JIPK. Volume 14 No 2. November 2022
Sinta 1 (Decree No: 158/E/KPT/2021)
e-ISSN:2528-0759; p-ISSN:2085-5842
DOI=10.20473/jipk.v14i2.33103
Available online at https://e-journal.unair.ac.id/JIPK/index

JIPK
(JURNAL ILMIAH PERIKANAN DAN KELAUTAN)
Scientific Journal of Fisheries and Marine

Short Communication

Concentrations of Heavy Metals in Three Brown Seaweed (Phaeophyta: Phaeophyceae) Collected from Tourism Area in Sanur Beach, Coast of Denpasar, Bali and Public Health Risk Assessment

I Wayan Rosiana[1], Anak Agung Ayu Putri Permatasari[1], I Made Murna[1], Putu Angga Wiradana[1], Yesha Ainensis El G. Pelupessy[1], Matius Victorino Ola Dame[1], Agoes Soegianto[2], Bambang Yulianto[3], and I Gede Widhiantara[1*]

[1]Study Program of Biology, Faculty of Health, Science and Technology, University of Dhyana Pura, Badung Regency, Bali, 80351. Indonesia
[2]Department of Biology, Faculty of Science and Technology, University of Airlangga, Surabaya, East Java, 60115. Indonesia
[3]Department of Marine Sciences, Faculty of Fisheries and Marine Sciences, University of Diponegoro, Semarang, Central Java, 50275. Indonesia

OPEN ACCESS

ARTICLE INFO

Received: January 22, 2022
Accepted: April 12, 2022
Published: April 15, 2022
Available online: August 30, 2022

*) Corresponding author:
E-mail:
widhiantara@undhirabali.ac.id

Keywords:
Brown Seaweed
Heavy Metals
Carcinogenic Effect
Health Risk
Sanur Beach

Abstract

Marine brown seaweed are known as one of the potential biological agents to be developed as functional food and medicinal sectors. This study aims to examine the concentration of heavy metals (Pb, Cd, Hg, and As) in brown algae *(Sargassum aquifolium*, *Padina australis*, and *Turbinaria ornata*.) and the possible exposure to health risks caused by consumption. Heavy metal concentrations were determined using Atomic Absorption Spectroscopy (AAS) on brown seaweed samples obtained from three different sites. The average concentration of heavy metals in the dry weight of brown seaweed remains within the guidelines established by The Food and Drug Supervisory Agency (BPOM) Number 32 of 2019 concerning the Safety and Quality of Traditional Medicines, which is then used to calculate the estimated daily intake (EDI), target hazard quotient (THQ and TTHQ), and target cancer risk (TCR) for arsenic associated with food exposure to potentially toxic metallic elements. Each species of brown seaweed has a THQ and TTHQ level of <1, indicating that one or more toxic metal elements in the same meal provide no significant non-carcinogenic risk. The TCR for arsenic in these seaweeds are all less than 1 x 10^{-4}, indicating no cancer risk. There are no chronic health hazards related with the ingestion of brown seaweed harvested from the coast of Sanur Beach at Denpasar, Bali.

Cite this as: Rosiana, I. W., Permatasari, A. A. P., Murna, I. M., Wiradana, P. A., Ainensis, Y. E. G., Pelupessy., Dame, M. V. O., Soegianto, A., Yulianto, B., & Widhiantara, I. G. (2022). Concentrations of Heavy Metals in Three Brown Seaweed (Phaeophyta: Phaeophyceae) Collected from Tourism Area in Sanur Beach, Coast of Denpasar, Bali and Public Health Risk Assessment. *Jurnal Ilmiah Perikanan dan Kelautan*, 14(2):327-339. http://doi.org/10.20473/jipk.v14i2.33103

1. Introduction

The consumption of seaweed has long been a cultural practice in many Asia countries (Hwang *et al*., 2019), as well as in several marine areas in Europe and America (Monagail and Morrison, 2020; Pérez-Lloréns, 2019). Coastal communities in Malaysia, the Philippines, and Thailand, for example, use different forms of fresh seaweed as a dietary element (Gomez-Zavaglia *et al*., 2019). Seaweed is extensively used to improve products in Japan and South Korea, mostly as a taste dietary supplement and to benefit from algal elements such as natural minerals (Hwang *et al*., 2019). Consumption and production of seaweed have been commonly recorded in Indonesia (Rimmer *et al*., 2021). Currently, seaweed cultivators focus on increasing the cultivation of red (Rhodophyceae) and green (Chlorophyceae) seaweeds and are still not aware of the potential of brown algae (Phaeophyceae) as functional food raw materials and biomaterials in the future (Permatasari *et al*., 2022).

The large potential of seaweed encourages the Ministry of Maritime Affairs and Fisheries (KKP) of the Republic of Indonesia to continue to increase productivity, especially through studies of suitable types of seaweed in Indonesia (Zaw *et al*., 2020; Wiradana *et al*., 2021). To support this goal, a national plan for the growth of the seaweed sector has been drawn up from 2018 to 2021 to encourage the economy, community empowerment, and national food and nutrition security (Presidential Regulation No. 33/2019) (Rimmer *et al*., 2021). Until now, brown seaweed (Phaeophyceae) has not been widely used as a consumer market by coastal communities in Indonesian waters (Sudarwati *et al*., 2020). Several types of brown seaweed, including *Padina* spp., *Sargassum* spp., *Laminarian* spp., and *Turbinaria* spp., thrive in the coastal waters of Indonesia. Brown seaweed is high in carbohydrates, protein, vitamins (B1, B2, B6, B16, C, and niacin), and minerals (calcium, sodium, magnesium, potassium, iodine, iron) (Choudhary *et al*., 2021). Some elements of this brown seaweed contain important bioactivity for humans. Brown seaweed polysaccharides (alginate, fucoidan, and laminarin) (Piñeiro-Ramil *et al*., 2022; Samsonchi *et al*., 2022; Vijayakumar *et al*., 2021) and polyphenols (Mekinić *et al*., 2019), for example, not only do they have promising antibacterial, antifungal, and antiviral properties, but they also have the potential to prevent several chronic diseases such as cardiovascular disease, cancer, obesity, hyperlipidaemia, and diabetes (Reyes *et al*., 2020; Yamagata, 2021). Recently, the capacity of brown seaweed biopolymers to function as more effective and efficient natural drug delivery systems in releasing certain bioactive constituents in the body has been highlighted (Cunha and Grenha, 2016; Zhong *et al*., 2020).

Heavy metals in marine environment present a significant concern and have an impact on human health (Zaynab *et al*., 2022). Pollution from tourism and industrial activities are the biggest threat to biological systems in many countries (Lloret *et al*., 2021), including Indonesia. Long-term exposure to stressors, on the other hand, can decrease the bioactive components of seaweed, resulting in the formation of reactive oxygen species and other oxidizing agents (Roleda *et al*., 2019). In addition, seaweed accumulates metals from fluctuating mineral concentration densities, and heavy metals can accumulate in body functions if consumed for long periods of time (Chen *et al*., 2018). Heavy metals accumulate in marine and coastal waters from both anthropogenic and lithogenic sources (Anbazhagan *et al*., 2021).

Toxicology is the study of the potential harmful consequences of exposure to chemicals on living organisms (Costa and Teixeira, 2014). It establishes a comprehensive explanation of the symptoms, bio-mechanisms, and detection of hazardous chemicals, especially the effects of poisoning after consumption (Ganesan *et al*., 2020). If an ingredient is to be produced as a raw material for functional and therapeutic foods, these activities must be completed to highlight safe concentrations/doses (Wasilah *et al*., 2021). In addition, bioindicators, in this case seaweed that collects pollutants in the environment, can be used to evaluate and monitor the levels of heavy metal pollution concentration in the marine environment (Rakib *et al*., 2021). Sanur Beach is one of the water areas in the Province of Bali which is often used as a tourist attraction, it has large marine biological resources in marine habitats (intertidal and near the coast) (Suartika, 2015; Turak and Devantier, 2013). The seagrass beds to the coral reefs of Sanur Beach are considered as one of the core conservation areas. The main causes of heavy metal pollution on the Sanur coast of Denpasar City according to this study include uncontrolled industrial and domestic wastewater runoff, tourism activities, ports and ships, runoff of oil, chemicals, and ship metal waste, as well as fisheries and other related activities.

In fact, no information on the accumulation of heavy metals in brown seaweed collected from the waters of Sanur Beach has been reported to date. As a result, the aim of this study was on the determination of heavy metals (Pb, Cd, Hg, and As) in brown seaweed collected from the waters of Sanur Beach in Denpasar, Bali. This research also focuses at the health risks of ingesting brown seaweed from Sanur Beach in Denpasar, Bali. The results of the analysis of heavy metals in brown seaweed were compared with the quality standards related to herbal medicines stipulated by the Regulation of the Food and Drug Supervisory Agency (BPOM) No. 32 of 2019 and the risk of consumption based on the provisions of the World Health Organization (WHO). The findings of this study are important because brown seaweed is significant in bioactive ingredients, which can help answer the challenges of food security and provide nutraceutical products to meet the needs of the food and drug industries in the future, thereby increasing the productivity of coastal communities.

2. Materials and Method

2.1 Study Site

We selected three sampling locations: (1) Mertasari Beach (8°42'48.5"S 115°15'02.9"E to 8°42'45.6"S 115°15'06.4"E), (2) Semawang Beach (8°42'27.1"S 115°15'46.2"E to 8°42'13.8"S 115°15'52.3"E), and (3) Sindhu Beach (8°41'02.1"S 115°15'54.5"E to 8°40'57.1"S 115°15'53.5"E) included in the Sanur Coast region to assess heavy metal contamination in brown seaweed (Figure 1).

The major activities of Mertasari Beach are fishing, recreational (including swimming), and water sports, which generate a large number of tourists to the pier. Furthermore, the estuary transports residential wastewater from the Denpasar City region. This residential garbage runs via canals and rivers before ending up at the coast of Mertasari Beach. Semawang Beach is both a recreational location and an area prone to pollution runoff from hotels in the region. Furthermore, boating, fishing, and tourist rubbish pollute the coastal aquatic environment in this region. Sindhu Beach's popular destinations are water recreation and water sports, which attract a large number of visitors. There is an effluent that approaches the coastal region from hotels near Sindhu Beach and discharges its waste into the beach, exposing marine biota and visitors to polluting contaminants. Crabs, isopods, bivalves, reef fish, seagrass, and gastropods are among the species that approach the three coastal regions vacation.

2.2 Sampling

In September - October 2021, brown seaweed samples were taken from three locations along the Sanur coastline (Mertasari – Semawang) (Figure 1). At each of the three sites, 500 grams fresh weight of three different types of brown seaweed collected at low tide in the

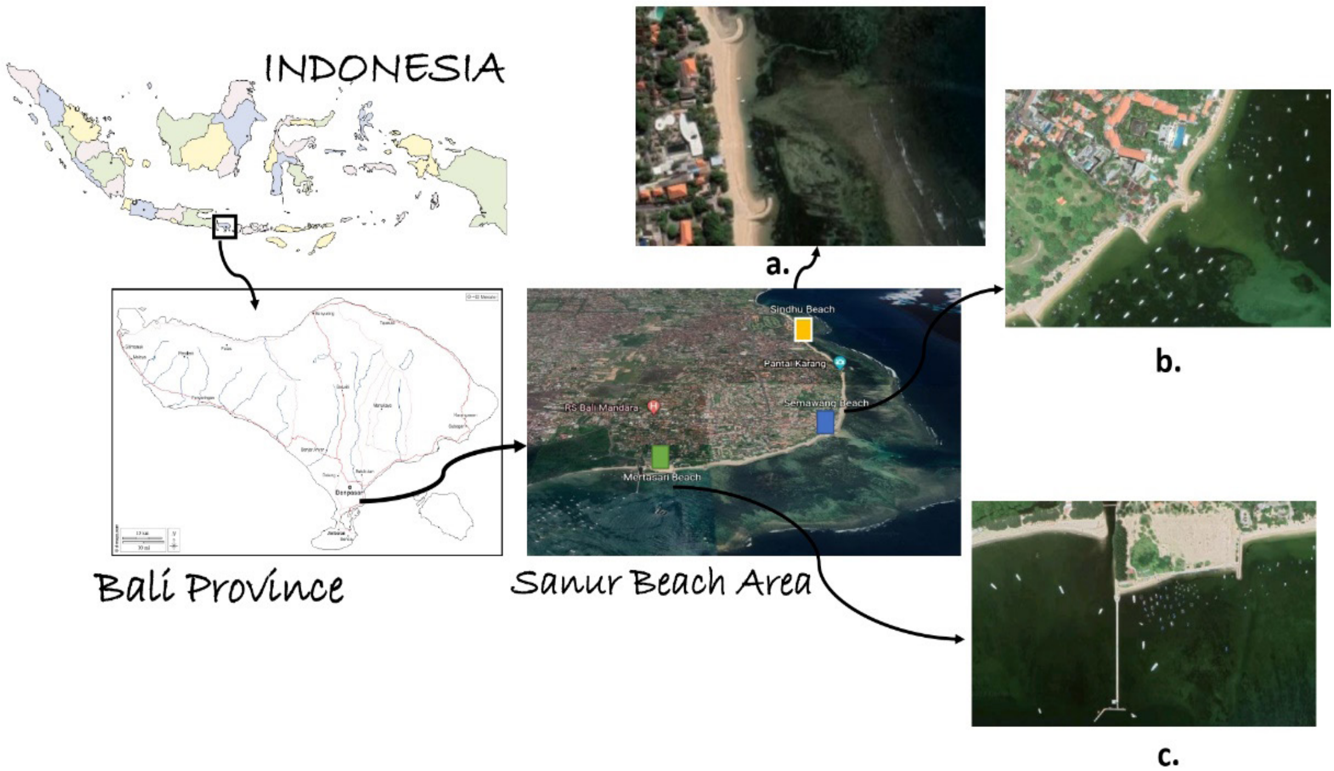

Figure 1. Map of the brown seaweed sampling location along the Sanur Beach Coast, Denpasar City, Bali Province. a) Sindhu Beach, b) Semawang Beach, and c) Mertasari Beach

intertidal zone. Sampling of seaweed at each location was carried out using the random composite sampling method in order to obtain three composites (n = 3)/ sample/location (Lancaster and Keller-mcnulty, 1998). At the collection point, samples were cleaned with brine, packed in sterile plastic bags, and transported to the laboratory in a refrigerator (4°C). The brown seaweeds were washed in fresh water to remove sand and epiphytes in the laboratory, oven-dried at 80°C to maintain a consistent weight, and put in for processing (Khaled *et al.*, 2014). The three types of brown seaweed were identified using key determinations conducted at the Central Oceanographic Laboratory of the National Research and Innovation Agency (BRIN), Jakarta, Indonesia.

2.3 Determination of Heavy Metals

A determination of heavy metal levels was carried out at the Environmental Health Laboratory, Sub-Division of Clinical Pathology, Sanglah Provincial General Hospital (RSUP), Denpasar, Bali. For the quantitative measurement of heavy metals in all samples, standard techniques were used. The Indonesian National Standard was used to quantify the levels of heavy metals in brown seaweed samples (cadmium (Cd), lead (Pb), mercury (Hg), and arsenic (As). The seaweed is ground into a fine powder using a mortar and pestle. One gram of homogenized brown seaweed sample was added to 10 mL of reagent combination containing 70% nitric acid, 70% perchloric acid, and 98% sulfuric acid in a 5:2:1 ratio. The mineralization procedure was performed on a heated plate at 50°C until the sample was virtually dry. After that, 10 mL of 2N HCl was added, and digestion was continued for 30 minutes. The solution was filtered using Whatman No.1 filter paper, and up to 25 mL of sterile distilled water was added before being kept at room temperature for subsequent examination (FAO/ SIDA, 1983).

For heavy metal analysis, an Atomic Adsorption Spectrophotometer (Shimadzu, Japan) model AA-7000 was utilized. Cd (228.8 nm), Pb (217.00 nm), Hg (253.7), and As are the working wavelengths (231.9 nm). The detection limits for the four heavy metals are based on BPOM Regulation 32 of 2019 concerning Safety and Quality Requirements for Herbal Medicines: Cd (≤0.3 ppm), Pb (≤10 ppm), Hg (≤0.05 ppm), and As (≤5 ppm). Blanks and reference standards were included during analysis, and samples were repeated three times. Standard heavy metal measurements were prepared using double-distilled water and analytical components purchased from Merck in the United States.

2.4 Public Health Risk Assessment of Heavy Metals in Brown Seaweed

2.4.1 Estimated Daily Intake (EDI)

The estimated daily intake (EDI) of each heavy metal (Cd, Pb, Hg, and As) was evaluated using the average concentrations in each type of brown seaweed sample and the daily consumption in grams of each food product. The estimated daily intake (EDI) was calculated using the following equation to determine the daily limit for brown seaweed consumption:

$$EDI = \frac{C \times C\ Cons}{Bw}$$

Where *C* represents the heavy metal content in seaweed (ppm/dry weight), *C cons* is the national average daily consumption of seaweed (8.54 g/day BW), and *Bw* represents body weight (adults = 50 kg; children = 15 kg).

2.4.2 Target Hazard Quotients (THQ)

The Target Hazard Quotient (THQ) is the ratio of hazardous element exposure to the reference doses, which is the highest level at which no impact are observed after ingesting a dietary component. Cd (0.0005 ppm), Pb (0.0035 ppm), As (0.00008 ppm), and Hg (0.0001 ppm) are the specific reference doses (FIR) for each identified heavy metal (Kerr *et al.*, 1998; U.S. EPA, 1997). The THQ formula calculates the non-carcinogenic health problems posed by the harmful compounds of each heavy metal. Non-carcinogenic health consequences are not predictable if THQ < 1. However, if THQ > 1, it is possible that significant health implications could occur. A THQ value greater than 1 does not indicate an absolute probability that an adverse non-carcinogenic health consequence will occur, but can be used to refer to the relevant agency. THQ is calculated using the United States Environmental Protection Agency (U.S. EPA) approach using the following equation:

$$THQ = \frac{EF\ x\ ED\ x\ FIR\ x\ C}{RfD\ x\ W\ x\ ATn} \text{ x } 10^{-3}$$

Where *EF* is the frequency of exposure (365 days/year), *ED* is the duration of exposure (71.5 years) equivalent to the average lifespan in Indonesia, *FIR* is the level of food consumption (in grams per person per day) the average daily consumption of seafood including seaweed in Indonesia reaches 85.4 g/day, *C* is the concentration of metals in food (ppm), *RfD* is the oral reference dose of each heavy metal (ppm/day), *W* is the average body weight - average in Indonesia (Adults:

50 kg and Children: 15 kg), and *ATn* is the average exposure time for non-carcinogens (365 days/year i.e. the number of years of exposure assuming 71.5 years in this study adjusting for life span of average population in Indonesia). It is assumed that cooking or certain processes do not affect the toxicity of heavy metals in seaweed (Antoine *et al*., 2017).

Furthermore, the effects of exposure to two or more polluting substances can result in additive effects or adverse interactions on the body. Thus, in this study, the cumulative health risk was also evaluated by adding up the THQ values of each metal and expressing them as Total THQ (TTHQ) (Hallenbeck, 1993) with the following:

$$TTHQ = THQ\ (toxicant\ 1) + THQ\ (toxicant\ 2) + \cdots THQ\ (toxicant\ n)$$

The higher the TTHQ value, the higher the toxic effect that may be caused (Ullah *et al*., 2017).

2.4.3 Target cancer risk for arsenic

Target cancer risk (TCR) was used to assess the potential for the occurrence of risks associated with exposure to carcinogenic agents over a period of lifetime exposure to metallic arsenic. The equation for the TCR of arsenic is as follows:

$$TCR = \frac{E_{FR} \times E_D \times F_{IR} \times C \times CPS_o}{BWa \times ATc} \times 10^{-3}$$

Where E_{FR} is the frequency of arsenic exposure (365 days), E_D is the duration of exposure (71.5 years), F_{IR} is the level of consumption of seafood including seaweed in Indonesia in grams/day, *C* is the concentration of arsenic in seaweed dry weight, CPS_O is the baseline factor for oral cancer for inorganic arsenic (1.5 ppm/day) (Antoine *et al*., 2017), and BWa is the reference body weight. AT_C is the average period of carcinogen exposure (365 days 71.5 years), and 10^{-3} is the unit conversion factor. The standard TCR value refers to previous studies, which showed values between 10^{-6} - 10^{-4} and considered acceptable, values less than 10^{-6} were negligible, and values greater than 10^{-4} were at increased risk for cancer (Shaheen *et al*., 2016; U.S. EPA, 1997). The carcinogenicity of Pb, Cd, and Hg has not been established to date according to the U.S. EPA (ATSDR, 1999).

2.5 Data Analysis

To calculate the average and standard deviation of the seaweed sample data replication using SPSS Version 23.0 software (IBM, USA) (Hussain *et al*., 2021). Graphs were processed using GraphPad software Version 8.0 (GraphPad, USA) (Widhiantara *et al*., 2021). Tabulation of EDI, THQ, TTHQ, and target cancer risk of As data using Ms. Software. Excel 2019 (Microsoft, USA) (Wasilah *et al*., 2021).

3. Results and Discussion

3.1 Content of Heavy Metals on Brown Seaweed

The highest to lowest Pb content are as following: *T. ornata*. (0.5737 ppm), *P. australis* (0.3687 ppm), and *S. aquifolium* (0.2690 ppm). Cd content ranged from 0.2367 ppm in *T. ornata*, 0.2123 ppm in *P. australis*, and 0.1630 ppm in *S. aquifolium* (Figure 2). Meanwhile, *S. aquifolium* and *P. australis* had the same Hg level of 0.00533 ppm, and in *T. ornata* of 0.00333 ppm. Furthermore, the highest and lowest As levels were shown in *S. aquifolium* (4,316 ppm), *T. ornata* (3.7973 ppm), and *P. australis* (3,421 ppm) (Table 1). All values are reported as dry weight. All concentrations of heavy metals contained in the three samples of brown seaweed are still safe based on BPOM No. 32 of 2019 concerning Safety and Quality Requirements for Herbal Medicines.

This work provides a significant overview of the challenges leading to the development of seaweed which will be further investigated as a pharmaceutical ingredient, especially as an herbal medicine in the future (Widhiantara and Jawi, 2021). However, because heavy metals act as limiting factors, besides having important functions in plant physiology, including seaweed as a source of micronutrients for growth, it becomes dangerous if the amount is above the tolerance threshold (Esposito *et al*., 2018; Lentini *et al*., 2018). The presence of non-essential heavy metals Pb and Cd in this study is thought to be related to domestic waste and the presence of hotels in the coastal area of Sanur which subsequently accumulates in seaweed. The marine environment (estuarine/coastal) is more vulnerable to heavy metal exposure from a variety of sources, including agricultural waste that uses synthetic pesticides irresponsibly, household and tourism waste (Zn, Cu, Cd, Ni, and Pb), and industrial activities (Hg, As, zinc, and hydrocarbons) (Tayeb *et al*., 2015).

Cd and Pb accumulation events have also been documented in seaweeds collected from the Gulf Coast of Mannar, India, the source of which has been linked to anthropogenic activities off the coast of Tuticorin, India (Anbazhagan *et al*., 2021). It should be highlighted that changes in heavy metal accumulation in each seaweeds are influenced by a variety of variables, including the type, age, and environment/habitat for algae development. Young tissue absorbs metal elements from the environment rapidly than old tissues, however metal elements are discharged more rapidly as the surrounding water concentration decreases/in low tide environments (Stengel and Dring, 2000).

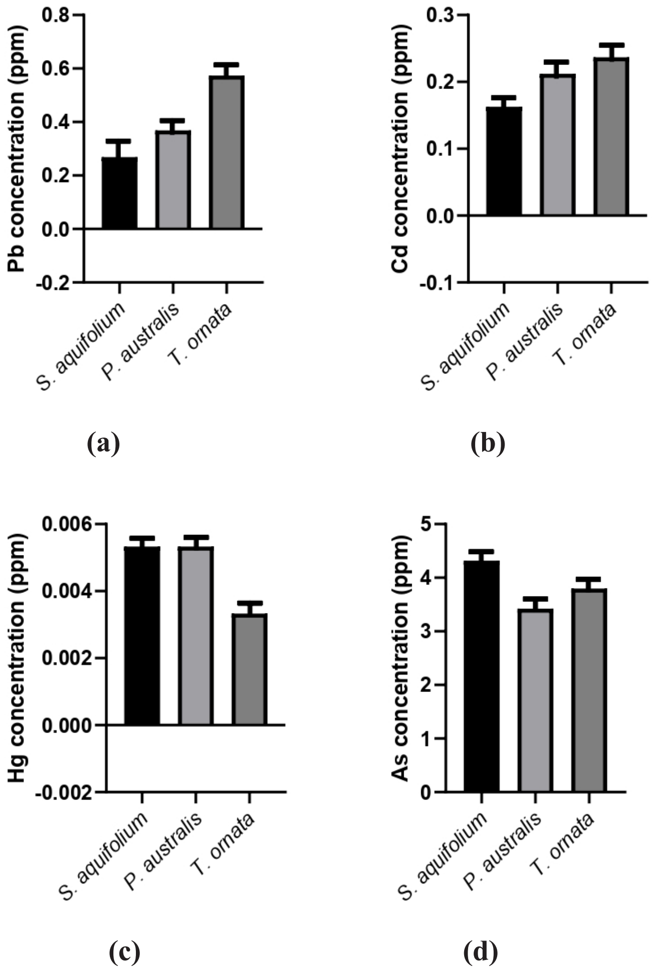

Figure 2. Average concentrations of Pb (a), Cd (b), Hg (c), and As (d) in brown seaweed collected from the waters of Sanur Beach, Denpasar-Bali. The concentration of metal elements in this study is below the BPOM quality standard No. 32 of 2019 regarding the Safety and Quality Requirements for Herbal Medicines.

This research found Hg in brown seaweed, which is still below the 2019 BPOM No. 32. According to the earlier research, the Hg concentration in Phaeophyceae collected from various seafood markets in Italy was 0.001 ppm, as well as many varieties of seaweed collected from Spain (0.011 – 0.017 ppm), Korea (0.006 – 0.026 ppm), and China (0.005 – 0.011 ppm) (Filippini *et al*., 2021). However, further study on the prevalence of this metal from varied sources in the environments is recommended (including season factor). Because naturally occurring Hg is produced continually throughout the weathering process and subsequently migrates to aquatic areas (Spyropoulou *et al*., 2022). However, it has been recognized that human activities contribute for around 30% of total Hg that enters the atmosphere each year and then impacts the surface environment (soil, fresh water, and seas) (Streets *et al*., 2011). Bacteria in marine habitats may convert inorganic mercury (Hg) to methylmercury, which can progressively accumulate in aquatic species such as seaweed. Phytoplankton, including seaweed, may absorb mercury greater than other aquatic organisms (Le Faucheur *et al*., 2014). Greater trophic level organisms (including humans) will have a higher mercury concentration than lower trophic level organisms.

Based on BPOM No. 32 of 2019, the As metal measurement findings in the three species of brown algae in this investigation were remained within the allowable limits. A recent research revealed that the As value of seaweed taken from South Korean seas varied from 1.72 to 9.46 ppm (Ryu *et al*., 2009), but the reported As value of seaweed in Japan was greater at 46.4 – 147 ppm (Narukawa *et al*., 2012). Interestingly, multiple investigations have shown that the Sargassum family contains up to 72 % arsenic as inorganic arsenic (Almela *et al*., 2002). The levels of As (III) and As (V) in the family Sargassaceae obtained from the Gulf of South Korea were 2.35 ppm and 5,347 ppm, respectively, similar to this findings (Khan *et al*., 2015).

3.2 Public Health Risk Assessment

3.2.1 Estimated daily intake (EDI)

The EDI findings are based on a variety of criteria (Table 2). The EDI in this research is based on food balances derived from Food and Agriculture Organization data (FAO). However, the inclusion of numerous criteria in the assessment of EDI may result in an overestimation to quantify the non-carcinogenic risk caused by ingestion of brown seaweed as in this study. As a result, further study is required to understand the non-carcinogenic factors that may result from the intake of this brown seaweed.

T. ornata exhibited a significant concentrations of Pb consumption in adults and children, with values of 0.027 mg/kg bw/day and 0.093 mg/kg bw/day, respectively. *T. ornata* had the greatest amount of Cd consumption, with 0.080 mg/kg bw/day (adults) and 0.269 mg/kg bw/day (children). *P. australis* adults (0.091 mg/kg bw/day) and children (0.30 mg/kg bw/day) had the greatest amount of daily Hg intake.

Table 1. Heavy metal concentrations in brown seaweed species collected from different locations were compared to values in this study

No.	Species	Location	Heavy metals (ppm)				Reference
			Pb	Cd	Hg	As	
1	*Sargassum polycystum*	Gulf of Mannar, Kerala Coast, India	12,36	0,38	-	-	(Anbazhagan *et al*., 2021)
2	*Cystoseira crinita*	Marsa-Matrouh beaches, Egypt, Mediterranean Sea	16.32 – 19.75	0.34 – 0.83	-	-	(Khaled *et al*., 2014)
3	*Laminaria* sp.	Seaweed market in Italy	0,11	0,21	0,03	7,14	(Filippini *et al*., 2021)
4	*Padina pavonica*	Wandoor area, Southern Andaman Island,	-	0,006	-	-	(Kaviarasan *et al*., 2018)
5	*Sargassum aquifolium*.	Sanur Beach Coast, Denpasar, Bali Province	0,269	0,163	0,005	4,316	This study
6	*Padina australis*	Sanur Beach Coast, Denpasar, Bali Province	0,368	0,212	0,005	3,421	This study
7	*Turbinaria ornata*	Sanur Beach Coast, Denpasar, Bali Province	0,573	0,236	0,003	3,797	This study
Quality Standards			**≤ 10**	**≤ 0.3**	**≤ 0.5**	**≤ 5**	BPOM No. 32 year of 2019

Table 2. Estimated daily intake of Pb, Cd, Hg, and As through consumption of brown seaweed collected from the waters of Sanur Beach, Denpasar-Bali

Heavy metals	Species	EDI	
		Adult (mg/kg bw/days)	Children (mg/kg bw/days)
	Sargassum aquifolium	0,013	0,043
Pb	*Turbinaria ornata*	0,027	0,093
	Padina australis	0,017	0,059
	Sargassum aquifolium	0,055	0,185
Cd	*Turbinaria ornata*	0,04	0,269
	Padina australis	0,02	0,241
	Sargassum aquifolium	0,03	0,03
	Turbinaria ornata	0,056	0,018
Hg	*Padina australis*	0,091	0,03
	Sargassum aquifolium	0,739	2,466
	Turbinaria ornata	0,584	
As	*Padina australis*	0,648	2,161

Table 3. THQ and TTHQ values for heavy metals were analyzed in brown seaweed collected from the coastal waters of Sanur, Denpasar-Bali

Heavy metals	Species	Population	THQ (mg/kg/days)
Pb	*S. aquifolium*	Adult	0.00612
		Children	0.00184
	T. ornata	Adult	0.00131
		Children	0.00392
	P. australis	Adult	0.00839
		Children	0.00137
Cd	*S. aquifolium*	Adult	0.00260
		Children	0.00779
	T. ornata	Adult	0.00377
		Children	0.00113
	P. australis	Adult	0.00338
		Children	0.00102
Hg	*S. aquifolium*	Adult	0.00425
		Children	0.00127
	T. ornata	Adult	0.00266
		Children	0.00797
	P. australis	Adult	0.00425
		Children	0.00127
As	*S. aquifolium*	Adult	0.00424
		Children	0.00130
	T. ornata	Adult	0.00335
		Children	0.00100
	P. australis	Adult	0.00372
		Children	0.00110
Total Target Hazard Quotients (TTHQ)			**0.07902**

Table 4. Target Cancer Risk (TCR) for arsenic analysis in brown seaweed in this study

Species	Adult	Children
S. aquifolium	7.56×10^{-6}	2.52×10^{-7}
P. australis	6.63×10^{-6}	2.21×10^{-7}
T. ornata	5.97×10^{-6}	1.99×10^{-7}

The same quantity of Hg was detected in *S. aquifolium* for adults and children - 0.030 mg/kg bw/day. *S. aquifolium* had the largest daily As consumption, namely in adults (0.739 mg/kg bw/day) and children (2.466 mg/kg bw/day).

Based on this observation, the heavy metal intake in each brown seaweed studied in this research may be utilized as a reference value for customers or relevant authorities who produce brown algae as a raw material for herbal medicines. This is associated because seaweed is an essential component of food additives (Leandro *et al*., 2020) and, on the other hand, is thought to be suited for use as an objective bioindicator of aquatic environments (Hasselström *et al*., 2018). The accumulation of heavy metals in the food chain as a result of human pressure has increased the value of information that highlights the estimated daily consumption of a food item from public waterways (Ali *et al*., 2019).

3.2.2 Target Hazard Quotients (THQ)

THQ and TTHQ were approved to examine potential non-carcinogenic impact (Table 3). THQ was calculated using the average heavy metal content of brown seaweed samples. THQ value of each metal is less than one, indicating that the general public or consumers will not face major health hazards because they just consume individual heavy metals from each variety of seaweed (Table 3) (Ullah *et al*., 2017). Additionally, the TTHQ in this study was 0.07902 mg/kg/day (<1), indicating that there is no possible health risk from consuming seaweed containing a combination of the four heavy metals examined (Wasilah *et al*., 2021).

3.2.3 Carcinogenic Risk for Arsenic (As)

The value of our findings ranged from 5.97×10^{-6} in *T. ornata* up to 7.56×10^{-6} on *S. aquifolium* which is this range for target adults. Arsenic for the target children, the range from 1.99×10^{-7} in *T. ornata* and 2.52×10^{-7} on *S. aquifolium* (Table 4). This results are important because the consumption of foodstuffs contaminated with heavy metals such as As in the long term throughout life every day can be considered a carcinogenic effect. According to the U.S. EPA (i) if the value of cancer risk is between 10^{-6}-10^{-4}, it is considered to be acceptable; (ii) if the value is less than 10^{-6}, it may be disregarded; and (iii) if the value is more than 10^{-4}, it is unacceptable (Hussain *et al*., 2021). According to the findings of this study, the arsenic found in three types of brown seaweed does not pose a major hazard to human health. However, further studies are needed to establish the carcinogenic effects demonstrated in in vivo trials (Shaheen *et al*., 2016).

4. Conclusion

In summary, according to the BPOM No. 32 of 2019 concerning the criteria for the Safety and Quality of Herbal Medicines, the evaluated brown seaweed had permissible levels of heavy metal elements. In the case of EDI, THQ, and TTHQ, there is no unacceptable danger of hazardous health consequences to consumers, since there is no carcinogenicity. Although further information is required to confirm this conclusion, the examined brown seaweed was under the tolerable cancer risk level for arsenic. Future research is required to be more comprehensive in evaluating the effect value on many other seaweed species, particularly in Bali Province. This is important because seaweed is a biological resource with high quality value in the pharmaceutical sector, and the situation of Bali's waters, which is exploited as a tourist industry, allows for the establishment of increasing anthropogenic exposure in coastal waters.

Acknowledgment

The researcher would like to appreciate to the Oceanography Laboratory of the National Research and Innovation Agency (BRIN), Jakarta for the support of scientific and technical assistance in this research.

Author's Contribution

I Wayan Rosiana and I Gede Widhiantara; conceptualization, supervision, grant administration. Putu Angga Wiradana and Anak Agung Ayu Putri Permatasari; data analysis, writing, editing, and drafting the manuscript. Yesha Ainensis El G. Pelupessy and Matius Victorino Ola Dame; designed the sampling and collected the data. Agoes Soegianto and Bambang Yulianto; review and proofread the manuscript. All authors discussed the results and contributed to final manuscript.

Conflict of Interest

The authors declare that they have no competing interests.

Funding Information

This research was partially supported by the Institute for Research and Community Service (LP2M)

University of Dhyana Pura (UNDHIRA) Bali through a Beginner Lecturer Grant with a contract number: 41/UNDHIRA-LPPM/PPM/2021.

References

Ali, H., Khan, E., & Ilahi, I. (2019). Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity, and bioaccumulation. *Journal of Chemistry*, 2019:1-14.

Almela, C., Algora, S., Benito, V., Clemente, M. J., Devesa, V., Súñer, M. A., Vélez, D., & Montoro, R. (2002). Heavy metal, total arsenic, and inorganic arsenic contents of algae food products. *Journal of Agricultural and Food Chemistry*, 50(4):918-923.

Anbazhagan, V., Partheeban, E. C., Arumugam, G., Arumugam, A., Rajendran, R., Paray, B. A., Al-Sadoon, M. K., & Al-Mfarij, A. R. (2021). Health risk assessment and bioaccumulation of metals in brown and red seaweeds collected from a tropical marine biosphere reserve. *Marine Pollution Bulletin*, 164:112029.

Antoine, J. M. R., Fung, L. A. H., & Grant, C. N. (2017). Assessment of the potential health risks associated with the aluminium, arsenic, cadmium and lead content in selected fruits and vegetables grown in Jamaica. *Toxicology Reports*, 4:181-187.

ATSDR. (1999). Agency for toxic substances & disease registry, Toxicological Profile.

Chen, Q., Pan, X. D., Huang, B. F., & Han, J. L. (2018). Distribution of metals and metalloids in dried seaweeds and health risk to population in Southeastern China. *Scientific Reports*, 8(3578):1-7.

Choudhary, B., Chauhan, O. P., & Mishra, A. (2021). Edible seaweeds: A potential novel source of bioactive metabolites and nutraceuticals with human health benefits. *Frontiers in Marine Science*, 8:740054.

Costa, S., & Teixeira, J. P. (2014). Encyclopedia of toxicology. *Reference Module in Biomedical Science*, 718-720.

Cunha, L., & Grenha, A. (2016). Sulfated seaweed polysaccharides as multifunctional materials in drug delivery applications. *Marine Drugs*, 14(3):42.

Esposito, S., Loppi, S., Monaci, F., Paoli, L., Vannini, A., Sorbo, S., Maresca, V., Fusaro, L., Asadi karam, E., Lentini, M., De Lillo, A., Conte, B., Cianciullo, P., & Basile, A. (2018). In-field and in-vitro study of the moss *Leptodictyum riparium* as bioindicator of toxic metal pollution in the aquatic environment: Ultrastructural damage, oxidative stress and HSP70 induction. *PLoS ONE*, 13(4):e0195717.

FAO/SIDA. (1983). Manual of methods in aquatic environmental research, Part 9. Analyses of spatial distribution of trace elements in macroalgae species from the Todos los Santos Bay, Bahía Brazil. *Marine Pollution Bulletin*, 64:2238-2244.

Filippini, M., Baldisserotto, A., Menotta, S., Fedrizzi, G., Rubini, S., Gigliotti, D., Valpiani, G., Buzzi, R., Manfredini, S., & Vertuani, S. (2021). Heavy metals and potential risks in edible seaweed on the market in Italy. *Chemosphere*, 263:127983.

Ganesan, A. R., Subramani, K., Balasubramanian, B., Liu, W. C., Arasu, M. V., Al-Dhabi, N. A., & Duraipandiyan, V. (2020). Evaluation of in vivo sub-chronic and heavy metal toxicity of under-exploited seaweeds for food application. *Journal of King Saud University - Science*, 32(1):1088-1095.

Gomez-Zavaglia, A., Lage, M. A. P., Jimenez-Lopez, C., Mejuto, J. C., & Simal-Gandara, J. (2019). The potential of seaweeds as a source of functional ingredients of prebiotic and antioxidant value. *Antioxidants*, 8(9):406.

Hallenbeck, W. H. (1993). Quantitative risk assessment for environmental and occupational health (2nd ed.). Florida: CRC Press.

Hasselström, L., Visch, W., Gröndahl, F., Nylund, G. M., & Pavia, H. (2018). The impact of seaweed cultivation on ecosystem services - a case study from the west coast of Sweden. *Marine Pollution Bulletin*, 133:53-64.

Hussain, N., Ahmed, K. S., Asmatullah, Ahmed, M. S., Hussain, S. M., & Javid, A. (2021). Potential health risks assessment cognate with selected heavy metals contents in some vegetables grown with four different irrigation sources near Lahore, Pakistan. *Saudi Journal of Biological Sciences*, 29(3):1813-1824.

Hwang, E. K., Yotsukura, N., Pang, S. J., Su, L., & Shan, T. F. (2019). Seaweed breeding programs and progress in eastern Asian countries. *Phycologia*, 58(5):484-495.

Kaviarasan, T., Gokul, M. S., Henciya, S., Muthukumar, K., Dahms, H. U., & James, R. A. (2018). Trace metal inference on seaweeds in Wandoor Area, Southern Andaman Island. *Bulletin of Environmental Contamination and Toxicology*, 100(5):614-619.

Kerr, S. B., Bonczek, R. R., McGinn, C. W., Land, M. L., Bloom, L. D., Sample, B. E., & Dolislager, F. G. (1998). The risk assessment information system. United States: Oak Ridge National Lab (ORNL).

Khaled, A., Hessein, A., Abdel-Halim, A. M., & Morsy, F. M. (2014). Distribution of heavy metals in seaweeds collected along Marsa-Matrouh beaches, Egyptian Mediterranean Sea. *The Egyptian Journal of Aquatic Research*, 40(4):363-371.

Khan, N., Ryu, K. Y., Choi, J. Y., Nho, E. Y., Habte, G., Choi, H., Kim, M. H., Park, K. S., & Kim, K. S. (2015). Determination of toxic heavy metals and speciation of arsenic in seaweeds from South Korea. *Food Chemistry*, 169:464-470.

Lancaster, V. A., & Keller-mcnulty, S. (1998). A review of composite sampling methods. *Journal of the American Statistical Association*, 93(443):1216–1230.

Le Faucheur, S., Campbell, P. G. C., Fortin, C., & Slaveykova, V. I. (2014). Interactions between mercury and phytoplankton: Speciation, bioavailability, and internal handling. *Environmental Toxicology and Chemistry*, 33(6):1211-1224.

Leandro, A., Pacheco, D., Cotas, J., Marques, J. C., Pereira, L., & Gonçalves, A. M. M. (2020). Seaweed's bioactive candidate compounds to food industry and global food security. *Life*, 10(8):140.

Lentini, M., De Lillo, A., Paradisone, V., Liberti, D., Landi, S., & Esposito, S. (2018). Early responses to cadmium exposure in barley plants: effects on biometric and physiological parameters. *Acta Physiologiae Plantarum*, 40(178).

Lloret, J., Carreño, A., Carić, H., San, J., & Fleming, L. E. (2021). Environmental and human health impacts of cruise tourism: A review. *Marine Pollution Bulletin*, 173:112979.

Mekinić, I. G., Skroza, D., Šimat, V., Hamed, I., Čagalj, M., & Perković, Z. P. (2019). Phenolic content of brown algae (Pheophyceae) species: Extraction, identification, and quantification. *Biomolecules*, 9(6):244.

Monagail, M. M., & Morrison, L. (2020). The seaweed resources of Ireland: a twenty-first century perspective. *Journal of Applied Phycology*, 32(2):1287-1300.

Narukawa, T., Hioki, A., & Chiba, K. (2012). Aqueous extraction of water-soluble inorganic arsenic in marine algae for speciation analysis. *Analytical Sciences*, 28(8):773-779.

Pérez-Lloréns, J. L. (2019). Seaweed consumption in the Americas. *Gastronomica*, 19(4):49-59.

Permatasari, A. A. A. P., Rosiana, I. W., Wiradana, P. A., Lestari, M. D., Widiastuti, N. K., Kurniawan, S. B., & Widhiantara, I. G. (2022). Extraction and characterization of sodium alginate from three brown algae collected from Sanur Coastal Waters, Bali as biopolymer agent. *Biodiversitas Journal of Biological Diversity*, 23(3):1655-1663.

Piñeiro-Ramil, M., Flórez-Fernández, N., Ramil-Gómez, O., Torres, M. D., Dominguez, H., Blanco, F. J., Meijide-Faílde, R., & Vaamonde-García, C. (2022). Antifibrotic effect of brown algae-derived fucoidans on osteoarthritic fibroblast-like synoviocytes. *Carbohydrate Polymers*, 282:119134.

Rakib, M. R. J., Jolly, Y. N., Dioses-Salinas, D. C., Pizarro-Ortega, C. I., De-la-Torre, G. E., Khandaker, M. U., Alsubaie, A., Almalki, A. S. A., & Bradley, D. A. (2021). Macroalgae in biomonitoring of metal pollution in the Bay of Bengal coastal waters of Cox's Bazar and surrounding areas. *Scientific Reports*, 11(20999):1-13.

Reyes, M. E., Riquelme, I., Salvo, T., Zanella, L., Letelier, P., & Brebi, P. (2020). Brown seaweed fucoidan in cancer: Implications in metastasis and drug resistance. *Marine Drugs*, 18(5):232.

Rimmer, M. A., Larson, S., Lapong, I., Purnomo, A. H., Pong-Masak, P. R., Swanepoel, L., & Paul, N. A. (2021). Seaweed aquaculture in Indonesia contributes to social and economic aspects of livelihoods and community wellbeing. *Sustainability*, 13(19):10946.

Roleda, M. Y., Marfaing, H., Desnica, N., Jónsdóttir, R., Skjermo, J., Rebours, C., & Nitschke, U. (2019). Variations in polyphenol and heavy metal contents of wild-harvested and cultivated seaweed bulk biomass: Health risk assessment and implication for food applications. *Food Control*, 95:121-134.

Ryu, K. Y., Shim, S. L., Hwang, I. M., Jung, M. S., Jun, S. N., Seo, H. Y., Park, J. S., Kim, H. Y., Om, A. S., Park, K. S., & Kim, K. S. (2009). Arsenic speciation and risk assesment of hijiki (*Hizikia fusiforme*) by HPLC-ICP-MS. *Korean Journal of Food Science and Technology*, 41(1):1-6.

Samsonchi, Z., Karimi, H., Izadi, Z., Baei, P., Najarasl, M., Ashtiani, M. K., Mohammadi, J., Moazenchi, M., Tahamtani, Y., Baharvand, H., Hajizadeh-Saffar, E., & Daemi, H. (2022). Transplantation of islet-containing microcapsules modified with constitutional isomers of sulfated alginate in diabetic mice to mitigate fibrosis for long-term glycemic control. *Chemical Engineering Journal*, 432:134298.

Shaheen, N., Ahmed, M. K., Islam, M. S., Habibullah-Al-Mamun, M., Tukun, A. B., Islam, S., & Rahim, A. T. M. A. (2016). Health risk assessment of trace elements via dietary intake of 'non-piscine protein source' foodstuffs (meat, milk and egg) in Bangladesh. *Environmental Science and Pollution Research*, 23(8):7794-7806.

Spyropoulou, A. E., Lazarou, Y. G., Sapalidis, A. A., & Laspidou, C. S. (2022). Geochemical modeling of mercury in coastal groundwater. *Chemosphere*, 286(1):131609.

Stengel, D. B., & Dring, M. J. (2000). Copper and iron concentrations in *Ascophyllum nodosum* (Fucales, Phaeophyta) from different sites in Ireland and after culture experiments in relation to thallus age and epiphytism. *Journal of Experimental Marine Biology and Ecology*, 246(2):145-161.

Streets, D. G., Devane, M. K., Lu, Z., Bond, T. C., Sunderland, E. M., & Jacob, D. J. (2011). All-time releases of mercury to the atmosphere from human activities. *Environmental Science & Technology*, 45(24):10485-10491.

Suartika, G. A. M. (2015). Sand, sea and ceremony: conflict over the littoral public realm in Sanur, Bali. International Conference Green Architecture for Sustainable Living and Environment (GASLE). *Procedia - Social and Behavioral Science*, 179(2015):128-140.

Sudarwati, W., Hardjomidjojo, H., Machfud, & Setyaningsih, D. (2020). Literature review: potential and opportunities for the development of seaweed agro-industry. *IOP Conference Series: Earth and Environmental Science*, 472(1):012063.

Tayeb, A., Chellali, M. R., Hamou, A., & Debbah, S. (2015). Impact of urban and industrial effluents on the coastal marine environment in Oran, Algeria. *Marine Pollution Bulletin*, 98(1–2):281-288.

Turak, E., & Devantier, L. (2013). Biodiversity and conservation priorities of reef-building corals in Bali, Indonesia. BioOne Complete.

Ullah, A. K. M. A., Maksud, M. A., Khan, S. R., Lutfa, L. N., & Quraishi, S. B. (2017). Dietary intake of heavy metals from eight highly consumed species of cultured fish and possible human health risk implications in Bangladesh. *Toxicology Reports*, 4:574-579.

U. S. EPA (United States Environmental Protection Agency). (1997). Exposure factors handbook. Washington D.C.: U.S. EPA.

Vijayakumar, S., Chen, J., Kalaiselvi, V., Divya, M., González-Sánchez, Z. I., Durán-Lara, E. F., & Vaseeharan, B. (2021). Antibacterial and antibiofilm activities of marine polysaccharide laminarin formulated gold nanoparticles: An ecotoxicity and cytotoxicity assessment. *Journal of Environmental Chemical Engineering*, 9(4):105514.

Wasilah, Q. A., Mawli, R. E., Sani, M. D., Soegianto, A., Wiradana, P. A., & Pradisty, N. A. (2021). Determination of lead and cadmium in edible wedge clam (*Donax faba*) collected from North and South Coasts of Sumenep, East Java, Indonesia. *Pollution Research Journal*, 40(2):593-597.

Widhiantara, I. G., & Jawi, I. M. (2021). Phytochemical composition and health properties of Sembung plant (*Blumea balsamifera*): A review. *Veterinary World*, 14(5):1185-1196.

Widhiantara, I. G., Permatasari, A. A. A. P., Rosiana, I. W., Wiradana, P. A., Widiastini, L. P., & Jawi,

I. M. (2021). Antihypercholesterolemic and antioxidant effects of *Blumea balsamifera* L. leaf extracts to maintain luteinizing hormone secretion in rats induced by high-cholesterol diets. *The Indonesian Biomedical Journal*, 13(4):396-402.

Wiradana, P. A., Widhiantara, I. G., Pradisty, N. A., & Mukti, A. T. (2021). The impact of COVID-19 on Indonesian fisheries conditions: opinion of current status and recommendations. IOP Conference Series: *Earth and Environmental Science*, 718(1):012020.

Yamagata, K. (2021). Prevention of cardiovascular disease through modulation of endothelial cell function by dietary seaweed intake. *Phytomedicine Plus*, 1(2):100026.

Zaw, N. D. K., Wiradana, P. A., Naw, S. W., Nege, A. S., Alamsjah, M. A., Akbar, R. E. K., & Rosi, F. (2020). First report on molecular identification of Caulerpa green algae from Mandangin Island Indonesia using partial 18SrRNA genes. *Journal of Aquaculture and Fish Health*, 9(3):252.

Zaynab, M., Al-Yahyai, R., Ameen, A., Sharif, Y., Ali, L., Fatima, M., Khan, K. A., & Li, S. (2022). Health and environmental effects of heavy metals. *Journal of King Saud University - Science*, 34(1):101653.

Zhong, H., Gao, X., Cheng, C., Liu, C., Wang, Q., & Han, X. (2020). The structural characteristics of seaweed polysaccharides and their application in gel drug delivery systems. *Marine Drugs*, 18(12):658.

