

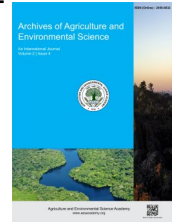


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ORIGINAL RESEARCH ARTICLE



Variation in heavy metals concentrations among seaweed species from Mkwiro seaweed farm, Kwale County, Kenya

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ABSTRACT

This study, conducted at the Mkwiro seaweed farm in Kwale County, Kenya, aimed to assess the concentrations of heavy metals (Cd and Pb) and essential elements (Na, Fe, Ca, and K) in selected edible seaweed species. The study used a cross-sectional, descriptive research design and probability sampling method to collect data. Seaweed samples of three selected species, Cottonii (*Kappaphycus alvarezii*), Sea lettuce (*Ulva lactuca*), and Bubble-green seaweed (*Boergesenia forbesii*) were collected in quadrants and subjected to chemical analysis. Statistical analyses were conducted using R Studio version 4.3.2, with a significance level set at $\alpha=0.05$. The Kruskal-Wallis test revealed significant differences in lead concentrations among seaweed types ($\chi^2(2) = 7.01, p = 0.03$). Cadmium concentrations did not show significant differences ($\chi^2(2) = 3.88, p = 0.14$). For calcium concentrations, ANOVA indicated no significant effect of seaweed type ($F(2,33) = 0.6381, p = 0.5347$). Iron concentrations differed significantly among seaweed types ($\chi^2(2) = 23.35, p = 0.00000849$), with *B. forbesii* having the highest median concentration. Potassium and sodium concentrations did not significantly vary among seaweed types ($p > 0.05$). The study uncovers elevated cadmium levels in seaweed, indicating potential contamination risks. However, concentrations of essential elements were lower. To address these findings, it is recommended to initiate regular monitoring and pollution control measures in seaweed farms. Additionally, diversifying cultivation with low-metal species can enhance product safety and quality.

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INTRODUCTION

Seaweeds, also referred to as macroalgae, are pivotal photosynthetic marine organisms sourced either from natural stocks or through cultivation methods (Lezcano *et al.*, 2018). By 2019, an overwhelming 97% of global mariculture production was attributed to cultivated seaweeds, with the remaining fraction stemming from natural stocks (Zhang *et al.*, 2022). Notably, Asian countries, primarily led by China and Indonesia, collectively contribute around 98% of the world's total seaweed production (Rogel-Castillo *et al.*, 2023). In recent years, European nations have displayed a growing interest in seaweed cultivation

and utilization (Campbell *et al.*, 2019). However, unlike the predominant cultivation methods prevalent in Asian nations, most of Europe's macroalgae production comes from natural seaweed beds (Zhang *et al.*, 2022). In stark contrast, Africa lags behind in both the production and utilization of macroalgae (Msuya *et al.*, 2022), contributing to only 0.4% of the world's total production (Rogel-Castillo *et al.*, 2023). Nevertheless, in the warm waters of Tanzania, Madagascar, and South Africa, some commonly farmed seaweeds include *Kappaphycus* spp., *Eucheuma* spp., *Ulva* spp., and *Gracilaria* spp. Seaweeds hold immense economic importance, with approximately 221 macroalgae species serving various purposes (Fernandes,

2020). Among these, about 145 species are employed for food, while approximately 110 species contribute to phycocolloid production (Chennubhotla et al., 2013). At an industrial scale, macroalgae serve as crucial sources of phycocolloids—algins, agars, and carrageenans—integral to gelling, thickening, and stabilizing processes in the food, pharmaceuticals, and cosmetics industries (Mantri et al., 2019; Häder, 2021). Phycocolloids play diverse roles, softening baked goods, facilitating water-oil emulsions for separation, and are integral in ice cream sauce production in the food and beverage sector (Busetti et al., 2017; Kim, 2012). Moreover, in the paper industry, alginate, derived from phycocolloids, is used for surface sizing, providing a continuous film surface (Bixler and Porse, 2011). Edible seaweeds possess rich nutrient profiles encompassing magnesium (Mg^{+2}), sodium (Na^+), calcium (Ca^{+2}), potassium (K^+), chloride (Cl^-), proteins, and carbohydrates (Circuncisão et al., 2018). Their chemical and nutritional compositions are influenced by various factors, such as species diversity and environmental conditions, including habitat, light exposure, water temperature, and salinity (Wong and Cheung, 2000).

Seaweeds, integral components of aquatic ecosystems and economically significant, exhibit a propensity for high bioconcentrations of heavy metals such as mercury, cadmium, arsenic, and lead from seawater (Chen et al., 2018). These heavy metals, even in minimal concentrations, pose a severe threat upon entry into living organisms, with their impact intensifying significantly at higher concentrations (Ankit and Korstad, 2022). Lead, for instance, induces irreversible brain damage, affecting human kidneys, reproductive systems, and the nervous system (Fatima et al., 2019). Similarly, cadmium, recognized as carcinogenic, triggers liver damage, blood-related complications, renal issues, and bone degeneration (Obasi and Akudinobi, 2020). Regulatory bodies like the French Agency for Food, Environmental, and Occupational Health and Safety (ANSES) establish upper limits (UL) for heavy metals in edible seaweeds, setting lead at 5.0 mg/Kg and cadmium at 0.5 mg/Kg, based on dry weight (ANSES, 2020). Moreover, variations in heavy metal concentrations exist not only between different seaweed species but also within species, indicating site-specific variations (ANSES, 2020). The rise in heavy metal contamination in aquatic environments, in tandem with industrialization, raises significant environmental concerns owing to the high toxicity of these elements, even in trace amounts, and their persistent bio-accumulative nature (Filippini et al., 2021; Ankit and Korstad, 2022; Pasumpon et al., 2023). A multitude of sources contribute to heavy metal presence in marine settings, including rock weathering, industrial discharge, sewage discharge, offshore oil exploration, agricultural runoff, marine transportation, and recreational activities (El-Sorogy et al., 2018; Shah et al., 2021). Within aquatic ecosystems, heavy metals tend to accumulate in aquatic life and ascend the food chain, a process known as bioaccumulation, biomagnification, and bioconcentration (Ahmad et al., 2020). The uptake and concentration of heavy metals within seaweed species are markedly affected by distinct seaweed varieties and their geographic locales (Hashim and Chu, 2004; Baghazadeh et al.,

2020). These pivotal factors substantially contribute to the divergence observed in heavy metal content among various seaweed types, underscoring the critical significance of factoring in both the specific species and geographical location during assessments of heavy metal levels.

Despite the economic and ecological importance of seaweeds, particularly in the Kenyan coastal regions, there remains a dearth of information regarding the variations in heavy metal concentrations among cultivated species, notably those sourced from the Mkwiro Seaweed Farm. Gaining insight into the specific concentrations of heavy metals in these seaweeds holds significant potential to ensure their safe utilization and bridge this research gap. This study sets out to assess the levels of essential and non-essential metals present in seaweeds cultivated at the Mkwiro Seaweed Farm along the Kenyan coast. Through a comprehensive evaluation of heavy metal concentrations in these seaweeds, the research aims to deepen the understanding of contamination risks. This information will assist policymakers in devising strategies for the secure cultivation and utilization of seaweed, i.e., the selection of species with lower metal concentrations.

MATERIALS AND METHODS

Area of study

This study was conducted at Mkwiro seaweed farm [(Figure 1) 04° 39.513" S, 039° 23.790" E] in Kwale County, along the Kenyan south coast region. Mkwiro, with an approximate of about 1000 inhabitants (van Hoof and Steins, 2017), is a primarily subsistence fishing village located 2 kilometers off the Kenyan coast. Wasini Island in general supports an array of biodiversity which includes mangrove wetlands, coastal forests, estuaries, white sandy beaches and sand dunes, coral reefs, and seagrass beds that support a host of marine and coastal species. Kenyan coastline is characterized by Northeast Monsoon (NEM) and the Southeast Monsoon [(SEM) Nyamora et al., 2018 which affect the physical and chemical conditions of sea water (Church and Obura, 2004). The sites are sheltered from strong wave action and tidal currents by a fringing reef (Kimathi et al., 2018). Generally, reef communities here are dominated by Porites spp. assemblages in calm waters and Acropora spp. assemblages in high energy environments (McClanahan and Obura, 1984).

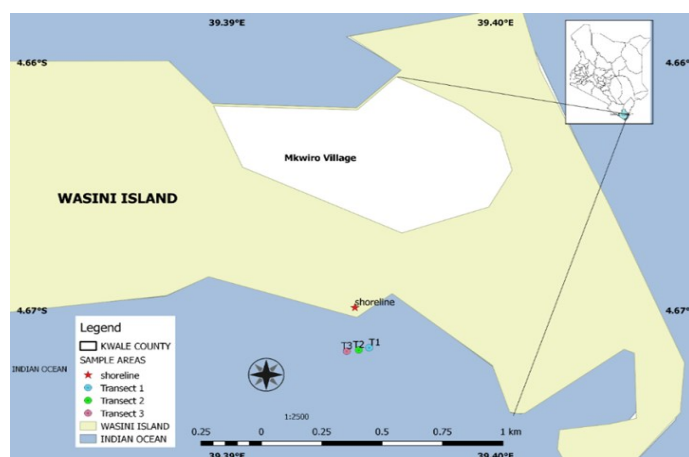


Figure 1. Map representation of the study area, Mkwiro seaweed farm, in Kwale County, Kenya.

Additionally, Mkwiro seaweed farm plays a vital role in the local economy, providing livelihoods to surrounding communities' members. The farm is managed by self-help groups consisting of 74 women and 6 men with the help of important stakeholders including local NGOs, Kwale County government and the government of Kenya through the Kenya Marine and Fisheries Policy and Legislation Act, 2016, Integrated Coastal Zone Management (ICZM) policy, 2013 and Maritime Zones Act, 2012. Fisheries Research Institute. The farm adheres to several policy frameworks including their seaweed cultivation. This location was chosen for the study due to its significance in the regional seaweed industry and its potential impact on the environment and local communities.

Study design

This study employed a cross-sectional, descriptive research design. This design provides a snapshot of the conditions being studied at a specific point in time (Spector, 2019). To ensure a representative sample, we used probability sampling, following the recommendations of Melsasail et al. (2018) This approach involves the random selection of elements from a defined population, minimizing bias and allowing for generalization of findings (Trochim and Donnelly, 2001). These design choices helped in ensuring the validity and reliability of our study's results. The use of random sampling also helps minimize potential selection bias, as it guarantees that each seaweed species has an equal chance of inclusion.

Data collection

Seaweed samples of three selected species, namely Cottonii (*Kappaphycus alvarezii* (Doty) Doty ex P.C. Silva), Sea lettuce (*Ulva lactuca*), and Bubble-green seaweed (*Boergesenia forbesii*), were collected in triplets per quadrant. The following steps were followed to ensure proper handling and processing of the samples.

Sample collection

Three 100-meter-long line transects, namely T1 (04° 39.513 S, -039° 23.790E), T2 (04° 40.527 S, -039° 23.717E), and T3 (04° 40.515 S, -039° 23.415E), were systematically placed 500 meters apart, each with quadrats at 25-meter intervals (Plate 1). Seaweed species including Cottonii (*Kappaphycus alvarezii* (Doty) Doty ex P.C. Silva), Sea lettuce (*Ulva lactuca*), and Bubble-green seaweed (*Boergesenia forbesii*) were purposefully sampled. Triplets of samples were collected from each quadrant using a clean stainless-steel pair of scissors. To eliminate any unwanted materials, each sample was cleaned with seawater, transferred into a dry polythene specimen bag, and properly labeled.

Sample transportation and preparation

All collected samples were promptly transferred to a cooler box packed with ice and transported to Chuka University laboratory in Tharaka-Nithi County within 24 hours. In the laboratory, the seaweed samples were thoroughly washed with ultrapure water and subsequently dried in an oven (Mermert) for 24 hours at



Plate 1. Layout-out of line transects as set at Mkwiro seaweed farm in Kwale Count, Kenya.

Chuka University. The dried samples were then ground into a fine powder before undergoing digestion via the conventional wet acid digestion method. The resulting digested samples were carefully stored in well-labeled specimen bottles prior to chemical analysis.

Chemical analysis

In the chemical analysis process, stock solutions of cadmium (Cd), lead (Pb), iron (Fe), sodium (Na), Calcium and Potassium (K) were prepared by dissolving precise amounts of analytical grade metal salts, sourced from Merck suppliers, in ultrapure water Milli-Q, USA). These solutions were then stored in securely sealed, labeled containers in controlled laboratory conditions. Subsequently, the 1000 ppm stock solutions underwent serial dilution to achieve concentrations, used as working standards. Calibration curves were generated by analyzing these working standards, encompassing a range of concentrations suitable for the anticipated levels of heavy metals and essential elements in the seaweed samples. The analysis of heavy metals (Cd, Pb) and essential element iron (Fe) were analyzed using a PG-990 (PG Instruments Ltd) Atomic Absorption Spectrophotometer in flame mode (FAAS) at Kenya Plant Inspectorate Services (KEPHIS) in Nairobi as described by Ghasemi et al. (2021). This instrument offered measurements of metal concentrations in solution through the absorption of specific wavelengths of light emitted by a flame. The essential elements sodium (Na), calcium (Ca) and potassium (K) were chemically analyzed using PerkinElmer Inductively Coupled Plasma Mass Spectrometer [(ICP-MS) NexION 2000B, USA] at KEPHIS in Nairobi using procedure used by Grainger et al. (2020).

Calibration and analysis

Before each analysis, the instrument was calibrated using the working standards to establish a linear relationship between absorbance or ion intensity and concentration. The prepared seaweed sample solutions were then introduced into the respective instrument, with optimized settings for maximum sensitivity and accuracy. Readings from the instruments were recorded and used to calculate the concentrations of the target heavy metals and essential elements in the seaweed samples.

To prevent any potential contamination, all glassware and equipment utilized in the analysis were thoroughly cleaned and rinsed with ultrapure water. Additionally, procedural blanks consisting of ultrapure water without sample were included in the analysis to monitor and correct for any background levels of the elements.

Statistical analysis

To evaluate the impact of seaweed types on heavy metal and essential element concentrations, a set of statistical analyses was used. Firstly, a Kruskal-Wallis test was conducted to assess potential differences among the seaweed types. This non-parametric test was chosen given its suitability for comparing multiple groups where the data does not meet normal distribution assumptions. For instance, when concentrations of Fe, Na, Pb, and Cd did not meet the normality assumption, a Kruskal-Wallis test was applied. Prior to this, an adjustment was made using Holm's method to account for multiple comparisons and maintain the overall significance level at $\alpha=0.05$. Next, for the analysis of calcium concentrations in the three types of seaweeds, a one-way analysis of variance (ANOVA) was performed. ANOVA was used in comparing means across multiple groups, where the data was found to meet normality and homogeneity of variance assumptions. To ensure the appropriateness of the ANOVA, normality assumptions were assessed using the Shapiro-Wilk test (significance level at $\alpha = 0.05$). This test provided a reliable assessment of whether the data follows a normal distribution. Additionally, Levene's test was used to examine the homogeneity of variances across the groups. This step was necessary to ensure that the variance within each group was consistent. All statistical analyses were performed using R Studio version 4.3.2, a widely used statistical software known for its robust capabilities in data analysis and visualization.

RESULTS AND DISCUSSION

The collected seaweed species, including *U. lactuca*, *K. alvarezii* (Cottonii), and *B. forbesii*, exhibited distinctive characteristics in their morphology and growth patterns. The *U. lactuca* displayed an erect growth pattern with translucent, tubular thalli, showcasing variations in color and attachment to the substrate. The *K. alvarezii*, identified as a red alga, featured densely entangled branches with pinkish-white tips. The *B. forbesii* demonstrated limited diversity, primarily linked to size variations corresponding to different developmental stages. This species was predominantly located in low-tide zones, gradually increasing in numbers toward subtidal areas. The clavate shape and upright growth pattern of *B. forbesii*, along with its attachment to the sea floor using a disc-shaped rhizomatous base or synthetic ropes in the seaweed farm, distinguished it in terms of morphology. The stipes of *B. forbesii* were compressed and stood vertically, supporting large vesicles with various upright, inflated, cartilaginous, and flexible air bladders, presenting a unique yellowish-orange hue (Plate 2).



Ulva lactuca

Kappaphycus alvarezii

Boergesenia forbesii

Plate 2. Representative Sea weeds observed at Mkwiro seaweed farm in Kwale Count, Kenya.

Effect of seaweed species on the concentration of heavy metals

The obtained results from the Kruskal-Wallis tests examining lead and cadmium concentrations among different seaweed species exhibit interesting variations. The test revealed a statistically significant effect ($\chi^2(2) = 7.01$, $p = 0.03$, adjusted using Holm's method) in lead concentrations among the seaweed types. Lead concentration in *B. forbesii* was significantly higher (Median = 0.57) and was significantly different from *K. alvarezii* (Figure 2). The differences observed among the seaweed species in their metal concentrations may have stemmed from their inherent properties in absorbing or retaining certain metals. It's worth noting that the lead levels observed in this study were comparatively lower than those reported by Baghazadeh *et al.* (2020) in a study conducted along the South coast of Iran. These observed discrepancies may be attributed to differences in geographical locations and varying environmental conditions, which are documented to influence heavy metal concentrations in seaweeds (Shah *et al.*, 2021). Furthermore, these variations may be associated with differences in anthropogenic activities, including industrial discharge, as well as the presence of natural sources of heavy metals. On the other hand, cadmium concentrations showed no significant ($\chi^2(2) = 3.88$, $p = 0.14$, adjusted using Holm's method), differences among the seaweed types. However, *B. forbesii* had slightly higher concentrations of cadmium (Median = 0.87167 ppm) as compared to *K. alvarezii* and *U. lactuca* respectively (Figure 3). The recorded concentrations of Cd align with previous studies (Huerta-Diaz, 2007; Chen *et al.*, 2021) from the Central West Coast of the Gulf of California and China. Nevertheless, our outcomes differ from Rajaram *et al.* (2020), who observed varying Cd concentrations among seaweed species along the Palk Bay in Southeastern India. Notably, the Cd levels we found were lower in comparison to Janadeleh and Jahangiri's (2016) findings regarding seawater contamination in the territorial waters of the Kingdom of Bahrain which again may be attributed to differences in the anthropogenic effects.

Effect of seaweed species on the concentration of essential elements

Minerals refers to inorganic substances present all body fluids and tissues. There are basically necessary in the maintenance of some physiochemical life essential processes (Riaz *et al.*, 2020). Marine macroalgae can accumulate minerals from their surrounding and contain about 10 - 20 times much the minerals

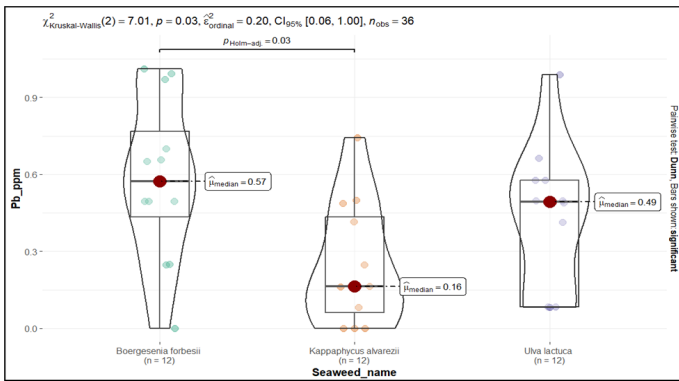


Figure 2. Kruskal-Wallis test for effect of seaweed type on the lead concentrations at Mkwiro Seaweeds farm in Kwale County, Kenya.

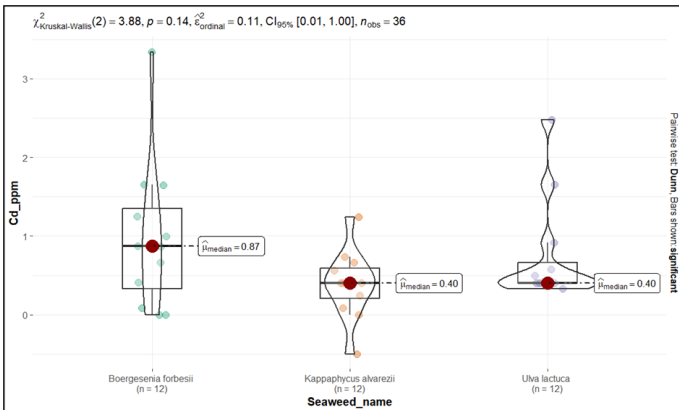


Figure 3. Kruskal-Wallis test for effect of seaweed type on the cadmium concentrations at Mkwiro Seaweeds farm in Kwale County, Kenya.

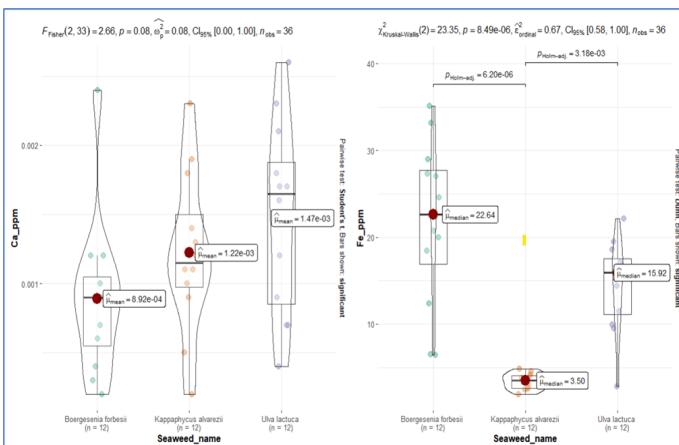


Figure 4. Kruskal-Wallis test for effect of seaweed type on the calcium (Ca_ppm) and iron (Fe_ppm) concentrations at Mkwiro Seaweeds in Kwale County, Kenya.

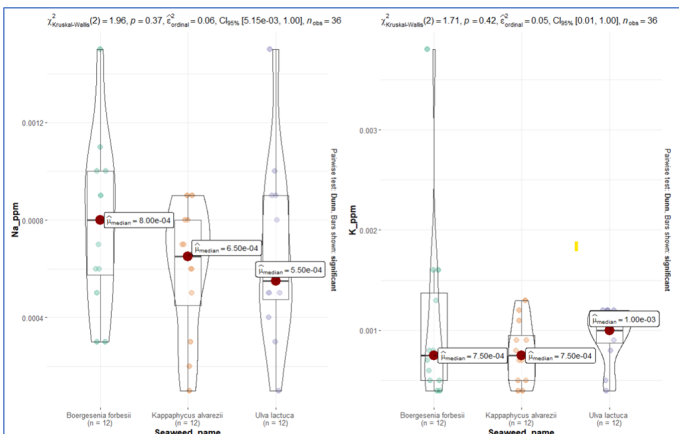


Figure 5. Kruskal-Wallis test for effect of seaweed type on the sodium (Na_ppm) and potassium (K_ppm) concentrations at Mkwiro Seaweeds in Kwale County, Kenya.

contained in land plants (Sadhukhan et al., 2019). The bioaccumulation capacity is dependent on the species type, life cycle stage as well as environmental conditions (Ji and Gao, 2021). In this study, a one-way analysis of variance (ANOVA) was conducted to examine the concentrations of calcium in three types of seaweeds collected from Mkwiro Seaweeds in Kwale County, Kenya. Fisher's exact test did not reveal a significant effect of seaweed type on calcium concentration ($F(2, 21.86) = 2.52, p = 0.08$). A post-hoc Tukey HSD test was not conducted due to the non-significant ANOVA result. However, calcium was slightly higher in *B. forbesii* (Mean = 0.0008.92 ppm) as compared to *K. alvarezii* and *U. lactuca* respectively (Figure 4). The calcium range observed in this study were lower compared to those reported by Rasyid (2017) in sea weeds from Indonesia and Xavier and Jose (2020) from Gulf of Manner in India.

Kruskal-Wallis test was conducted to compare the iron concentrations among the three seaweed types. The test statistic was significant ($\chi^2(2) = 23.35, p = 0.00001$, adjusted using Holm's method), indicating that there are significant differences in iron concentration among the groups (Figure 4). *Boergesenia forbesii* had significantly higher median of iron concentration (22.64 ppm) while *K. alvarezii* had significantly lower median [(3.5 ppm) Figure 4]. Our results differed with those from a similar study (Huerta-Diaz et al., 2007; Adharini et al., 2020) where metal concentrations including that of iron did not differ among the different seaweed species on the west coast of the Gulf of California and in Indonesia respectively. However, our results are in agreement with reports from other studies Filippini et al., 2021; Pasumpon et al., 2023). Variations on the results reported by different authors may have been due to difference in study time. For instance Villares et al. (2013) observed that seasons significantly influenced the concentrations of metals in seaweeds at a coastal embayment in northwest Spain. Further as explained by Huerta-Diaz et al. (2007), high vertical water mixing at the sea zones may cause nutrient surge or a decrease in elemental concentration. Kruskal-Wallis test did not reveal a significant effect ($\chi^2(2) = 1.71, p = 0.42$). This suggests that there are no significant differences in potassium concentrations among the three seaweed types. However, the concentration of potassium (K) was slightly lower in *Ulva lactuca* (Median = 0.001) as compared to concentrations in *Boergesenia forbesii* and in *Kappaphycus alvarezii* (Figure 5). The finding of this study differs with those of Thodhal et al. (2019) which reported significance difference of metals including potassium among the sea weeds. It's important to highlight that the K levels observed in our study were lower compared to findings in other studies (Farzanah et al., 2022). Kruskal-Wallis test yielded a non-significant result ($\chi^2(2) = 1.96, p = 0.37$), indicating that there are no significant differences in sodium concentrations among the three seaweed types. However, the concentration of sodium was slightly lower in *Ulva lactuca* (Median = 0.00055 ppm) as compared to concentrations in *Boergesenia forbesii* and in *Kappaphycus alvarezii* respectively (Figure 5).

Conclusion and recommendation

The study's findings indicate significant concentrations of cadmium, with levels surpassing recommended limits in a majority of the seaweed samples. This highlights a potential risk associated with heavy metal contamination in seaweed farms. Additionally, essential elements such as sodium, iron, calcium, and potassium were observed to be lower than reported in existing literature. This suggests the need for further investigation into the factors influencing essential element uptake and distribution in seaweeds. Notably, the study also revealed that both Iron and Lead have a direct influence on seaweed height, providing valuable insights into the complex interactions between metals and seaweed growth patterns. To safeguard the safety and quality of seaweed products, it is imperative to establish routine monitoring programs for heavy metal concentrations in seaweed farms, coupled with effective pollution control measures. This proactive approach will mitigate potential risks associated with heavy metal contamination. In addition, diversifying seaweed cultivation by exploring a range of species with lower heavy metal uptake and higher nutritional value is crucial. This strategy not only contributes to more resilient and sustainable practices but also enhances overall product quality and safety for consumers. By harmonizing these efforts, the seaweed industry can ensure a safer and more diverse range of nutritional options.

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Authors contribution

Conceptualization, writing—original draft preparation P.L.L., J.G. and E.C.N.; Resources, methodology, data collection, writing—review and editing, all authors; Data curation, analysis and visualization, F.O.O.; Supervision, J.G. and E.C.N. All authors have read and agreed to the published version of the manuscript.

Conflict of interest: The authors declare no conflict of interest.

Ethical approval: Not applicable.

Data availability: The data that support the findings of this study are available on request from the corresponding author.

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Conflict of interest: The authors of this manuscript declare no conflicts of interest.

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