Arab Universities Journal of Agricultural Sciences
(2022) 30 (1) 97-105
Website: http://ajs.journals.ekb.eg

Element Composition and Toxicity of Seaweed Algae *(Ulva lactuca, Laurencia obtuse* and *Padina pavonica*) Toward Shrimp

Ahmed MH Abd El-ghfar1[1]*, Ibrahim M Hassan[2], Manar T Ibrahim[2], Hayam M Ibrahim[1]

1- Food Science & Technology Department, National Research Centre, Cairo, Egypt

2- Food Science Department, Faculty of Agriculture Ain Shams University, P.O. Box 68, Hadayek Shoubra 11241, Cairo, Egypt

*Corresponding author: dr.ahmed771990@gmail.com

http://10.21608/AJS.2022.100471.1426

Received 19 October 2021; Accepted 1 February 2022

Keywords:

Algae minerals,
Laurencia obtusa,
Ulva lactuca,
Padina pavonica
Brine shrimp method,
Cytotoxicity

Abstract: This study examined the macro and trace element contents as well as the cytotoxicity of *Laurencia obtusa*, *Ulva lactuca*, and *Padina pavonica* algae collected from the Mediterranean coast of Egypt (Alexandria coast). The results indicated that the seaweed samples contained high concentrations of Ca, Cl, Mg, K, Na, P and Fe. The Cu and Zn contents of the algae were in the ranges of 0.39-0.95 mg/100 g dw and 0.11-0.49 mg/100 g dw respectively. Fresh red algae contained the highest level of Zn and Cu, whereas the iodine level was 1.52, 1.27 and 1.01 mg/100g in fresh each of *Padina pavonica*, Laurencia obtuse and *Ulva lactuca* respectively. Fresh red, fresh green, dry green, dry red, in addition to fresh and dried brown algae had maximum lead (Pb) levels of 0.16, 0.14, 0.13, 0.12, 0.04 and 0.02 g/100 g respectively. Fresh red algae contained the highest Cd and Mn levels, whereas dried brown algae exhibited the lowest Cd and Mn concentration. Different extracts of fresh and dried brown algae exhibited significant larvicidal activity against brine shrimp. LC_{50} values indicated that dry samples were more cytotoxic than fresh samples.

1 Introduction

Seaweeds are primitive macrophytic algae with no true roots, stems, or leaves. They belong to the Phaeophyta (brown), Chlorophyta (green), and Rhodophyta (red) seaweed families (Ganesan et al 2020). In many countries, seaweeds are important coastal resources for human consumption and the environment. Seaweeds were widely consumed fresh, dried, or added to prepared foods, particularly in Asian countries (Peñalver et al 2020).

There are 250 macroalgae species that are commercially used around the world and 150 of them are consumed by humans (Veena et al 2007). Seaweed, or marine macroalgae, is an important biological resources, as it contains a diverse range of bioactive compounds. Many marine macroalgae have long been used as medicines and food ingredients in various parts of the world (Mišurcová et al 2011). Because of their low lipid content, high polysaccharide concentration, mineral content, polyunsaturated fatty acid content, vitamins, and dietary fiber, seaweeds are considered a healthy food source. The beneficial properties of seaweed primarily due to result from polysaccharides, sterols, polyphenols, and other bioactive molecules. These compounds have antioxidant, anti-inflammatory, anti-cancer, and anti-diabetic properties (Peñalver et al 2020). The nutritional value of seaweed is estimated by their mineral content, which includes magnesium, sodium, potassium, phosphorous, iron, iodine, and zinc (Bocanegra et al 2009). Because of their marine habitat, seaweed has a high and diverse mineral content. Seaweed contains all of the essential and trace minerals that humans require (Vashuki et al 2020). Thus, seaweed is an ideal candidate for use as ingredients in the food industry for the development of functional foods (Circunciso et al 2018). Seaweed may be used as a NaCl substitute in processed foods to reduce sodium consumption along with increased levels of potassium and other minerals that would otherwise be lacking in NaCl-salted foods.

The high mineral content (8%–40%) in seaweed is linked to several factors including the seaweed phylum, its geographic location, and the season. It is also associated with environmental and physiological changes. Public awareness of health issues has increased in recent years. As a result, the demand for seaweed as a source of food and biochemical resources has steadily increased (Vashuki et al 2020). Adults may consume edible seaweeds as a food supplement to obtain their daily macro, micro, and trace element needs (Rupérez 2002).

Ash content varies from 21 to 32 g/100 g, whereas 20 mg/100 g is considered high and is associated with mineral elements. Furthermore, the presence of high mineral levels in seaweed has important implications in the food industry (Ganesan et al 2020). Seaweed accumulates heavy metals because the marine environment contains a wide range of mineral concentrations, and heavy metals can accumulate in organs after long-term consumption (Abirami and Kowsalya 2012).

In addition to the cell wall of seaweeds, other chemical and physical parameters (oxidation of minerals, seawater temperature, pH, salinity, light, etc.) may influence mineral uptake. Most of these variables influence macroalgae chemical composition, are seasonally dependent, and influence nutrient uptake mechanisms. The morphological properties and life phase of macroalgae must also be considered (Circunciso et al 2018). Regulatory bodies should establish guidelines for the amount and quality of seaweed for consumption, as well as potential drug-seaweed interactions (Kumar and Sharma 2020).

The goal of study was to examine the content of macro elements (Na, Ca, K, Mg, Fe, CI, and P), trace elements (Zn, Cu, and I), and toxic elements (Cd, Pb, Co, Mn, and Cr) in three different seaweeds from the Mediterranean coast of Egypt including red (*Laurencia obtusa*), green (*Ulva lactuca*), and brown (*Padina pavonica*) (Alexandria coast) varieties.

2 Materials and Methods

2.1 Sample collection and preparation

Different macroalgae species representing different classes, including red (*Laurencia obtusa* family Rhodomelaceae), green (*Ulva lactuca* family Ulvaceae), and brown (*Padina pavonica* family Dictyotaceae, were collected from three locations (Abu Qir Bay) along the Egyptian Mediterranean coast (Alexandria) during June 2019. To remove foreign particles, sand particles, and epiphytes, the seaweed was collected by hand and immediately washed with sea water. The samples were then preserved in an ice-box and transported to the NRC laboratory, where they were thoroughly washed with tap water to remove salt on the surface. The samples were identified in Faculty of Science, Alexandria University according to morphological inspection **(**Aleem 1993).

Dry seaweed prepared by drying in an air oven (SHELLAB - Model 1350FX - Made in USA) at 40°C ± 2°C (about 4 h) until dryness. The dried samples were ground and sieved with a 1 mm size mesh. The ground algae samples were stored at room temperature in plastic bags until further analysis.

2.2 Estimation of mineral content

Ash contents were determined according to methods reported by the AOAC (2005). Mineral contents of Na and K were determined in diluted solutions of ash samples with an emission flame photometer (Brown and Lilleland 1946). The following nutrient minerals (%dry weight) were measured: Nitrogen (N) was determined by the modified micro-Kjeldahl method (Pregl 1945); P-was determined by Chapman and Pratt (1961). Cu, Fe, Zn, I, and Mg in ppm were estimated using atomic absorption spectrometry (spectronic 21 D) as described by Jackson (1973). Co, Cd, Mn, Cr, and Pb were measured by ICP-MS using Thermo Elemental X 7 instrument.

2.3 Cytotoxicity bioassay

2.3.1 Algae extract preparation

To determine the cytotoxicity of non-polar and polar components, dried algae was extracted sequentially with four different solvents: petroleum ether, chloroform, isopropanol, and water. The algae were soaked overnight in shaking incubator under aseptic conditions and then filtered through Whatman filter paper 1. Using a rotary evaporator, the extracts were concentrated at low temperature (40°C) and pressure.

2.3.2 Brine shrimp lethality bioassay (BSLBA)

The cytotoxicity of the algae extracts was investigated using a brine shrimp lethality bioassay (BSLBA). The BSLBA is a simple method for determining the toxicity of various substances. The lethality bioassay for brine shrimp is a predictor of cytotoxicity and harmful activity (Krishnaraju et al 2005). This in vivo lethality assay has been used in a registered study of cytotoxic and antitumor agents with great success.

Brine shrimp eggs were a gift from the Ramsis Damian AquaLab in Hurgada, Egypt. The brine shrimp (Artemia salina) were hatched for 48 h in a vessel filled with sterile artificial seawater (dissolve 38 g of sea salt, NaCl in 1000 mL distilled water, adjust pH to 8.5 using 1N sodium hydroxide). The active shrimp were collected and used for the experiment (Krishnaraju et al 2005).

Small amounts of dry Artemia salina cysts were sprinkled into a dark-covered-beaker. After 24 hours, a 0.06% yeast solution was added to a hatching beaker for every liter of saltwater to feed the Artemia larvae, otherwise, they would die during the 3rd or 4th instar stage (Parra et al 2001). The temperature (37°C) as well as the food quantity and quality influence molting into the next larval phase (Lalisan et al 2014). The active nauplii free of eggshells were transferred (10 each) using a Pasteur capillary pipette into a tube in the light after 36 hours of incubation at room temperature (25°C–28°C) (Sharma et al 2013). Each tube was used to test one concentration and one type of extract. It is necessary to use nauplii from identical generations for all concentrations of the tested compound (Ogugu et al 2012).

Brine (4.5 mL solution) was added to all test tubes. A set of dilutions were prepared for the tested substance (extract) and 0.5 mL was added to each tube. After drawing 10 active shrimp into each test tube with a glass capillary tube, the solution was thoroughly mixed with a cyclo-mixer. The surviving larvae were counted after incubating at 25°C for 24 h and the lethality concentration (LC_{50}) was calculated.

2.3 Statistical analysis

All measurements were performed in triplicate and the data are presented as the mean ± SD. The data were subjected to an analysis of variance. The analysis was carried out using the proc-ANOVA procedure based on ASSISTAT Version 7.7 beta (2014).

3 Results and Discussion

3.1 Mineral composition of algae

The necessary nutrients required for human nutrition may be found in marine algae (Mabeau and Fleurence 1993). The ash content of food provides a general overview of its mineral value. The ash content of various algae samples in our study ranged from 21.91 to 28.94 g/100 g **(Table 1)**.

The major minerals, trace elements (g/100g dw), and toxic elements (mg/100g dw) in the three algae, *Ulva lactuca*, *Laurencia obtusa*, and *Padina pavonica,* are listed in Table 1. The results indicated that the samples contained high levels of Ca, Cl, Mg, K, Na, P, and Fe. It is known that the element content of algae may be influenced by a variety of environmental factors, such as element concentration in water (Andrade et al 2004), interactions between minerals, light intensity, pH, salinity, and metabolic factors, such as element dilution caused by algae growth. In addition, metabolic requirements regulate element concentration in seaweed to a large extent (Zbikowski et al 2006).

All of the species examined exhibited high levels of macro elements. *Padina pavonica* (fresh brown seaweed) had the highest Ca content (3.00 g/100 g dw), followed by *Ulva lactuca* (fresh green algae), whereas *Laurencia obtusa* had the lowest (1.71 g/100 g dw) (dry red algae). Fresh *Laurencia obtusa* had the highest Cl content (1.69 g/100 g dw) and dry *Ulva lactuca* had the lowest (1.52 g/100 g dw).

Calcium was found to be the most important mineral in seaweeds with a much higher concentration compared with terrestrial foods according to MacArtain et al (2007). Mineral content varies by species, wave exposure, season, physiological factors, environmental factors, processing type, and mineralization methods (Mabeau and Fleurence 1993). According to MacArtain et al (2007), 8 g dw of *U. lactuca* yields 260 mg of calcium or approximately 37% of the RNI values for adult males.

Table 1. Ash and mineral contents in the three algae, *Ulva lactuca, Laurencia obtusa,* and *Padina pavonica*

Elements	**Sea Water**	***Ulva lactuca***		***Laurencia obtusa***		***Padina pavonica***	
		Dry	**Fresh**	**Dry**	**Fresh**	**Dry**	**Fresh**
Ash %		21.91f ± 0.002	22.20e ± 0.02	28.41b ± 0.002	28.94a ± 0.02	25.00d ± 0.01	25.11c ± 0.01
g/100g							
Ca	3.88	2.10	2.21	1.71	1.84	2.88	3.00
Na	0.49	0.31	0.35	0.37	0.38	0.39	0.41
Cl	2.65	1.52	1.64	1.68	1.69	1.59	1.62
K	0.68	0.59	0.60	0.47	0.49	0.60	0.61
Mg	2.34	2.19	2.22	2.37	2.40	2.01	2.09
P	0.19	0.12	0.12	0.10	0.11	0.14	0.15
mg/100 g							
Fe	176.55	91.11	93.92	94.88	99.89	103.95	109.66
Zn	0.87	0.13	0.16	0.45	0.49	0.11	0.13
Cu	1.11	0.51	0.54	0.78	0.95	0.39	0.42
I	2.01	0.59	0.82	0.97	1.01	1.27	1.52
	µg/100 g						
Mn	1.01	0.13	0.14	0.33	0.39	0.12	0.17

Fresh and dried brown algae had the highest sodium content (0.41 and 0.39 mg/100 g dw, respectively), whereas dried and fresh green algae exhibited the lowest (0.31 and 0.35 mg/100 g dw, respectively) (Table 1). In the present study, fresh brown algae had high levels of K and P (0.61 and 0.15 mg/100 g dw, respectively), whereas low levels of K and P were found in dry red algae (0.47 mg/100 g dw and 0.10 mg/100 g dw, respectively).

Brown seaweed accumulates an enormous variety of elements and are an excellent source of major, minor, and trace elements. Thus, brown seaweed is known for its effectiveness as a dietary supplement or as part of a well-balanced diet. In all of the algae studied; the Na/K ratios ranged from 0.53 to 0.77. Olives and sausages, however, had Na/K ratios of 43.63 and 4.89. This is significant from the perspective of nutrition because NaCl consumption and diets with a high Na/K ratio are associated with hypertension. Magnesium is a co-factor in a variety of enzyme-dependent biochemical reactions that occur in the body. It is important for brain and liver function as well nerve calmness, because it is involved in ATP-dependent metabolic reactions. Magnesium also contributes to cell growth, tissue elasticity, and neuromuscular functions (Vashuki et al 2020).

Fresh *Laurencia obtuse* had a high level of magnesium (2.40 g/100 g dw), whereas dry *Padina pavonica* had 7.64 mg/100 g dw **(Table 1)**. Ferrous levels ranged from 91.11 mg/100 g dw (dry green algae) to 109.66 mg/100 g dw (wet green algae). The DRI for Thai males and females aged 19 to 50 years was 10.2 mg/100 g for males and 24.7 mg/100 g for females, respectively.

Copper (Cu) and zinc (Zn) levels in the tested algae ranged from 0.39 to 0.95 mg/100 g and 0.11 to 0.49 mg/100 g, respectively, for the trace elements (Table 1). Fresh red algae exhibited the highest copper and zinc concentrations. Copper intake in the diet should not exceed 10,000 mg/100 g for females and 12,000 mg/100 g for males. Mabeau and Fleurence (1993) and Rupérez (2002) reported that the concentration of the elements were within the ranges found in other seaweed studies. Fresh *Padina pavonica*, dried *Padina pavonica*, fresh Laurencia obtuse, dried Laurencia obtuse, fresh *Ulva lactuca*, and dried *Ulva lactuca* contained 1.52, 1.27, 1.01, 0.97, 0.82, and 0.59 mg/100 g dw of iodine, respectively. Iodine intake is recommended at 0.15 mg per day worldwide (Roleda and Hurd 2019).

Iodine is required for the synthesis of thyroid hormones such as thyroxine and triiodothyronine, which are important regulators of human physiological processes (Haldimann et al 2005). Iron, like iodine, plays an important role in various cellular functions, particularly in oxygen transport and it is a component of several enzymes involved in electron transport and DNA synthesis (Puntarulo 2005).

The toxic elements (µg/100g dw) in the three algae studied *(Ulva lactuca, Laurencia obtusa,* and *Padina pavonica*) are listed in Table 2. Fresh red, fresh green, dry green, dry red, fresh, and dried brown algae had maximum lead (Pb) values of 0.16, 0.14, 0.13, 0.12, 0.04, and 0.02 µg/100 g, respectively **(Table 2**). Almela et al (2006) measured 1 mg Pb/100 g.

Fresh red seaweed (0.59 µg/100 g dw) had highest Cd content. In contrast, dried brown algae had the lowest Cd (0.12 µg/100 g dry weight). Laurencia obtuse (0.39 µg/100 g dw) was found to have a high manganese content. *Padina pavonica*, a dried brown algae, had a concentration of 0.12 µg/100 g dw. The highest levels of Mn were found in red seaweed according to Circunciso et al (2018). The relatively high concentrations of some heavy metals in seaweed reflect two factors: the high metal concentration in the study area and the ability of algae to absorb them (Karez et al 1994).

3.2 Toxicological evaluation of Algae

BSLA has been widely used to assess the toxicity of a wide range of plant compounds over the last three decades (Hamidi et al 2014). To screen the toxicity of marine natural products, BSLA is used as an alternative bioassay technique (Carballo et al 2002). The lethality assay for brine shrimp (Artemia sp.) may be used to detect toxic compounds in extracts. The cytotoxicity of aqueous extracts can be efficiently screened using this low-cost and simple assay (Hamidi et al 2014). Carballo et al (2002) proposed a brine shrimp lethality assay based on the ability of cytotoxic compounds to inactivate nauplii in laboratory hatched brine shrimp (Artemia sp). This method was developed to determine the bioactive and cytotoxic effects of synthetic compounds in a safe, practical, and inexpensive manner (Almeida et al 2002). Moreover, several studies have shown consistency with the LC_{50} values obtained from the BSLA and other assays (Hamidi et al 2014).

Different extracts of fresh and dry brown algae (*Padina pavonica*) exhibited good larvicidal activity against brine shrimp **(Table 3 and Fig 1)**. In isopropanol extracts of fresh and dry brown algae, the LC_{50} values were 840 mg dw/L and 510 mg dw/L respectively. The lowest LC_{50} values were found in various extracts of fresh and dried red algae (*Laurencia obtusa*). The isopropanol extract from fresh *Laurencia obtusa* had an LC_{50} value of 260 mg dw/L, whereas dry *Laurencia obtuse* yielded 220 mg dw/L. According to Premarathna et al (2020), the red seaweed species had significantly lower LC_{50} values when compared to all other brown and green algae samples. *Ulva lactuca* from Negombo (northwestern coastal area) had the highest LC_{50} value of all the green seaweed samples tested.

These findings revealed that the LC_{50} in the fresh samples were higher than those in the dry samples. As a result, the higher LC_{50} value, the better the algae from a health and nutritional standpoint. An LC_{50} value of 840 mg dw/L is required for brine shrimp mortality. Clarkson's toxicity criterion classifies plant extracts with LC_{50} values greater than 1000 g/mL as non-toxic, extracts with an LC_{50} between 500 and 1000 g/mL as low toxic, extracts with an LC_{50} between 100 and 500 g/mL are medium toxic, and extracts with an LC_{50} value of 0 - 100 g/mL as highly toxic (Hamidi et al 2014).

Based on the tested concentrations, *Ulva lactuca* (green seaweed), Caulerpa racemosa, and Caulerpa ser tularioides, and the brown seaweed species, Padina antillarum, did not cause mortality. In L929 cells, the majority of the aqueous algae extracts exhibited no cytotoxic effects. Migration and cell proliferation were observed in L929 cells treated with the majority of the aqueous brown seaweed extracts. Furthermore, with the exception of the red seaweed, Jania adhaereus, almost all of the tested aqueous extracts of brown, green and red seaweed had no cytotoxic effects on L929 cells (Premarathna et al 2020). For the quantities that are commonly consumed, none of the seaweed studied would present a significant risk of toxicity. When compared to other fruits and vegetables, algae contain a variety of valuable minerals and carbohydrates that benefit human health. Thus, seaweed may be part of a balanced and healthy diet (Smith et al 2010).

Table 2. Toxic elements (µg/100 g) in three algae: *Ulva lactuca*, *Laurencia obtusa,* and *Padina pavonica*

Elements	Sea Water	*Ulva lactuca*		*Laurencia obtusa*		*Padina pavonica*	
		Dry	**Fresh**	**Dry**	**Fresh**	**Dry**	**Fresh**
Cd	0.91	0.15	0.17	0.48	0.59	0.12	0.15
Pb	0.81	0.13	0.14	0.12	0.16	0.02	0.04
Cr	0.72	0.00	0.00	0.00	0.00	0.00	0.00
Co	0.79	0.00	0.00	0.00	0.00	0.00	0.00

Table 3. LC$_{50}$ values in mg/L of extract for different algae samples after incubating for 24 h. The 50% lethal concentration for brine shrimp nauplii was considered the LC$_{50}$ value

Solvent/Material	**Moisture**	**Petroleum ether**	**Chloroform**	**Iso-propanol**	**Water**
Padina pavonica dried	$9.17^{f}\pm0.001$	400	370	510	470
Laurencia obtusa dried	$9.63^{e}\pm0.003$	150	130	220	200
Ulva lactuca dried	$10.20^{d}\pm0.003$	280	260	330	310
Padina pavonica fresh	$76.14^{c}\pm0.003$	780	720	840	810
Laurencia obtusa fresh	$82.45^{a}\pm0.001$	180	160	260	210
Ulva lactuca fresh	$81.20^{b}\pm0.001$	330	300	400	390

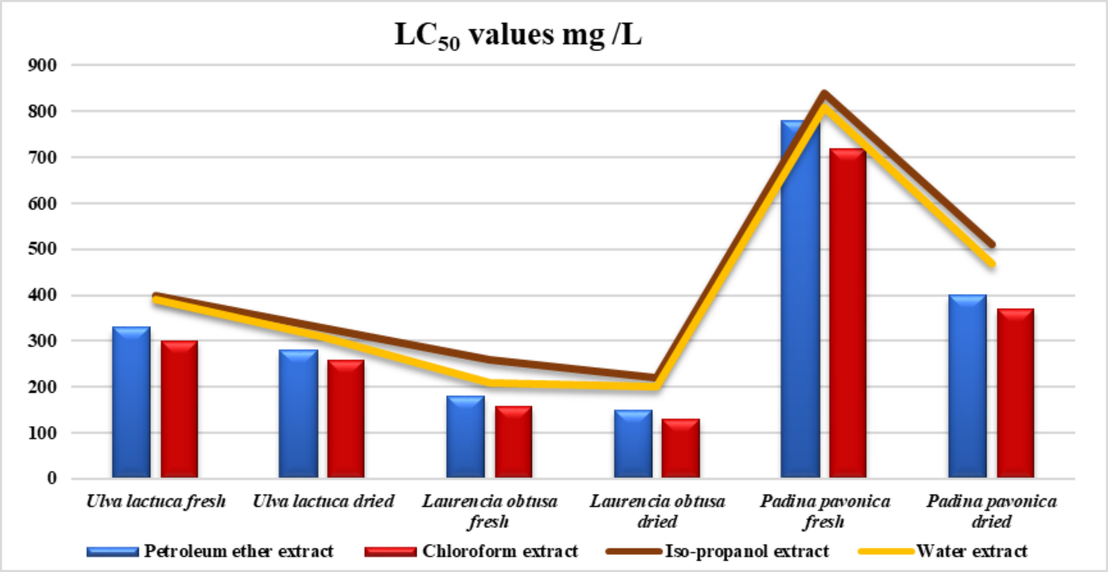

Fig 1. LC$_{50}$ values in mg/L of extracts of different algae samples after incubation for 24 h. 50% lethal concentration for brine shrimp nauplii was considered as LC$_{50}$ value

Padina pavonica

Ulva lactuca

Laurencia obtusa

4 Conclusion

In the present study, we found that edible brown, red, and green algae may be used as a food supplement to help meet the recommended daily intake of several macro, micro and trace elements. Seaweed is recommended as useful additive to functional ingredients and used for the development of functional food products.

References

Abirami RG, Kowsalya S (2012) Sub–chronic toxicity and heavy metal toxicity study on Kappaphycus alvarezii in albino rats. *Asian Pacific Journal of Tropical Biomedicine* 2, S1372–S1376. https://doi.org/10.1016/S2221-1691(12)60419-5

Aleem AA (1993). The marine algae of Alexandria, Egypt, Ed. *University of Alexandria, Egypt* 138, 1–55.

Almeida PA, Silva TMS, Echevarria A (2002) Mesoionic 5-alkyl-1,3-dithiolium-4-thiolates: Synthesis and brine shrimp toxicity. *Heterocyclic Communications* 8, 593–600. https://doi.org/10.1515/HC.2002.8.6.593

Almela C, Clemente MJ, Velez D, Montoro R (2006) Total arsenic, inorganic arsenic, lead and cadmium contents in edible seaweed sold in Spain. *Food and Chemical Toxicology* 44, 1901–1908. https://doi.org/10.1016/j.fct.2006.06.011

Andrade LR, Farina M, Amado Filho GM (2004) Effects of copper on *Enteromorpha flexuosa* (Chlorophyta) in vitro. *Ecotoxicology and Environmental Safety* 58, 117–125. https://doi.org/10.1016/S0147-6513(03)00106-4

AOAC (2005) Official Methods of Analysis, 18th ed. Association of Official Analytical Chemists AOAC International, Gaithersburg, MD, USA.

Bocanegra A, Bastida S, Benedí J, et al (2009) Characteristics and nutritional and cardiovascular-health properties of seaweeds. *Journal of Medicinal Food* 12, 236–258. https://doi.org/10.1089/jmf.2008.0151

Brown JD, Lilleland D (1946) Rapid determination of potassium and sodium in plant material and soil extract by flame photometery. *Proceedings of the American Society for Horticultural Science* 48, 331–346.

Carballo JL, Hernández-Inda ZL, Pérez P, García-Grávalos MD (2002) A comparison between two brine shrimp assays to detect in vitro cytotoxicity in marine natural products. *BMC Biotechnology* 2, 1-5. https://doi.org/10.1186/1472-6750-2-17

Chapman HD, Pratt PF (1961) Methods of Analysis for Soil, Plants and Water. University of California, *Division of Agricultural Science, Berkeley, CA, USA* 56–63. C.F. Taha RS (2016) Improving Salt Tolerance of Helianthus annuus (L.) Plants by Moringa oleifera Leaf extract. Egypt Journal Agronomy 38, 117-140. https://doi:10.21608/agro.2016.301

Circuncisão AR, Catarino MD, Cardoso SM, Silva AMS (2018) Minerals from macroalgae origin: Health benefits and risks for consumers. *Marine Drugs* 16, 1-30. https://doi.org/10.3390/md16110400

Ganesan AR, Subramani K, Shanmugam M, et al (2020) A comparison of nutritional value of underexploited edible seaweeds with recommended dietary allowances. *Journal of King Saud University–Science* 32, 1206–1211. https://doi.org/10.1016/j.jksus.2019.11.009

Haldimann M, Alt A, Blanc A, Blondeau K (2005) Iodine content of food groups. *Journal of Food Composition and Analysis* 18, 461–471. https://doi.org/10.1016/j.jfca.2004.06.003

Hamidi MR, Jovanova B, Panovska TK (2014) Toxicological evaluation of the plant products using Brine Shrimp (Artemia salina L.) model. *Macedonian Pharmaceutical Bulletin* 60, 9–18.

Jackson ML. (1973). Soil chemical analysis, Prentice Hall of India Pvt. Ltd., New Delhi, India, 498, 151-154.

Karez CS, Magalhaes VF, Pfeiffer WC, Amado Filho GM (1994) Trace metal accumulation by algae in Sepetiba Bay. Brazil. *Environmental Pollution* 83, 351–356. https://doi.org/10.1016/0269-7491(94)90157-0

Krishnaraju AV, Rao TVN, Sundararaju D, et al (2005) Assessment of bioactivity of Indian medicinal plants using brine shrimp (Artemia salina) lethality assay. *International Journal of Applied Science and Engineering* 3, 125–134.

Kumar MS, Sharma SA (2020) Toxicological effects of marine seaweeds: a cautious insight for human consumption. *Critical Reviews in Food Science and Nutrition* 61, 500–521. https://doi.org/10.1080/10408398.2020.1738334

Lalisan JA, Nuñeza OM, Uy MM (2014) Brine shrimp (Artemia salina) bioassay of the medicinal plant Pseudelephantopus spicatus from Iligan City, Philippines. *International Research Journal of Biological Sciences* 3, 47-50.

Mabeau S, Fleurence J (1993) Seaweed in food products: Biochemical and nutritional aspects. *Trends in Food Science and Technology* 4, 103–107. https://doi.org/10.1016/0924-2244(93)90091-N

MacArtain P, Gill CIR, Brooks M, Campbell R, Rowland IR (2007) Nutritional value of edible seaweeds. *Nutrition Reviews* 65, 535–543. https://doi.org/10.1111/j.1753-4887.2007.tb00278.x

Mišurcová L, Machů L, Orsavová J (2011) Seaweed minerals as nutraceuticals. *Advances in Food and Nutrition Research* 64, 371–390. https://doi.org/10.1016/B978-0-12-387669-0.00029-6

Ogugu SE, Kehinde AJ, James BI, Paul DK (2012) Assessment of cytotoxic effects of methanol extract of Calliandra portoricensis using brine shrimp (*Artemia salina*) lethality bioassay. *Global Journal of Bio-Science and Biotechnology* 2, 257-260.

Parra LA, Yhebra RS, Sardiñas IG, Buela LI (2001) Comparative study of the assay of Artemia salina L. and the estimate of the medium lethal dose (LD50 value) in mice, to determine oral acute toxicity of plant extracts. *Phytomedicine* 8, 395-400. https://doi.org/10.1078/0944-7113-00044

Peñalver R, Lorenzo JM, Ros G, et al (2020) Seaweeds as a Functional Ingredient for a Healthy Diet. *Marine Drugs* 18, 301. https://doi.org/10.3390/md18060301

Pregl F (2013) Quantitative Organic Microanalysis. In H. Leicester (Ed.), A Source Book in Chemistry, 1900-1950. Cambridge, MA and London, England: Harvard University. pp. 6-8 https://doi.org/10.4159/harvard.9780674366701.c6.

Premarathna AD, Ranahewa TH, Wijesekera SK, et al (2020) Preliminary screening of the aqueous extracts of twenty-three different seaweed species in Sri Lanka with in-vitro and in-vivo assays. *Heliyon* 6, e03918. https://doi.org/10.1016/j.heliyon.2020.e03918

Puntarulo S (2005) Iron, oxidative stress and human health. *Molecular Aspects of Medicine* 26, 299–312. https://doi.org/10.1016/j.mam.2005.07.001

Roleda MY, Hurd CL (2019) Seaweed nutrient physiology: Application of concepts to aquaculture and bioremediation. *Phycologia* 58, 552–562. https://doi.org/10.1080/00318884.2019.1622920

Rupérez P (2002) Mineral content of edible marine seaweeds. *Food Chemistry* 79, 23–26. https://doi.org/10.1016/S0308-8146(02)00171-1

Sharma N, Gupta PC, Singh A, Rao CV (2013) Brine shrimp Bioassay of *Pentapetes phoenicea* Linn. and Ipomoea carnea Jacq. leaves. *Der Pharmacia Lettre* 5, 162-167.

Smith JL, Summers G, Wong R (2010) Nutrient and heavy metal content of edible seaweeds in New Zealand. *New Zealand Journal of Crop and Horticultural Science* 38, 19-28. https://doi.org/10.1080/01140671003619290

Vasuki S, Kokilam G, Babitha D (2020) Mineral composition of some selected brown seaweeds from Mandapam region of Gulf of Mannar, Tamil Nadu. *Indian Journal of Geo-Marine Sciences* 49, 63-66.

Veena CK, Josephine A, Preetha SP, Varalakshmi P (2007). Beneficial role of sulfated polysaccharides from edible seaweeds Fucus vesicuolosus in experimental hyperoxaluria. *Food Chemistry* 100, 1552-1559. https://doi.org/10.1016/j.foodchem.2005.12.040

Zbikowski R, Szefer P, Latała A (2006) Distribution and relationships between selected chemical elements in green alga *Enteromorpha* sp. from the southern Baltic. *Environmental Pollution* 143, 435–448. https://doi.org/10.1016/j.envpol.2005.12.007