <70 μg/kg/day, with intake levels between 100 and 110 μg/kg/day potentially causing subclinical damage.

4.2. Analysis of contamination sources and patterns

Our correlation analysis indicated the existence of significant interrelationships between various REEs and toxic metals, suggesting the presence of common contamination sources. Indicated the existence of significant interrelationships between various REEs and toxic metals, suggesting the presence of common contamination sources. These findings are consistent with those of Bergsten-Torralba et al. (Bergsten-Torralba et al., 2020), who demonstrated similar contamination patterns and inter-element correlations in various microalgae species exposed to REEs. The positive correlation between Mn and As, along with the negative correlations with other elements, suggests that contamination behaviors are complex and influenced by specific environmental factors or anthropogenic activities. This complexity is further supported by the findings of Koppel et al. (Koppel et al., 2018), who explored the interactive effects of metal mixtures on marine microalgae and identified similar element-specific interactions. The research conducted by Mustafa et al. (Mustafa et al., 2021) has demonstrated significant correlations between the concentrations of heavy metals present in microalgae and the levels of environmental pollution, thereby highlighting the influence of anthropogenic sources. Spearman's correlation analyses elucidated relationships among elements within microalgal samples, indicating that the occurrence of Mn is likely influenced by natural processes such as rock weathering. Conversely, the presence of strong positive correlations among Pb, Cd, Cr, Hg, Co, As, and Se indicates a probable common anthropogenic source, which is consistent with the pollution characteristics observed in the sediment of Chinese rivers.

The capacity of microalgae to adsorb heavy metals is well documented, giving rise to concerns about elevated heavy metal levels in many microalgal products and their implications for safety (Cavalletti

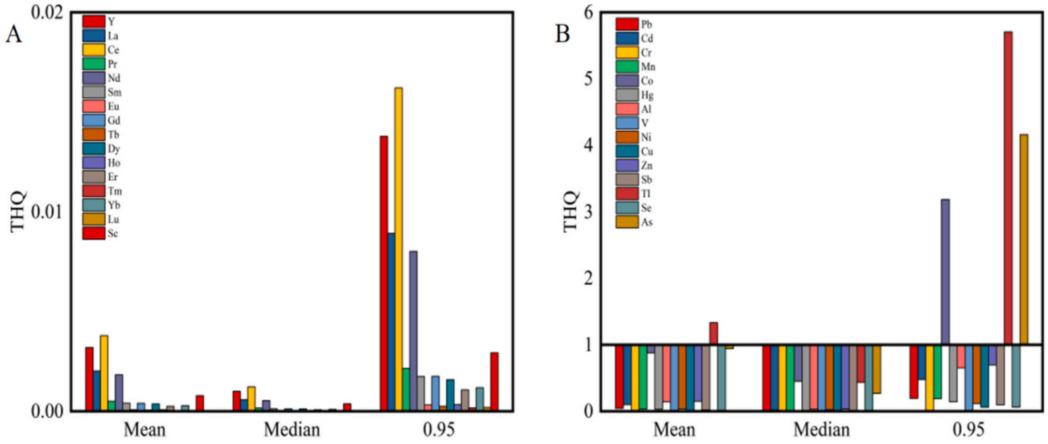

Fig. 3. THQ values of major REEs and Heavy metals and toxic elements in microalgae (A: REEs; B: Heavy metals and toxic elements).

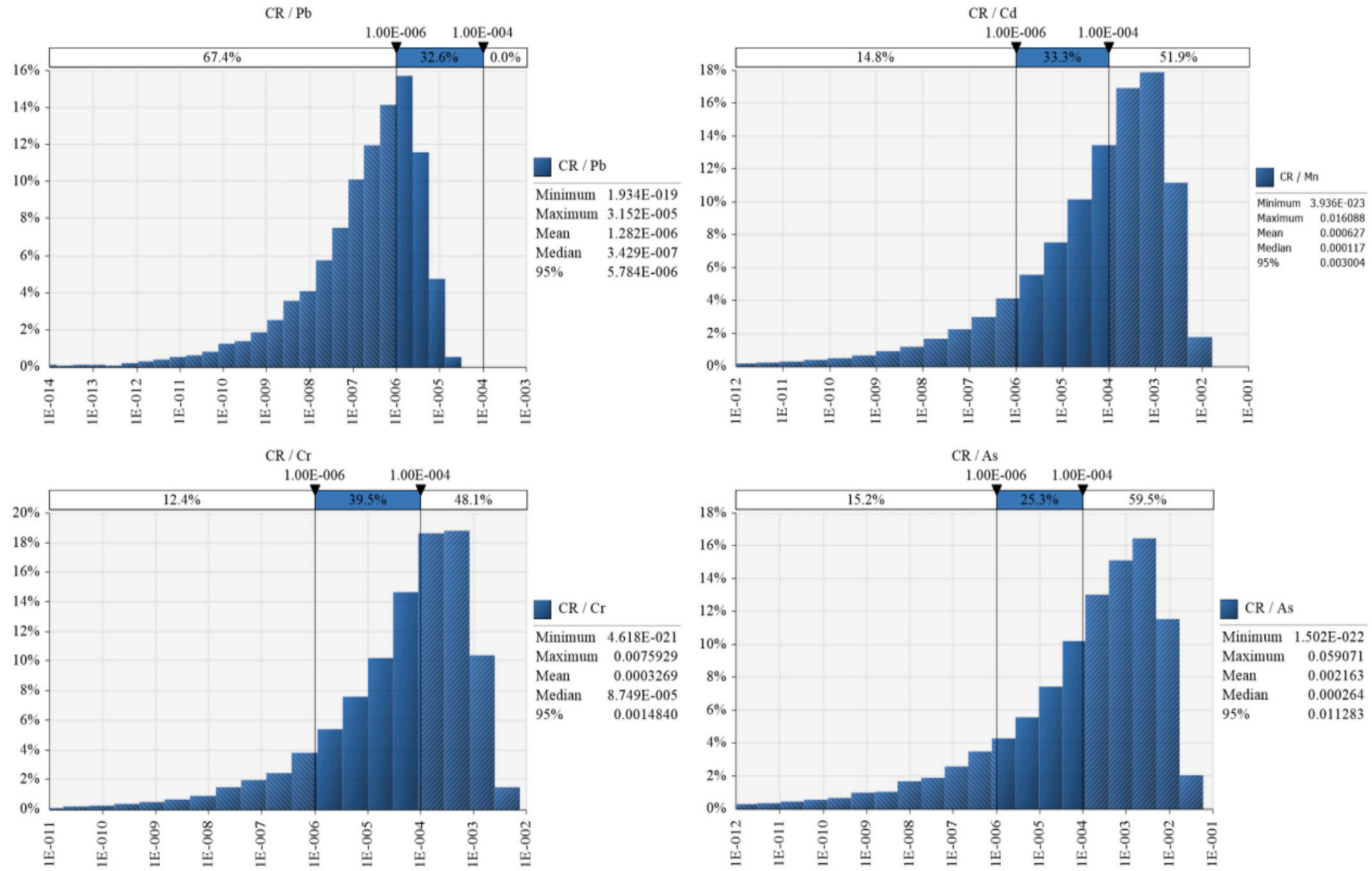

Fig. 4. Probability distribution of cancer risk (CR) values for Heavy metals in microalgae.

et al., 2021). These findings underscore the need for stringent measures to ensure the safe consumption of microalgal products. These findings highlight the necessity for rigorous measures to guarantee the safe consumption of microalgal products. The contamination of Chinese aquatic environments, particularly lakes, is primarily driven by agricultural, industrial, and domestic discharges, which present a significant environmental challenge (Tang et al., 2022).

4.3. Health risk assessment and regulatory implications

Our health risk assessment, using Monte Carlo simulations, revealed that the cumulative non-carcinogenic risk of REEs, as indicated by the hazard index (HI), exceeded the safety threshold, particularly for cobalt (Co), thallium (Tl), and arsenic (As). These findings align with Malhotra et al. (Malhotra et al., 2020) which reported significant health risks associated with REEs exposure through contaminated aquatic organisms. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established provisional tolerable daily intake (PTDI) thresholds for Pb, Cd, Cr, Hg, Al, and As. We calculated the estimated daily intake (EDI) values to assess long-term health impacts. Our findings revealed that Al (273.97 mg/kg) and As (2.8 mg/kg) levels in microalgal powders substantially exceed their respective PTDI values, highlighting potential health risks. While aluminum is generally considered to have low toxicity, prolonged exposure could potentially lead to adverse effects, underscoring the need for further investigation into the health implications of aluminum and other heavy metals in microalgal products (Suomi & Tuominen, 2023).

The Target Hazard Quotients (THQ) for Co, Tl, and As were found to exceed 1, indicating potential non-carcinogenic risks. The cumulative HI, predominantly influenced by Co, Tl, and As, surpassed 1, accounting for 82.87% of the total non-carcinogenic risk. These findings suggest that microalgal powder consumption poses manageable health risks, provided the intake of these specific elements is carefully controlled. Recent risk assessments have highlighted non-carcinogenic risks associated with arsenic in various aquatic foods, such as crayfish (Peng et al., 2022), bivalves, shellfish (Jin et al., 2023), and algae (Liu et al., 2022), aligning with our findings. Additionally, our study broadens the discussion on non-carcinogenic risks to include Tl and Co. Thallium, known for its high toxicity even at trace levels, presents significant health concerns, particularly due to its increased prevalence from industrial activities such as coal combustion and metal smelting. Enhanced regulatory measures and monitoring are necessary to mitigate the impact of these heavy metals on public health.

Cobalt, despite its low-to-moderate toxicity, is increasingly used in battery production, especially in China, raising concerns about its non-carcinogenic risks. Cd, Cr, and As were identified as presenting a high carcinogenic risk in microalgal powder consumption, with probabilities of exceeding the 10^{-4} threshold at 51.9% for Cd, 48.1% for Cr, and 59.5% for As. Previous carcinogenic risk assessments for these metals in aquatic products like fish (Mendoza et al., 2023), shrimp, bivalves, and algae (Liu et al., 2022). found minimal carcinogenic risk, but this study constitutes the first analysis of carcinogenic risks posed by heavy metals in various commercial microalgal powders. This assessment highlights the importance of rigorous quality control and safety assessments in microalgal food product production and processing to safeguard public health and promote the responsible growth of the microalgae industry.

4.4. Recommendations for future research and regulatory measures

Our findings highlight the urgent need for comprehensive regulatory measures to ensure the safety of microalgal food products. Future research should focus on developing standardized monitoring protocols and exploring the mechanisms of bioaccumulation and toxicity of REEs and heavy metals in microalgae. Studies such as those by Zhang et al. (Zhang et al., 2019), which explored the co-flocculating microalgae's efficiency in removing nitrogen and other contaminants from wastewater, provide valuable insights into potential bioremediation strategies, which explored the co-flocculating microalgae's efficiency in removing nitrogen and other contaminants from wastewater, provide valuable insights into potential bioremediation strategies. In conclusion, our study underscores the significant contamination levels of REEs and toxic metals in microalgal food products, highlighting the need for stringent safety monitoring and regulatory frameworks. These findings, supported by comparisons with previous research, provide a comprehensive understanding of the contamination risks and their implications for consumer health.

CRediT authorship contribution statement

Xiaopan Wu: Writing – original draft, Software, Formal analysis. **Xiaole Zhao:** Visualization, Methodology. **Jiayong Hu:** Validation, Methodology, Investigation. **Shiwen Li:** Project administration, Investigation, Data curation. **Xiao Guo:** Writing – original draft, Software, Formal analysis. **Qiao Wang:** Validation, Project administration. **Yan Liu:** Investigation, Conceptualization. **Zhiyong Gong:** Supervision, Resources. **Yongning Wu:** Supervision, Resources. **Min Fang:** Writing – review & editing, Visualization, Validation. **Xin Liu:** Writing – review & editing, Visualization, Supervision, Resources, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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