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Assessing the levels of heavy metals Cd, Cr, and Pb in the naturally occurring red seaweed *Gracilariopsis heteroclada* in Iloilo Province, Philippines, for potential mass cultivation

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Abstract. *Beup RA, Felongco SGE, Cailin SJC, Guanzon Jr NG, Fantonalgo RN, Pakingking Jr RV. 2024. Assessing the levels of heavy metals Cd, Cr, and Pb in the naturally occurring red seaweed* Gracilariopsis heteroclada *in Iloilo Province, Philippines, for potential mass cultivation. Nusantara Bioscience 16: 149-153.* The Philippines, a leading exporter of seaweed, has incorporated strategies in its Philippine Industry Seaweed Roadmap (2022-2026) to boost seaweed production. Therefore, to achieve this, potential sites for seaweed cultivation must undergo assessment for heavy metal content. A pilot study assessed heavy metals Cd, Cr, and Pb levels in red seaweed (*Gracilariopsis heteroclada*) sourced from three locations in Iloilo Province, Philippines: Site 1, a fishpond in Brgy, Nabitasan, Leganes; Site 2, along Dumangas Port coastline in Brgy, Sapao, Dumangas; and Site 3, a fishpond in Brgy, Talokgangan, Banate. Flame atomic absorption spectrometry was employed to analyze heavy metal concentrations. The mean concentrations of Pb in *G. heteroclada* from Sites 1, 2, and 3 were 5.0129±0.0896, 5.0388±0.8749, and 3.5119±0.9262 mg/kg, respectively; for Cr, they were 3.3002±0.1436, 4.0464±0.3800, and 2.8027±0.1436 mg/kg, respectively. The mean Cd concentrations were also 0.8518±0.0349, 0.8115±0.0202, and 0.5423±0.0419 mg/kg, respectively. Although Cd levels met safety standards, Pb and Cr concentrations exceeded the maximum permissible levels set by the European Union (EU) and China Food and Drug Administration (CFDA), respectively. This suggests that the examined sites may not be suitable for large-scale cultivation due to potential health risks from Pb and Cr exposure through seaweed consumption. Continued monitoring of toxic metal levels in these areas is therefore imperative.

Keywords: Cadmium, chromium, *Gracilariopsis heteroclada,* lead, Philippines, red seaweeds

INTRODUCTION

Gracilaria is a versatile species commonly found in brackish water, mangrove swamps, and the sea, owing to its broad tolerance for changing environmental conditions, including varying salinities. Certain *Gracilaria* species exhibit rapid growth and can absorb high levels of nutrients, making them a valuable source of bioactive substances for multiple products (Wu et al. 2018). *Gracilaria* spp. are utilized in various ways, serving purposes in healthcare and acting as food, fodder, feed, and fertilizer. In Hawaii, fresh *Gracilaria* spp. has been collected and sold as a salad vegetable for many years, and coastal communities in Indonesia, Malaysia, the Philippines, and Vietnam have been gathering *Gracilaria* for food (Mahadevan 2015). Regarding trade, seaweed exports are expanding moderately, with the Philippines as one of the top export sources alongside Indonesia, Ireland, Chile, Korea, and China. The Philippines, following China, is the second-largest supplier of semi-processed and processed carrageenan in Asia (Bureau of Fisheries and Aquatic Resources 2022). According to the Bureau of Fisheries and Aquatic Resources (2022), there has been a noticeable increase in the Philippines' seaweed exports from 1996 to 2019. Specifically, there was a 29% growth observed, with total volumes reaching 37,148 metric tons in 1996 and 48,026 metric tons in 2019.

Seaweeds, including *Gracilaria* spp., can absorb heavy metals from the aquatic environment due to erosion, industrial activities, and domestic sewage (Sadhasivam et al. 2012). The accumulation of these contaminants over time can lead to elevated levels of heavy metals in seaweed, indicating a higher risk of heavy metal exposure during consumption. Sadhasivam et al. (2012) found that eight seaweed species could absorb seven types of heavy metals in varying quantities based on the species and collection areas. Additionally, prolonged ingestion of seaweed, even at low concentrations, may pose potential health risks, as heavy metals are considered non-essential. Consequently, consuming significant quantities of seaweed could result in adverse gastrointestinal, neurological, and carcinogenic effects (Asensio et al. 2021).

Gracilaria spp. play a crucial role in Philippine aquaculture, contributing significantly to both domestic and international production. Among these species, *Gracilariopsis heteroclada* stands out as particularly abundant, thriving in various natural habitats such as fishponds, estuaries, and bays, typically in intertidal or shallow subtidal areas less than 1 meter deep, where it attaches to rocks or floats freely. Despite its importance, pertinent information regarding the heavy metal contents of *G. heteroclada* in the Philippines is scarce. Therefore, with its prevalence in specific municipalities of Iloilo Province and its widespread utilization as a bioproduct, it is imperative to assess the presence of heavy metals in *G. heteroclada* due to its ability to accumulate these contaminants. Recognizing the Philippines as a leading seaweed exporter, the government has integrated strategies into its Philippine Industry Seaweed Roadmap (2022-2026) to enhance seaweed production (Bureau of Fisheries and Aquatic Resources 2022). This initiative underscores the need to evaluate potential heavy metal content cultivation sites. In the current study, a pilot investigation aimed at determining the concentrations of lead (Pb), cadmium (Cd), and chromium (Cr) in *G. heteroclada* samples collected from selected sites in the municipalities of Leganes, Dumangas, and Banate within Iloilo Province was conducted. Resolute results generated from this study would be crucial in assessing the suitability of these sites for large-scale cultivation of red seaweeds.

MATERIALS AND METHODS

Sampling sites

G. heteroclada specimens were gathered from three distinct locations within the Iloilo Province, Philippines. These selected sites included: (i) Site 1, a fishpond in Brgy. Nabitasan, municipality of Leganes, Iloilo (10° 47' 6.828" N, 122° 38' 13.4376" E), (ii) Site 2, positioned along Dumangas Port in Brgy. Sapao, municipality of Dumangas, Iloilo (10° 46' 50.4372" N, 122° 42' 31.6476" E), and (iii) Site 3, a fishpond located in Brgy. Talokgangan, municipality of Banate, Iloilo (11° 0' 52.4952" N, 122° 49' 58.1952" E) (Figure 1). These sites were selected primarily based on the abundance of naturally occurring red seaweeds and their proximity to nearby establishments, which could potentially serve as sources of heavy metal contamination in the surrounding water bodies.

Water physicochemical parameters

In-situ temperature, pH, and salinity measurements were conducted using a portable multiparameter tool (WalkLAB Professional pH and Temperature Meter HP 9010) and a handheld refractometer (RHS-100ATC), respectively. Dissolved oxygen analysis of water samples was performed at the Chemistry Laboratory of the Central Philippine University.

Collection of seaweed samples

Four kilograms of fresh weight of *G. heteroclada* were gathered from each of the three sampling sites, following a method adapted from Khaled et al. (2014) to ensure an adequate quantity of thalli. Wild thalli found within the sampling areas were manually collected and placed in plastic ziplock bags. Only mature thalli were selected for sampling, and collection occurred during low tide at the shoreline of Dumangas Port. The collected samples were promptly transported to the Research Laboratory at Central Philippine University for preparation. Upon arrival, the seaweed samples underwent thorough washing with running tap water to eliminate sand, salts, and other external particles adhering to the surface. Subsequently, distilled water was used for a final rinse. Drying and homogenization procedures followed the methods outlined by Baghazadeh et al. (2021) and Khandaker et al. (2021), with slight modifications. Briefly, the fresh weight of the samples was recorded before they were divided based on their sampling areas. Air drying was conducted at room temperature (28°C) for 72 hours, followed by further drying in a closed system microwave oven set at 70°C for two days to eliminate remaining moisture. The dried samples were then blended using a heavy-duty blender, resulting in homogenized seaweed powder stored in ziplock bags at room temperature until further analysis.

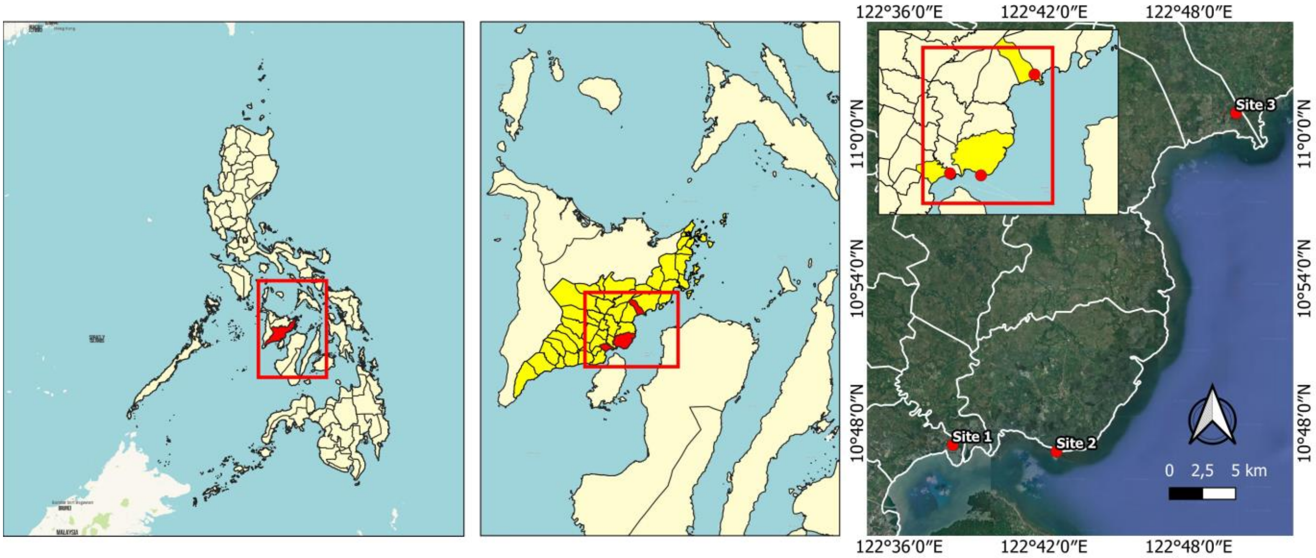

Figure 1. Map of the Philippines indicating the locations of the three sampling sites within the Iloilo Province, Philippines

Heavy metal analysis

The dry-weight replication method outlined by Khaled et al. (2014) was employed with slight modifications. Ten grams (10 g) of dried seaweed from each sample were placed into a crucible in triplicate and weighed using a top-loading analytical balance. The ash extraction technique for seaweed samples described by Rasyid (2017) was slightly modified. Briefly, to determine ash content, *G. heteroclada* samples were heated in a muffle furnace at 450-550°C for 2 hours, followed by cooling at room temperature after removal from the furnace.

Sample digestion was conducted following the method detailed by Qari (2015) and Uddin et al. (2016), utilizing the nitric-hydrochloric acid digestion 1:3 method. Triplicate samples of seaweed species (10 g each) were digested in a freshly prepared acid mixture of one (1) mL 65% HNO_3, 3 mL 37% HCl, and 50 mL H_2O. The resulting mixture was gently boiled over a water bath (95°C) for 5 minutes. Following digestion, the cooled solutions were filtered using Whatman filter paper (No. 41) and transferred to volumetric flasks, with distilled water added to reach a final volume of 50 mL for instrumental analysis. Blank flasks underwent the same treatment using identical volumes of acid and distilled water, with the digestion process repeated for each replicate.

Furthermore, following the procedure outlined by El-Said and El-Sikaily (2013), all digested solutions were analyzed in triplicate for Pb, Cd, and Cr using a flame atomic absorption spectrometer (Agilent 55 AA Spectrometer G8430A). Standard precautions for element analysis were adhered to throughout the process, and analytical-grade reagents were utilized to prepare calibration curves. The detection limits for the studied metals were as follows: Pb (0.1 ppm), Cr (0.1 ppm), and Cd (0.01 ppm).

Statistical analysis

The gathered data underwent statistical analysis employing One-Way Analysis of Variance (ANOVA) to assess potential variations in heavy metal levels among the three sampling sites. After identifying significant differences among the sampling sites, Post hoc analysis was conducted using Duncan's Multiple Range Test (DMRT). The level of significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

Moreover, to effectively achieve the objectives outlined in the Philippine Industry Seaweed Roadmap (2022-2026), which aims to enhance seaweed production in the country, it is imperative to conduct assessments for heavy metal content at potential seaweed cultivation sites in the Philippines. In our current investigation, we delved into the presence of three common heavy metals (Pb, Cr, and Cd) in seaweeds, alongside analyzing the water parameters at three designated sampling sites. Our findings, as depicted in Table 1, reveal notable concentrations of Pb and Cr exceeding the maximum permissible limits required by both the European Union (EU) (European Commission 2020) and China Food and Drug Administration (CFDA) (USDA FAS 2017). Conversely, Cd concentrations remained within acceptable regulatory thresholds. Across the three sampling sites, Pb concentrations ranged from 3.5119±0.9262 mg/kg to 5.0388±0.8749 mg/kg, with Dumangas (Site 2) displaying the highest mean and Banate (Site 3) exhibiting the lowest. Similarly, Cr concentrations varied from 2.8027±0.1436 mg/kg to 4.0464±0.3800 mg/kg, with Dumangas showcasing the highest level and Banate registering the lowest mean compared to the other sampling sites. Cd concentrations ranged from 0.5423±0.0419 mg/kg to 0.8518±0.0349 mg/kg, with Leganes (Site 1) recording the highest mean and Banate the lowest among the three sampling sites. Our findings suggest that while Cd pollution levels were relatively lower than Pb and Cr across all sampling sites, the presence of Pb and Cr remains a concern. pH values across the sampling sites fell within the normal range, while salinity, dissolved oxygen, and water temperature exhibited expected variations typical of marine environments, as shown in Table 2. The lower levels of dissolved oxygen in the bodies of water may have been attributed to a combination of factors such as the presence of fertilizers, excess amounts of nutrients, and organic matter, all of which exacerbate oxygen depletion. Additionally, samples were collected early in the morning, hence the amount of light to drive photosynthesis may be limited.

The elevated levels of Pb and Cr, particularly observed at Dumangas and Leganes, are likely attributed to nearby anthropogenic sources such as local businesses, residences, and maritime activities. Improper waste disposal and industrial processes in these areas could contribute significantly to heavy metal contamination. For instance, maritime vessels operating near Dumangas Port could introduce substantial amounts of fuel, paint, coatings, and other pollutants, contributing to the observed high lead and chromium content in seaweed samples. Similarly, observations at Leganes pointed to potential sources of heavy metal contamination, including effluents from local businesses, residences, and educational institutions. Additionally, agricultural practices near the fishpond and the possibility of corroded leaded pipelines in residential areas may further exacerbate lead contamination in seaweed samples collected from Leganes. Comparatively, Banate exhibited the lowest heavy metal concentrations among the three sites, attributed to its more secluded location with less vehicular and marine traffic. However, despite the relatively lower concentrations, the presence of Pb and Cr still exceeded maximum limits. Our current findings plausibly corroborate with the research conducted by Gangoso et al. (2022), which highlighted that industrial processes, improper waste management, fuel combustion, and the degradation of leaded pipelines were the primary sources of Pb contamination identified in three specific sampling locations in the southern Philippines. Similarly, the elevated levels of Cr could originate from natural sources like weathering rocks containing Cr, as well as from domestic pollution and the prolonged deposition of Cr. Furthermore, the study reported by Gangoso et al. (2022) indicated that Cd, particularly sludge, can travel long distances, contaminating surface waters and accumulating in sediments. Additionally, our findings are consistent with the study conducted by Karthikeyan et al. (2020), which examined the concentrations of priority metals (Cr, Cd, and Pb) in water, sediment, and biota in the Ennore estuary along the southeast coast of India. Karthikeyan et al. (2020) observed higher levels of these metals than other estuaries in India, suggesting significant bioaccumulation of non-essential metals such as Cd and Pb in marine organisms. The presence of these heavy metals was attributed to various anthropogenic activities, including industrial operations, dredging, thermal power plants, petrochemical industries, vehicular emissions, port activities, and urban expansion, encompassing both residential and fishing communities.

The current study shows that the three sampling sites contained high levels of Pb and Cr but relatively low levels of Cd, indicating that *G. heteroclada* can accumulate substantial quantities of heavy metals over time. Notably, following safety standards, concentrations of Pb and Cr surpassed the maximum permissible limits established by the European Union (EU) (European Commission 2020) and the National Food Safety Standard for Maximum Levels of Contaminants in Foods (GB 2762-2017) of the China Food and Drug Administration (CFDA) (USDA FAS 2017). Consequently, even at low concentrations, prolonged consumption of seaweed may pose health risks due to the toxicity of heavy metals, which are non-essential elements. Lead, for instance, is a toxic heavy metal that adversely affects various bodily systems, including the kidneys, hematopoietic, and nervous systems (Charkiewicz and Backstrand 2020). Assi et al. (2016) reported a range of physiological, biochemical, and neurobehavioral effects associated with Pb ingestion, with severe consequences on the cardiovascular, reproductive, hematopoietic, and peripheral and central nervous systems, as well as organs like the liver, bone, and kidneys. Chakraborty et al. (2022) noted its accumulation in organs, such as the lungs, liver, and kidneys, with detrimental effects on human health. Similarly, increased Cr accumulation in organs has been linked to deteriorating organ functions and overall human health. Chromium exposure is associated with liver and kidney damage, widespread dermatitis, gastrointestinal tract disorders, respiratory tract cancers, cardiovascular disorders, and alterations in DNA methylation status (Chakraborty et al. 2022). At lower levels, cadmium can harm the liver, kidneys, skeletal, and cardiovascular systems. Additionally, Genchi et al. (2020) noted that environmental Cd exposure may elevate the risk of osteoporosis. Epidemiological data suggest that occupational and environmental exposure to Cd may be related to various cancers, including those affecting the breast, lung, prostate, nasopharynx, pancreas, and kidneys (Genchi et al. 2020). Given the adverse effects of consuming contaminated seaweed, it is clear that harvesting and distributing red seaweed from these sampling sites is not recommended. However, large-scale efforts can be targeted towards bioremediation of the polluted sampling sites instead. For example, Rahhou et al. (2023) extensively documented the bioaccumulation and restoration capabilities of *Ulva lactuca*. This species exhibits a remarkable affinity for a wide array of metals, including iron (Fe) and manganese (Mn), as well as essential nutrients like nitrogen and phosphorus. Such characteristics render *U. lactuca* effective in remediating heavy metal contamination and mitigating eutrophication, underscoring its significant potential for bioremediation purposes (Rahhou et al. 2023).

Table 1. Concentrations of heavy metals in *G. heteroclada* collected from the three sampling sites in Iloilo Province, Philippines

Heavy metal	**Mean (±SD) concentration (mg/kg)***			**Maximum permissible limit (mg/kg)**	
	Site 1. Brgy. Nabitasan, Leganes, Iloilo	**Site 2. Brgy. Sapao, Dumangas, Iloilo**	**Site 3. Brgy. Talokgangan, Banate, Iloilo**	**European Union**[a]	**China Food and Drug Administration**[b]
Pb	5.0129±0.0896^{a}	5.0388±0.8749^{a}	3.5119±0.9262^{a}	1.5	1.5
Cr	3.3002±0.1436^{b}	4.0464±0.3800^{a}	2.8027±0.1436^{c}	NA	2.0
Cd	0.8518±0.0349^{a}	0.8115±0.0202^{a}	0.5423±0.0419^{b}	3.0	2.0

Note: *Limit of detection (mg/kg dry weight): Pb: 0.1; Cr: 0.1; Cd: 0.01. Different letter superscripts denote significant differences at $P < 0.05$. (NA): Not available. [a]European Commission (2020). [b]United States Department of Agriculture Foreign Agricultural Service (2017)

Table 2. Water physicochemical characteristics (mean±SD) of the three sampling sites

Site	**Water physicochemical parameters**			
	pH	**Salinity (‰)**	**Temperature (°C)**	**Dissolved Oxygen (mg/L)**
Site 1 (Brgy. Nabitasan, Leganes, Iloilo)	8.04±0.00	34±0.00	26.0±0.00	4.0±0.20
Site 2 (Brgy. Sapao, Dumangas, Iloilo)	8.14±0.23	29±0.00	25.8±0.20	4.9±0.15
Site 3 (Brgy. Talokgangan, Banate, Iloilo)	8.12±0.00	29±0.00	24.4±0.23	3.9±0.15

Our current study presents preliminary data on heavy metals Pb, Cr, and Cd concentrations in red seaweeds collected from selected sites in Iloilo province, identified as potential areas for large-scale cultivation. *G. heteroclada* has shown the ability to gradually absorb and accumulate heavy metals from its aquatic surroundings, making it a valuable bioindicator for heavy metal contamination. Our current data, therefore, emphasizes the necessity for ongoing investigation and regular monitoring of seaweed cultivation areas due to the frequent association between high seaweed consumption and human poisoning. Distribution and consumption of *G. heteroclada* from the sampled sites are strongly discouraged, given the elevated heavy metal levels indicating widespread contamination across water bodies. Consequently, these sites studied are presently unsuitable for extensive production and consumption due to high levels of Pb and Cr in the red seaweeds naturally growing in these sites. Therefore, to augment our understanding of trace metal concentrations comprehensively, future investigations should prioritize the exploration of pollution indices, particularly to elucidate the pollution status within the sediment and water of the presently studied sites. Continuous monitoring of heavy metal content in harvested *G. heteroclada* is therefore recommended to ensure safety. Local Government Units (LGUs) should proactively educate communities about the risks associated with consuming metal-contaminated seaweed and encourage alternative sourcing for businesses and restaurants. Collaboration between government agencies and the private sector is essential to enforce regulations and screening measures for water and environmental quality, addressing concerns highlighted by this study within the seaweed industry. Ultimately, concerted efforts from LGUs and the private sector are imperative to tackle pollution sources in water bodies, securing the long-term sustainability of the seaweed sector in the Philippines.

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