Original Article

Assessment of heavy metals and aflatoxins in cottonii and spinosum seaweeds from Tanzania

Sarah F. Maro[1,2], Lillian D. Kaale[1]*

[1] Department of Food Science and Technology, University of Dar es Salaam, PO Box 35134, Dar es Salaam, Tanzania

[2] Tanzania Bureau of Standards, PO Box 9524, Sam Nujoma Road, Dar es Salaam, Tanzania

Western Indian Ocean JOURNAL OF Marine Science

Open access

Citation:
Maro SF, Kaale LD (2025) Assessment of heavy metals and aflatoxins in cottonii and spinosum seaweeds from Tanzania. Western Indian Ocean Journal of Marine Science 24(1): 01-09 [doi: 10.4314/wiojms.v24i1.1]

Received:
August 18, 2024

Accepted:
December 11, 2024

Published:
March 13, 2025

*** Corresponding author:**
elykaale@gmail.com

Abstract

There has been little research on heavy metals and aflatoxins in Tanzanian seaweeds. Concentrations of lead (Pb), cadmium (Cd), total mercury (THg), and methyl mercury (MeHg) were measured in cottonii (*Kappaphycus alvarezii*) and spinosum (*Eucheuma denticilatum*) seaweeds from Tanzania. Seaweeds were collected in February 2024 from Tanga, Pwani, and North Unguja. Inductively coupled plasma optical emission spectroscopy (ICP-OES) was used to assess Cd and Pb, a direct mercury analyzer (DMA-80) was used to analyze THg and MeHg, and high-performance liquid chromatography was used to determine anti-aflatoxigenic activity. Heavy metal concentrations were highest for Cd, followed by Hg, Pb and MeHg, and varied significantly (p<0.05) between species and sampling locations. The concentrations of Cd, Pb, and MeHg were below safety thresholds set by the European Union and Codex Standard, while THg concentrations exceeded Codex limits but were within European Union limits, and can therefore safely be consumed. Aflatoxins were not detected in the seaweed samples. Further research on heavy metal concentrations and moisture stability in Tanzanian seaweeds is recommended, over a period of at least a year.

Keywords: Rhodophyta, cadmium, lead, mercury, moisture stability, aflatoxins

Introduction

Seaweeds, or marine macroalgae, are a broad category of photosynthetic organisms found in marine habitats. They are essential to marine ecosystems because they support primary productivity and offer habitat for a variety of marine species. Seaweeds are divided into three major categories according to the pigments they contain: Rhodophyta (red), Ochrophyta (brown), and Chlorophyta (green) algae (Selmi *et al*., 2021). Currently there are about 10,000 seaweed species worldwide (de Souza Celente *et al*., 2023; Thiviya *et al*., 2022). In Tanzania, there are around 428 species of Rhodophyta, Chlorophyta and Ochrophyta, but few are commercially cultivated (Msuya, 2020). These include the red seaweeds *Eucheuma denticulatum* (spinosum) and *Kappaphycus alvarezii* (cottonii) (Msuya *et al*., 2022).

Edible seaweeds can be eaten in a variety of forms, including fresh, fermented, dried, frozen, whole, powdered into flakes, and granules (Zhang *et al*., 2022). They can also form an ingredient in foods including bread, pasta, pastries, and drinks (Zhang *et al*., 2022). Seaweeds are rich in protein, polysaccharides, dietary fiber, minerals and vitamins (MacArtain *et al*., 2007). They are also rich in bioactive compounds such as antioxidants, vitamin C and E, polyphenols, sulphated polysaccharides, carotenoids, sterols, phlorotannins, catechins, flavonols, and phlorotannins (Fernández-Segovia *et al*., 2018; Lomartire *et al*., 2021).

Seaweeds are used in a wide various of culinary and non-food industries, including textiles, pharmaceuticals, nutraceuticals, food additives, animal feed, bio-packaging, biofuel, and biofertilizer/biostimulants (Pangestuti and

Kim, 2015). Seaweeds are also known as the "Medical Food of the 21st Century" because of their numerous uses, including in laxatives, medicinal capsules, cancer treatment, goiter treatment, bone-replacement therapy, and cardiovascular surgery (Reddy *et al*., 2023).

Due to the significant economic value of seaweeds and their high demand, the seaweed business is growing on a global scale. For instance, the production of seaweed tripled worldwide from 11.8 million tonnes in 2000 to 35.76 million tonnes in 2019, with Asian countries leading the way accounting for 97 % (FAO, 2021). Tanzania is among the top ten countries worldwide in seaweed production with estimates of 102,960 tonnes per year (FAO, 2021), which has provided some of the most marginalized people in the community jobs and income, especially women.

However, little research has been done on seaweed safety and quality, including aflatoxin and heavy metal concentrations, despite the wealth of production-related data. Since metals cannot be broken down, they remain in the environment and can cause health issues and environmental degradation if they are present at high concentrations (Foday *et al*., 2021). The majority of aquatic organisms have the capacity to accumulate heavy metals to levels higher than those found in their immediate environment (Mshana and Sekadende, 2014). Toxic metals are found in marine ecosystems because of contaminated household and industrial waste discharge from coastal towns and cities. Occasionally, these wastes are dumped into the marine environment untreated. When heavy metals enter the human body in high concentrations via food, inhalation, or other pathways, they may disrupt physiological and metabolic processes (Jaishankar *et al*., 2014).

Seaweeds are perishable, and their high moisture content and nutritious components are the main causes of microbial growth damage and short shelf life (López-Hortas *et al*., 2022). Mould and fungi, like *Aspergillus flavus* and *Aspergillus parasiticus*, thrive in high moisture environments, contaminating food during and after harvest. These fungi produce toxins, such as aflatoxins, that are carcinogenic to animals and humans (Kumar *et al*., 2021; Kaale *et al*., 2021). Studies on aflatoxin concentrations in seaweeds are scarce. There are advantageous components in algal extracts that have a variety of bioactivities. One of these is the capacity to inhibit the growth of microorganisms that cause food spoiling, such as *Aspergillus* sp., *Fusarium* sp., or *Penicillium* sp., thereby functioning as an antifungal agent (Fraga-Corral *et al*., 2023). Moreover, antioxidant and chelating properties of algal extracts can lessen the toxicity of mycotoxins. Mycotoxins can be made less toxic by antioxidants, but because of their high protein or polysaccharide content, algal bio-adsorption may be able to bind the toxins, halt their degradation, and encourage their systemic release (Fraga-Corral *et al*., 2023).

The objective of this research was to measure the concentrations of heavy metals (cadmium (Cd), lead (Pb), total mercury (THg), and methyl mercury (MeHg)) in the red seaweeds *Eucheuma denticulatum* and *Kappaphycus alvarezii* that are grown on Tanzania's mainland and islands. Since excess moisture promotes microbial growth in food, which compromises product integrity and the health of consumers, the levels of aflatoxins at different moisture contents in the seaweed was also assessed.

Materials and methods

Study area and sample collection

Seaweed samples were collected in February 2024 from seven villages across three regions in Tanzania, namely Mlingotini in Pwani, Kigunda, Kiwengwa, Mchangani in North Unguja, and Moa, Mwambani and Ushongo in Tanga (Fig. 1). Seaweeds are cultivated along the coast, and more than five farmers can work on a farm, each of them owning a portion. Seaweed samples were collected from three distinct locations within the same farm: at one end, in the middle and at the other end of the farm. The samples from each location were combined to form a sample for each farm. Samples were placed in opaque polypropylene bags with saltwater to maintain wetness and freshness, labeled, and transported in a cooler box to the University of Dar es Salaam's Food Science and Technology laboratory for analysis. The seaweeds were inspected by hand to remove debris. Seawater was used to rinse away any remaining sand and debris.

Chemicals and reagents

The reagents used were of laboratory analytical quality. The mineral acids HNO_3 and HCl were from Loba Chemie PVT LTD, distilled water for standard and sample dilution were from the Tanzania Bureau of Standards, multielement standards from LGC, toluene from Fisher Scientific UK, l-cysteine from the British Drug Houses LTD, methanol from Sigma-Aldrich, HPLC grade methanol from Chem-Lab NV, and acetonitrile from Merck Kgae. Aflatoxin standards were from Sigma-Aldrich.

Sample preparation

Sample preparation followed the procedures outlined by Selmi *et al.* (2021), with slight modification. Following a distilled water wash, 1.2–1.5 kg of wet seaweed was dried for 48 h at 40 °C in a multifunctional dryer.

Sample preparation and microwave assisted digestion

Microwave-assisted digestion of seaweeds followed the procedures outlined by Thodhal Yoganandham *et al.* (2019). All apparatus was soaked in 10 % HNO_3

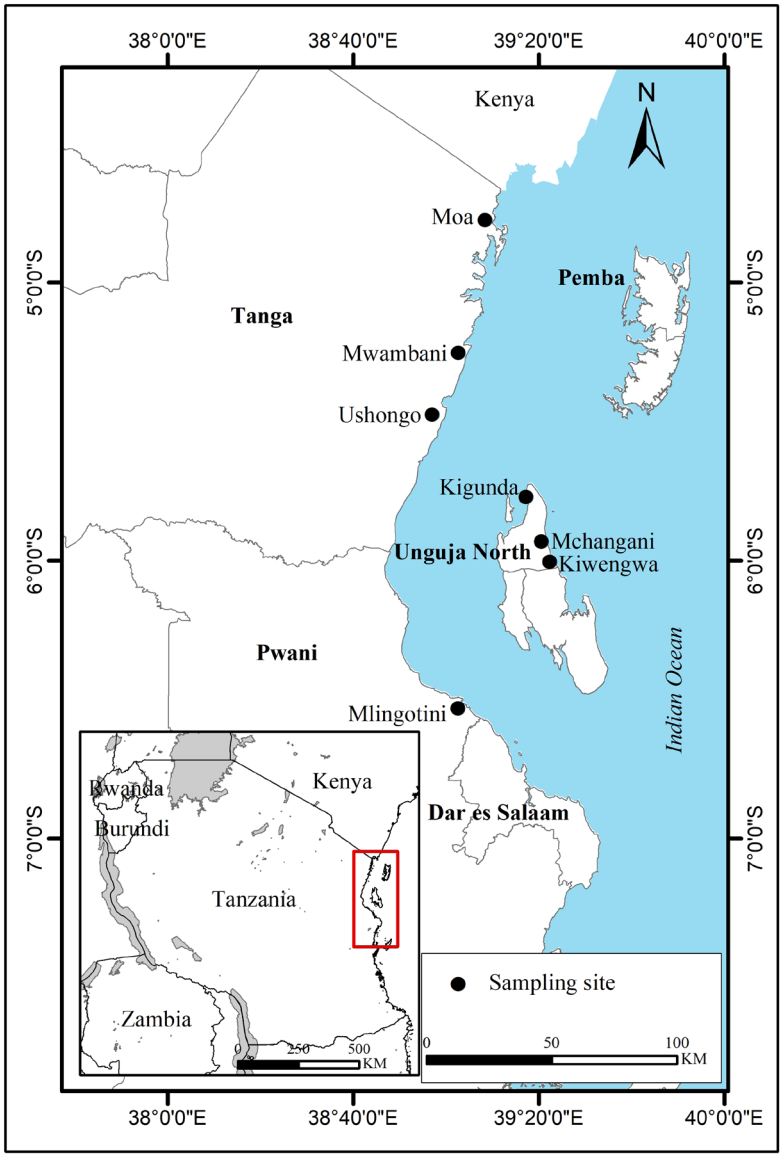

Figure 1. Maps showing the positions of seaweed sampling sites in Tanzania.

A Silver Crest powder grinder was used to grind the sample into a fine powder, at a rotation speed of 28 000 rpm for 2.5 min. The powder was placed into an opaque plastic bag pending analysis.

Determination of Cd and Pb

Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) was used for the analysis of Cd and Pb, following the procedures outlined by Selmi *et al.* (2021).

for 24 h and then rinsed with deionized water. About 0.5 g of dried and powdered seaweed sample was weighed and placed into a Teflon vessel, to which 5 ml HNO_3 was added. The mixture was left for 20 min to digest at ambient temperature. The Teflon vessels were sealed and the samples digested further using an Ethos Easy Advanced Microwave Digestion system. The samples were heated to 200 ℃ over a period of about 20 min, and then maintained at this temperature for 15 min. The samples were cooled at ambient

temperature and filtered through 0.45 µm membrane filters. The filtrate was transferred to a 50 ml conical flask and diluted to the mark with deionized water. Analysis of the samples was carried out using an Agilent 5900 ICP-OES set to operate at wavelengths (nm) of 214.439 and 220.353 for the measurement of Cd and Pb, respectively.

Quality control and method validation

The ICP-OES was calibrated by running a blank solution and five calibration standards (0.05 ppm, 0.10 ppm, 0.35 ppm, 0.50 ppm and 0.75 ppm) prepared from a 10 ppm multi-element standard solution, and a five-point standard calibration curve was prepared for Cd and Pb. Initial calibration (0.1 ppm QC) and continuous calibration (0.35 ppm QC) were performed to confirm the ICP-OES was functioning properly and generating accurate findings. Cd and Pb recovery was determined by spiking seaweed samples with 0.1 ppm of a multi-element standard. The samples were treated in the same manner as other samples. Recovery was determined as:

$$Recovery\ (\%) = \left(\frac{Concentration\ measured\ in\ spiked\ sample}{Concentration\ expected\ in\ spiked\ sample}\right) x100$$

A 0.1 ppm multi-element standard was analyzed seven times to obtain the standard deviation, which was then used to compute the limit of detection (LOD) and limit of quantification (LOD and LOQ), as:

LOD = (3 × standard deviation)/(slope of calibration curve)
LOQ = (10 × standard deviation)/(slope of calibration curve)

Determination of THg and MeHg

Total Hg and MeHg were analyzed using a DMA-80 direct mercury analyzer (Milestone Srl, Italy) as previously reported by Maggi *et al.* (2009), Diabagaté *et al.* (2021), and Nava *et al.* (2023). The determination of THg required no sample pretreatment. About 0.1 g of dried and powdered seaweed was weighed into cuvettes and placed in a sample holder. The cuvettes were automatically introduced to the DMA-80 and combusted at 200 – 650 ℃ for 4 – 5 min. A wavelength of 253.54 nm was used to detect THg.

MeHg extraction

About 2 g of dried and powdered seaweed sample was weighed and mixed with 10 ml of 6 M HCL. The samples were placed in a vertical shaker for 5 min and centrifuged (Hunan Xiangyi Laboratory Instrument Development CO., LTD) at 2400 rpm for 10 min. The liquid phase was discarded. The remaining semisolid phase was mixed with 20 mL of toluene, shaken using a vertical shaker for 20 min, and centrifuged at 2400 rpm for 20 min. The liquid phase was collected in falcon tubes. The remaining semisolid phase was again mixed with 15 ml of toluene, shaken, and centrifuged to ensure maximum extraction of MeHg. The second supernatant was collected and combined with the first, and then mixed with 6 mL of 1 % (v/w) L-cysteine aqueous solution to strip MeHg from the toluene. The mixture was centrifuged (2400 rpm for 20 min) and the upper layer containing toluene was separated and again mixed with 6 ml of 1 % (v/w) L-cysteine aqueous solution and centrifuged. The top layer containing toluene was discarded and the remaining L-cysteine extract was analyzed using the DMA-80.

Quality control and method validation

Calibration of the DMA-80 was done by preparing concentrations of 0.525, 1.03, 2.08, and 3.12 ppm from a 1000 ppm Hg standard. A four-point calibration curve was prepared to obtain a slope for calculating the LOD and LOQ. The reference material IAEA 461 (Marine Biota Sample) with a known concentration of 0.39 mg/kg of THg and 0.0623 mg/kg of MeHg was used to determine recovery, as:

$$Percentage\ recovery\ (\%) = \left(\frac{Concentration\ measured\ in\ reference\ material}{Expected\ concentration\ in\ reference\ material}\right) x100$$

The reference material was analyzed five times for THg and MeHg to obtain standard deviations to calculate the LOD and LOQ, as:

LOD = (3 × standard deviation)/(slope of calibration curve)
LOQ = (10 × standard deviation)/(slope of calibration curve)

Determination of moisture content

The moisture content of the seaweed samples was determined by oven drying as outlined by Jose and Xavier (2020). A clean crucible was placed in an oven (5E-MHG6090 Model, China) for 30 min at 105C, cooled in a desiccator for 30 min, and weighed on an analytical balance (JF Series, JF2204, max. 220 g) to obtain the mass of the empty, dried crucible, W_1. About 10 g of sample was added to crucibles in triplicate per sample, recorded as W_2, and then placed in oven at 105C for 4 h. The samples were cooled in a desiccator for 30 min and placed in oven for further drying for 30 min until constant weight, W_3.

The moisture content was calculated as:

$$\text{Moisture content (\%)} = \frac{W_2 - W_3}{W_2 - W_1} x100$$

Moisture content adjustment

The moisture contents were adjusted to the desired content as per Zasypkin and Lee (1998).

$$\textit{Water to Add (kg)} = \textit{Weight of Material (kg)}\ x \left(\frac{\textit{Target Moisture \%-Initial Moisture (\%)}}{\textit{100-Target Moisture (\%)}}\right)$$

Explanation of the Terms

Weight of Material (kg): The weight of the material that needs moisture adjustment.

Initial Moisture (%): The current moisture content of the material (as a percentage).

Target Moisture (%): The desired moisture content of the material (as a percentage).

Aflatoxin analysis

Analysis of aflatoxins was done using High-Performance Liquid Chromatography (HPLC), as outlined by Mohamed *et al*. (2017) and Kyei-Baffour *et al*. (2021), with minor modifications. In this instance, seaweed samples maintained at room temperature for 90 days and assessed for moisture stability at three different moisture contents (12 %, 20 %, and 35 %) were examined for aflatoxins.

Sample preparation

To obtain moisture stable samples, 800 g of seaweed was cleaned, dried in a multipurpose drier at 40℃ for 48 h, and ground to fine powder using a Silver Crest powder grinder at 28 000 rpm for 150 s. The sample was divided into three portions, with sample moisture content of 12 %, 20 % and 35 %, and packed in opaque plastic bags and stored at ambient temperature for 90 days.

Extraction of aflatoxins and sample clean up

About 12.5 g of ground seaweed was mixed with 25 ml of 80 % methanol in 250 ml conical flasks. The mixture was shaken (Shaking Incubator, LFZ-TSI-200D) at 250 rpm for 30 min. The mixture was filtered through Whatman #1 filter paper into a 100 ml conical flask. Four ml of the filtrate was drawn and mixed with 8 ml distilled water and shaken for 30 s. The mixture was subjected to solid phase extraction by passing it through an Immuno-affinity cleanup column (Aflastar R 500), followed by washing the column with 10 ml of distilled water. Elution with 0.5 ml HPLC grade methanol was done twice, collecting the eluents in amber glass vials.

Quantification of aflatoxins using HPLC

Aflatoxin quantification in standards and samples was conducted using HPLC with Post-Column Photochemical Derivatization. A Waters HPLC (Singapore) with an auto-sampler (E15SM7573A), pump, column oven (E15SMH 970G, ISM GRP1 CLASS B), photochemical derivatizer (PhCR Photochemical Reactor Box, Code No. 6000001222, Singapore), and fluorescence detector (C15475 467G) was used. Analysis was done using a mobile phase ratio of 65:30:5 distilled water, methanol (Chem-Lab NV), and acetonitrile (Merck Kgae). A column (Waters Spherisorb S5 ODS1 5 µm, 4.6 mm × 200 mm) was used to separate AFB1, AFB2, AFG1 and AFG2 at a temperature of 30 °C, flow rate of 0.8 ml/min, and injection volume of 10 µL. Detection was done using a fluorescence detector at an emission wavelength of 465 and excitation wavelength of 355 nm.

Quality control and method validation

The linearity of the method was evaluated by the establishment of a linear relationship between HPLC peak area and different concentrations of aflatoxin B1, B2, G1, and G2 from a mixed standard solution (Sigma-Aldrich). To achieve this, concentrations of 4, 6, 8 and 10 ng/mL for aflatoxins B1 and G1, and 1.2, 1.8, 2.5 and 3.0 ng/mL for aflatoxins B2 and G2, were used.

Aflatoxin recovery was determined by spiking seaweed samples with 10 ng/g for Aflatoxin B1 and G1 and 3 ng/g for B2 and G2. Recovery was calculated as the ratio (%) of the observed concentration to the expected concentration. The LOD and LOQ were determined by multiplying the ratio of the standard deviation of the lowest concentration and the slope of the calibration curve by three and ten, respectively (NATA-National Association of Testing Authorities 2012).

Statistical analysis

Heavy metal results are expressed as mean ± standard deviation (SD) mg/kg based on three replicate measurements. Analysis of heavy metal and moisture content results was carried out in R programing language version 4.3.2 (R core 2023), as discussed by Nepper-Davidsen *et al*. (2023). Two-way analysis of variance (ANOVA) was used to identify if there were significant differences in heavy metal concentrations between samples from different geographical locations. To test if there is significant difference in variation, a 5 % level of significance was used. A Tukey's

Table 1. Analytical method validation summary for heavy metals.

Parameter	Unit	Cd	Pb	Hg	MeHg
Linearity of standard	-	0.99	0.99	0.99	0.99
Accuracy	%	99.16 ± 1.70	97.94 ± 1.07	99.83±0.20	99.26±0.19
Precision	%	0.10 ± 0.00	0.10 ± 0.00	0.39±0.00	0.06±0.00
Limit of detection	mg/kg	0.001	0.018	0.02	0.002
Limit of quantification	mg/kg	0.002	0.06	0.06	0.009
Recoveries	%	97.21	97.86	99.72	97.74

Honestly Significant Difference (HSD) test was used to identify mean values that differed significantly.

Results and Discussion

Quality control

The analytical methods were found to be accurate and reliable, as evidenced by the different quality control data for heavy metals (Table 1) and aflatoxins (Table 2).

For heavy metals and aflatoxins, the method linearity was found to have a coefficient of determination ≥ 0.99. Heavy metal recoveries were between 97-99 %, whereas aflatoxin recoveries were between 93-9 9%.

Heavy metal concentration in seaweeds

The mean concentrations of Cd, Pb, THg and MeHg in *Eucheuma denticulatum* (spinosum) and *Kappaphycus alvarezii* (cottonii) are provided in Table 3. The order of heavy metal concentrations was Cd > THg > Pb > MeHg. Comparing the Pb and Cd concentrations in cottonii and spinosum from this study to concentrations found in *Amphiroa* sp. and *Gracilaria* sp. from 13 Tanzanian locales by Ferletta et al. (1996) shows the concentrations in cottonii and spinosum were significantly lower. The results of the present study show that Pb concentrations in cottonii and spinosum are lower than concentrations measured in seaweeds at Unguja and Pemba by Suleiman *et al*. (2021), while Cd and THg concentrations in both seaweeds were higher than concentrations reported in *G. griffithsiae* and *A. taxiformis* by Selmi *et al*. (2021). Furthermore, the concentrations of Cd found in both seaweeds in the current study are significantly higher than Cd concentrations reported in *Kappaphycus alvarezii* by Kumar *et al*. (2022). The present study revealed that Pb and Cd concentrations in spinosum and cottonii were comparatively lower than concentrations reported by Riosmena-Rodríguez *et al*. (2010) in *Gracilaria textorii, Chondria nidifica, Gracilaria vermiculophylla, Gracilariopsis andersonii, Hypnea johnstonii, Laurencia pacifica* and *Sarcodiotheca gaudichaudii*. The present study also revealed that the concentration of Pb was lower than in the *Codium amplivesiculatum* and *Codium cuneatum* (Riosmena-Rodríguez *et al*., 2010), but the Cd concentration was higher than in these seaweeds. The Pb concentrations in cottonii reported by Asni and Najamuddin (2020) and Ajik and Tahiluddin (2024) are higher than the concentrations found in the current study, while the Cd concentrations found in this study are higher. The concentration of MeHg in seaweeds in the current study is higher than in Wakame, Sea spaghetti and Hijiki seaweeds (Jinadasa *et al*., 2021).

The findings of the present study show that there is a significant difference (p<0.05) in the heavy metal content between spinosum and cottonii at each geographical location. The level of heavy metals in seaweed can be affected by environmental factors such as salinity, pH, and light intensity. For instance, *S. boveanum* from Bandar-e-Lengeh and Busher had

Table 2. Analytical method validation summary for aflatoxins.

Parameter	Unit	B1	B2	G1	G2
Linearity of standard	-	0.99	0.99	0.99	0.99
Accuracy	%	99.11± 1.63	98.59 ± 1.06	98.01 ± 1.13	98.51 ± 1.05
Precision	%	3.89± 0.16	2.36 ± 0.01	3.84 ± 0.09	2.36 ± 0.03
Limit of detection	ng/mL	0.08	0.01	0.04	0.01
Limit of quantification	ng/mL	0.27	0.43	0.15	0.42
Percentage recoveries	%	98.93	99.6	98.55	93.33

Table 3. Concentration of heavy metals (mg/kg dry weight) in seaweed samples.

Region	Village	Cadmium		Lead		Mercury		Methyl mercury	
		Cottonii	Spinosum	Cottonii	Spinosum	Cottonii	Spinosum	Cottonii	Spinosum
Pwani	Mlingotini	0.42 ± 0.03^{cd}	0.38 ± 0.02^{de}	0.24 ± 0.05^{fgh}	0.02 ± 0.00^{j}	0.24 ± 0.01^{fg}	0.17 ± 0.01^{hgi}	0.11 ± 0.00^{ghi}	0.10 ± 0.00^{ij}
Tanga	Mwambani	0.51 ± 0.08^{cd}	0.64 ± 0.18^{b}	0.04 ± 0.00^{ij}	0.02 ± 0.00^{j}	0.35 ± 0.01^{ef}	0.35 ± 0.05^{ef}	0.07 ± 0.00^{ij}	0.17 ± 0.06^{ghi}
Tanga	Ushongo	0.51 ± 0.02^{bc}	0.45 ± 0.06^{cde}	0.00 ± 0.00^{j}	0.00 ± 0.00^{j}	0.38 ± 0.01^{de}	0.36 ± 0.02^{ef}	0.09 ± 0.00^{ij}	0.12 ± 0.01^{ghij}
Tanga	Moa	0.52 ± 0.03^{c}	NC	0.09 ± 0.02^{c}	NC	0.11 ± 0.00^{c}	NC	0.07 ± 0.00^{c}	NC
Unguja	Kigunda	0.50 ± 0.06^{cd}	0.34 ± 0.01^{ef}	0.00 ± 0.00^{j}	0.00 ± 0.00^{j}	0.36 ± 0.02^{ef}	0.43 ± 0.03^{cde}	0.12 ± 0.00^{ghi}	0.12 ± 0.01^{ghij}
Unguja	Mchangani	0.81 ± 0.06^{a}	0.84 ± 0.03^{a}	0.00 ± 0.00^{j}	0.00 ± 0.00^{j}	0.41 ± 0.03^{cde}	0.11 ± 0.03^{hij}	0.07 ± 0.00^{ij}	0.07 ± 0.00^{ij}
Unguja	Kiwengwa	NC	0.79 ± 0.01^{f}	NC	0.00 ± 0.00^{j}	NC	0.30 ± 0.02^{ghi}	NC	0.08 ± 0.03^{hi}

NC: Not Cultivated

Pb concentrations of 16.90 ppm and 18.39 ppm, respectively (Daryaii *et al*., 2020). These geographical locations had different salinities and pH levels (Daryaii *et al*., 2020). Daryaii *et al*. (2020) speculated that the difference may be related to the electronegativity values of the metals.

The heavy metal concentrations in dried seaweeds must adhere to Codex 193, under the Tanzania Standard of Dried Seaweed Specification (TZS 2750:2022). The levels of Cd, Pb, and MeHg were below the limits specified by Codex 193, and the European Union (2023). The THg concentrations measured in this study are higher than those indicated in Codex 193, but lower than those indicated by the European Union (2023). According to the European Union (2023), the seaweeds are still safe to be consumed.

Aflatoxins in seaweeds

Samples evaluated for moisture stability at three moisture contents (12 %, 20 %, and 35 %) were examined for Aflatoxin B1, B2, G1, and G2. No aflatoxins were found in the samples. It is possible the seaweeds naturally contain antifungal properties or chemicals that inhibit the growth of *Aspergillus* sp., preventing aflatoxin production.

Conclusions

The heavy metals Cd, Pb, THg, and MeHg were assessed in seaweeds collected from North Unguja, Tanga, and Pwani. Heavy metal concentrations varied significantly between cottonii and spinosum seaweed species, and between village locations. The concentrations of Cd, Pb, and MeHg were below limits set by the European Union and Codex Standard. Conversely, the THg concentration was higher than the Codex limit but lower than the European Union limit. According to the European Union (2023), the seaweeds are still safe to be consumed. No aflatoxins were found in the seaweed samples. Further research on determining heavy metal concentrations in other Tanzanian seaweeds and locations and seaweed moisture stability over a period of at least a year is recommended.

Acknowledgements

The authors are grateful to the Tanzania Bureau of Standards for its financial support.

References

Asni A, Najamuddin (2020) Pb heavy metal distribution patterns in seaweed (*Kappaphycus alvarezii*) cultivation locations by season in Bantaeng Waters, South Sulawesi. The 3rd International Symposium Marine and Fisheries (ISMF) [10.1088/1755-1315/564/1/012037]

Ajik KO, Tahiluddin AB (2023) Proximate composition, trace and macro element, and heavy metal content of edible seaweed *Solieria robusta* in Tawi-Tawi, Philippines. Food Bulletin 2: 23-28 [10.61326/foodb.v2i2.104]

Diabagaté D, Sébastien OK, Marcellin KY, Achille G, Vianney MH (2021) Determination of total merccury and methylmercury in mangrove oysters (*Crassostrea gasar*) from a Direct Mercury Analyzer (Dma-80). Journal of Earth Science and Climatic Change 12: 1-5

Daryaii LB, Samsampour D, Bagheri A, Sohrabipour J (2020) High content of heavy metals in seaweed species: A case study in the Persian Gulf and the Gulf of Oman in the southern coast of Iran. Journal of Phycological Research 4: 544-560

De Souza Celente G., Sui Y, Acharya P (2023) Seaweed as an alternative protein source: Prospective protein

extraction technologies. Innovative Food Science and Emerging Technologies 86: 103374 [10.1016/j.ifset.2023.103374]

European Union (EU) (2023) Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006. Official Journal of the European Union L119: 103-157

FAO (2021) Global status of seaweed production, trade and utilization. World Aquaculture Performance Indicators (WAPI) - Seaweed Innovation Forum Belize. pp 1-17

Ferletta M, Bråmer P, Semesi A, Björk M (1996). Heavy metal contents in macroalgae in the Zanzibar channel, an initial study. In: Björk M, Semesi AK, Pedersén M, Bergman B (eds). Current trends in marine botanical research in the Eastern African Region. Proceedings of the Symposium on the Biology of Microalgae, Macroalgae, and Sea rasses in the Western Indian Ocean, held 3-10 December 1995 at the University of Mauritius. Sida Marine Science Program, Stockholm. 407 pp

Fernández-Segovia I, Lerma-García MJ, Fuentes A, Barat JM (2018) Characterization of Spanish powdered seaweeds: Composition, antioxidant capacity and technological properties. Food Research International 111: 212-219 [10.1016/j.foodres.2018.05.037]

Foday EH, Bo B, Xu X (2021) Removal of toxic heavy metals from contaminated aqueous solutions using seaweeds: A review. Sustainability 13: 1-17 [doi: 10.3390/su132112311]

Fraga-Corral M, Otero P, Echave J, Pereira AG, Garcia-Oliveira P, Perez-Vazquez A, Donn P, Cassani L, Cao H, Simal-Gandara J, Prieto MA (2023) Algal extracts as preventive mechanism for mycotoxins development. Biology and Life Sciences Forum 24: 1-8 [10.3390/iect2023-14814]

Jaishankar M, Tseten T, Anbalagan N, Mathew BB, Beeregowda KN (2014) Toxicity, mechanism and health effects of some heavy metals. Interdisciplinary Toxicology 7: 60-72 [10.2478/intox-2014-0009]

Jinadasa KK, Herbello-Hermelo P, Peña-Vázquez E, Bermejo-Barrera P, Moreda-Piñeiro A (2021) Mercury speciation in edible seaweed by liquid chromatography - Inductively coupled plasma mass spectrometry after ionic imprinted polymer-solid phase extraction. Elsevier. pp 1-8 [10.1016/j.talanta.2020.121841]

Jose J, Xavier J (2020) Study of mineral and nutritional composition of some seaweeds found along the coast of Gulf of Mannar, India. Researchgate 7: 631-637 [10.14719/PST.2020.7.4.912]

Kaale LD, Kimanya ME, Macha IJ, Mlalila N (2021) Aflatoxin contamination and recommendations to improve its control: A review. World Mycotoxin Journal 14: 27-40 [10.3920/WMJ2020.2599]

Kumar A, Pathak H, Bhadauria S, Sudan J (2021) Aflatoxin contamination in food crops: causes, detection, and management: a review. Food Production, Processing and Nutrition 3: 1-9 [10.1186/s43014-021-00064-y]

Kyei-Baffour VO, Kongor JE, Anyebuno G, Budu AS, Firibu SK, Afoakwa EO (2021) A validated HPLC – FLD method for the determination of mycotoxin levels in sun dried fermented cocoa beans: Effect of cola nut extract and powder. Elsevier. pp 1-6 [10.1016/j.lwt.2021.111790]

Lomartire S, Marques JC, Gonçalves AMM (2021) An overview to the health benefits of seaweeds consumption. Marine Drugs 19 (6) [10.3390/md19060341]

López-Hortas L, Caleja C, Pinela J, Petrović J, Soković M, Ferreira ICFR, Torres MD, Domínguez H, Pereira E, Barros L (2022) Comparative evaluation of physicochemical profile and bioactive properties of red edible seaweed *Chondrus crispus* subjected to different drying methods. Elservier. pp 1-12 [10.1016/j.foodchem.2022.132450]

MacArtain P, Gill CIR., Brooks M, Campbell R., Rowland IR (2007) Nutritional value of edible seaweeds. Nutrition Reviews 65: 535-543 [10.1301/nr.2007.dec.535-543]

Maggi C, Berducci MT, Bianchi J, Giani M, Campanella L (2009) Methylmercury determination in marine sediment and organisms by Direct Mercury Analyser. Elservier 641: 32–36 [10.1016/j.aca.2009.03.033].

Mohamed HMA, Emeish WFA, Braeuning A, Hammad A (2017) Detection of -producing fungi isolated from nile tilapia and fish feed. EXCLI Journal 16:1 308-1318.[10.17179/excli2017-960]

Mshana JG, Sekadende B (2014) Assessment of heavy metal pollution in *Octopus cyanea* in the coastal waters of Tanzania. Journal of Health and Pollution 4: 10-17 [10.5696/2156-9614-4-6.10]

Msuya FE (2020) Seaweed resources of Tanzania. status, potential species, challenges and development potentials. Botanica Marina 63: 371-380 [10.1515/bot-2019-0056]

Msuya FE, Bolton J, Pascal F, Narrain K, Nyonje B, Cottier-Cook EJ (2022) Seaweed farming in Africa: current status and future potential. Journal of Applied Phycology 34: 985-1005 [10.1007/s10811-021-02676-w]

Nava V, Di Bella G, Fazio F, Potortì AG, Lo Turco V, Licata P (2023) Hg Content in EU and non-EU processed meat and fish foods. Researchgate 13: 1-11 [10.3390/app13020793]

Nepper-Davidsen J, Glasson CRK, Lawton RJ, Magnusson M (2023) High spatial and temporal variation in biomass composition of the novel aquaculture target *Ecklonia radiata*. Journal of Applied Phycology 35 (4): 1755-1768 [10.1007/s10811-023-02969-2]

Pangestuti R, Kim S (2015) Seaweed proteins, peptides, and amino acids. In: Seaweed Sustainability. Elsevier Inc. pp 125-140 [10.1016/B978-0-12-418697-2/00006-4]

Selmi A, Khiari R, Snoussi A, Bouzouita N (2021) Analysis of minerals and heavy metals using ICP-OES and FTIR techniques in two red seaweeds (*Gymnogongrus griffithsiae* and *Asparagopsis taxiformis*) from Tunisia. Biological Trace Element Research 199: 2342-2350 [10.1007/s12011-020-02335-0]

Reddy GG, Rao KVLS, Adelina M, Jaya Harsha MAJ, Venkateswarlu V (2023) Nutritional content of marine macroalgae (Seaweeds) from Kanyakumari coastal district, Tamil Nadu, India. International Journal of Fisheries and Aquatic Studies 11: 123-126 [10.22271/fish.2023.v11.i1b.2774]

Riosmena-Rodríguez R, Talavera-Sáenz A, Acosta-Vargas B, Gardner SC (2010) Heavy metals dynamics in seaweeds and seagrasses in Bahía Magdalena, B.C.S., México. Journal of Applied Phycology 22: 283-291 [10.1007/s10811-009-9457-2]

Suleiman AS, Salum KS, Miza AK, Atumaini AM, Fatma OK (2021) Assessment of heavy metal contamination in red seaweeds from the coastal areas of Zanzibar by using the EDXRF technique. Chemical Science International Journal 30: 1-9 [10.9734/CSJI/2021/v30i1030255]

Thiviya P, Gamage A, Gama-Arachchige NS, Merah O, Madhujith T (2022) Seaweeds as a source of functional proteins. Phycology 2: 216-243 [10.3390/phycology2020012]

Thodhal Yoganandham S, Raguraman V, Muniswamy GK, Sathyamoorthy G, Rajan Renuka R, Chidambaram J, Rajendran T, Chandrasekaran K, Santha Ravindranath RR (2019) Mineral and trace metal concentrations in seaweeds by microwave-assisted digestion method followed by Quadrupole Inductively Coupled Plasma Mass Spectrometry. Biological Trace Element Research 187: 579-585 [10.1007/s12011-018-1397-8]

Zhang L, Liao W, Huang Y, Wen Y, Chu Y, Zhao C (2022) Global seaweed farming and processing in the past 20 years. Food Production, Processing and Nutrition 4: 23 [10.1186/s43014-022-00103-2]

Zasypkin DV, Lee T (1998) Extrusion of soybean and wheat flour as affected by moisture content. Journal of Food Science 63 (6): 1058-1061