

Analysis of metals and metalloids present in Sri Lankan dried seaweeds and assessing the possibility of health impact to general consumption patterns

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Abstract – Seaweeds are considered as a functional food across many regions of the world and has an increasing consumption trend due to its health benefits. However, there is a concern regarding the amount of heavy metals and metalloids present in seaweeds. Therefore, the study aimed to assess the levels of metals present in specific seaweeds and its potential impact on consumption. Considered metal ions were Arsenic (As), Copper (Cu) Chromium (Cr), Nickel (Ni), Cadmium (Cd), Lead (Pb) and Mercury (Hg). At the assessment done at four different sites in the coastal regions of Sri Lanka for chlorophytes, rhodophytes and phaeophytes. Concentration of metals were analyzed using the ICPOES. According to the arrived results, concentration of metals varies as Cr > Ni > Cd > Cu > As > Pb = Hg with having zero concentration for Hg and Pb for all varieties and all sites. It was also found that the least amounts of metals were present at Jaffna site in phaeophytes (*Sargassum* sp.) and chlorophytes (*Ulva* sp.) When considering the Hazardous Index of the varieties, least was found in *Sargassum* sp. in Jaffna site. Studies were repeated for 2 seasons and there are significant differences ($p < 0.05$) between the dry season and wet season in the concentration of heavy metals present. However, since the seaweeds are grown for commercial purposes only in Jaffna area, it is evident that the chlorophyte and phaeophyte varieties claim very low health risk for potential heavy metals and are suitable for consumption purposes.

Keywords: Seaweeds / Heavy metals / Health / Consumption / seasonal variation

1 Introduction

Seaweeds are been considered as a collection of marine macroscopic algae which provide many benefits to a vast variety of industries. These majorly include human nutrition and nutraceuticals, animal feed and functional ingredients. Decades back, industries have emerged to grow from East Asian countries with giving an immense importance to seaweed culturing (FAO, 2020). Global seaweed production has increased remarkably, to 32.4 million tons per annum. Majority of the produce originates from Eastern Asian countries and have been used as food and ingredients and to lower extent as bioactive compounds and medicines (Busetti et al., 2017; Monagail et al., 2017). However, using seaweeds

for both human and animal consumption has raised certain concerns relating to heavy metal contaminations. Heavy metals are released to the ocean from mining, printing, electronic, petroleum industries and municipal wastes (Wang, 2013) and can get accumulated in seaweeds at different levels and metabolized and stored as organified, depending on the type of the metal. These hazardous accumulations may impact the sea flora and fauna thereby accumulations in greater dosages in food chains, including humans. Such known heavy metal accumulated in the food chains are Cu and Cd (Gaudry, 2007). Heavy metals like Cd, Hg and Pb can be toxic and detrimental when accumulated at higher dosages. Though no proper correlation was not found in the patterns of the accumulations of heavy metals in water, water sediments and seaweeds, it is clear that the accumulation in the seaweeds is higher than that of the respective water and sediment samples (Seralathan, 2008). Therefore, these accumulations need to be studied further.

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Heavy metals affect the human and animal body in various ways. Accumulation of heavy metal in the fatty tissue around the organs, will impact the central nervous system, thereby impairing its activities. When considering the Arsenic metalloid, inorganic Arsenic is known to have portrayed more toxicity than organic Arsenic and is known to be a genotoxic variety, impacting the development of cancers relating to liver, bladder and lungs (Rose et al., 2007). However, according to Taylor et al. (2017), it is evident that most of the seaweed accumulate Arsenic as virtually non-toxic arseno-sugars. More knowledge provides novel information on the speciation of arsenic in different species; such as *Hizikia fusiforme* (Laparra et al., 2003) and *Laminaria digitata* (Ronan et al., 2017). For this reason, levels of Arsenic present in seaweeds tend to be higher than that in the plants on land with the seaweeds ability to derive Arsenic from seawater. Having said this, many studies across the globe has declared levels of heavy metals present at unpolluted water sources. As per the respective studies, water sources generally contain less than 50 mg kg^{-1} of Zinc, 20 mg kg^{-1} of Copper, between 2 and 50 mg kg^{-1} of Lead, up to 100 mg kg^{-1} of Nickel, less than 6 mg kg^{-1} of Chromium and less than 1 mg kg^{-1} of Cadmium (Moore et al., 1984; Bryan and Langston, 1992). However, heavy metal levels in the sediments contrast with that of seaweeds and assessing heavy metal levels in sediments does not give an indication on the heavy metals accumulated in seaweeds, grown in the same area (Lorenzo, 2000).

As per Riget et al. (1997), lowest metal and metalloid concentration was found on *F. vesiculosus* grown in Western Greenland, with giving reference to Zinc ($7.2\text{--}17.3 \text{ mg kg}^{-1}$), Lead ($0.3\text{--}0.4 \text{ mg kg}^{-1}$) and Iron ($33\text{--}77 \text{ mg kg}^{-1}$). These values are comparatively low when comparing the levels of *F. vesiculosus* from other sites in North America and Europe, which are affected more with the heavy metal sources in the area. However, these values can vary depending on the availability and impact of the local metal sources of the area (Forsberg et al., 1988; Riget et al., 1997; Jayasekera and Rossbach, 1996; Preston et al., 1972). For example, Zinc level found in Southern North Sea in America, was 400 mg kg^{-1} (Dutton et al., 1973) while high Iron level of 600 mg kg^{-1} is observed in the same area (Struck et al., 1997). Also, as per the studies of Bryan and Hummerstone (1973) and Young (1975) on seasonal variation of heavy metal concentration of seaweeds, an increment of heavy metal accumulation was found in winter and spring while there is a reduction in concentration in summer and autumn. These findings were applicable for Mn, Fe, Zn, Cu, Cd, Co and Al. However, it is also predicted that this variation caused in the seasons is due to the differences of surface heavy metal sediments which are caused by the differences of the tidal regime and the capacity of incoming fresh water to carry suspended sediments during different seasons (Bryan and Hummerstone, 1973).

Nevertheless, studies stated above limit the sampling area to Europe and other continents except Asia. Therefore, the following study aims to assess the heavy metal and metalloid concentrations in coastal regions of Sri Lanka. Since seaweeds are grown in many of the coastal regions of the country for export processes, assessment of the results of this study will reveal the suitability of them for human and animal consumption purposes.

In Sri Lanka, seaweeds are occurring in three major communities in the sea, being seaweed vegetations, seagrass beds and mangrove forests. Seaweed vegetations are developed in rocky substrates in intertidal zone, spray zone and subtidal zone. Development of these seaweeds depend on the season and surf. Monospecific vegetations can occur in these seaweed vegetation sites, but in most of the instances, different genera could be intricated. Mid and low inter tidal pools contain continuously submerged seaweed vegetation which is different from the air exposed substrates at low tide. In sheltered areas with a sand substrate, seaweed growth is limited due to the shifting nature with the sand erosion. However, some species grow under these adverse circumstances as well, attached to shell or coral fragments.

In terms of seagrass communities, seaweeds are grown in surf sheltered subtidal biotopes. In Sri Lanka, these communities could be seen in Puttalam bay, Chillaw and Weligama lagoon. Larger sized species of seaweeds are seen to be grown between the seagrass plants and smaller species grow as epiphytes on the seagrass stipes and leaves (Durairatnam, 1961).

Mangrove based seaweeds are developed around lagoons. Some species develop in mangrove tide channels and some in muddy pools in mangrove vegetation. But, since the vegetation is rather small and are covered by the sediments most of the time, presence of the seaweed is not much noticeable. Additionally, some species grow on hard floating substrates such as boats and ropes which are wave swept continuously, and also on animals such as shells.

When considering the seasonality impacts on the growth of seaweeds in Sri Lanka, depending on the climatic condition of the country, seasonality comprises of four major seasons. First inter monsoon season falls in March –April months, Southwest monsoon season in May–September, second inter monsoon from October–November and North East monsoon from December–February (Report on climatic zonation of Sri Lanka, Department of Meteorology). Latter two seasons are commonly known as the wet season while the rest of the two seasons are referred as the dry season. In the wet season, exposed rocks show a dense population of seaweeds where in the dry season, the population density reduces due to the heat followed by desiccation. Mild to low inter tidal pools on the sheltered areas show different behaviours over the season. In wet season, seaweeds grown in these areas get flushed by sea water where the temperature and the salinity are balanced (Milledge et al., 2016). Seasonality of the algal species of Sri Lanka was first determined by Svedelius (1906) where the seasonality was studied on rocky outcrops in Dickwella. As identified in his study, seasonality developments in the rock outcrops are significant. However, some seaweeds from low intertidal and subtidal biotopes are less sensitive to seasonality since they are submerged in sea water and the temperature of the sea water does not vary drastically as the temperature of the air does.

2 Materials & methodology

2.1 Sample collection from sites

As Figure 1 highlights, seaweed collection was done from 04 sites of the coastal regions in Sri Lanka, including,

Jaffna-Valaipadu (Latitude: 9.6614° N, Longitude: 80.0255° E), Kalpitiya (Latitude: 8.2295° N, Longitude: 79.7596° E), Hikkaduwa (Latitude: 6.1313° N, Longitude: 80.1007° E) and Matara (Latitude: 5.9549° N, Longitude: 80.5550° E).

Samples were drawn in August 2019 (South Western Monsoon season) and February 2020 (Northeast Monsoon season). Seaweed samples included *Sargassum* sp.-Phaeophyta (Fig. 2a), *Ulva fasciata*-Chlorophyta (Fig. 2b) and *Gracilaria* sp.-Rhodophyta (Fig. 2c). A composite sample from each seaweed type was collected by combining 8–10 cm

of fronds removed from 20 to 30 numbers of randomly chosen plants. Though it was identified that growing tips contain metal concentrations more than the old stalky grown plants (Forsberg et al., 1988), the identification was not always true as per the study done on total arsenic level in *Ascophyllum* sp. (Ronan et al., 2017). Therefore, samples were selected to represent a combination of both life spans. Samples were cleaned at site with seawater to remove epiphytes and transported to laboratory in cold conditions for further analysis.

All seaweed samples were dried at 60 °C, stored at 4 °C afterwards and were immediately analyzed within 24 hours.

2.2 Analysis of metals and metalloids

All of the samples were to be digested before analysis and the digestion was carried out using the microwave digestion technique. About 0.25 g from each of the sample was measured to a digestion vessel and 4 ml of concentrated HNO₃ and 1 ml of H₂O₂ was added to the each. Afterwards, 0.1 ml of 50 ppm Au and Lu mix was also added to each digestion vessel. After placing vessels in the digestion system, temperature of the system was raised to 190 °C and was maintained for 15 minutes.

All the digested solutions were then analyzed using ICP-OES (AOAC 999.11:2012). Limits of detection (LOD) were defined as 3 times of the standard deviation of 10 rounds of blank measurement. Analyzed metals included As, Cu, Cr, Ni, Cd, Pb and Hg and their LODs were 1.8, 5.4, 2.3, 3.1, 0.5, 3.5 and 2.3 µg/kg respectively. For the purpose of evaluating the efficiency of extractions and internal quality control, NIST-SRM 3232 for Kelp powder (*Thallus laminariae*) was obtained and the element levels were analyzed using the same methodology which was used to analyze the seaweed samples and mean recoveries were also calculated. Analytical values obtained via this analysis were compared with the certified values of the standard.

2.3 Consumption data of seaweeds

Data were not available on the consumption levels of Sri Lankan seaweeds from the selected sites, since most of the harvest is been exported. Therefore, for the purpose of the study, general consumption levels are considered. General per capita consumption of fresh seaweeds per day is 25.31 g/day



Fig. 1. Selected sites in Sri Lanka for the analysis.



Fig. 2. Pictorial representation of varieties of seaweeds selected for the study.

Table 1. Results of elemental levels in SRM of *Thallus laminariae*.

Elements	Certified Value (mg/kg)	Measured Value (mg/kg)	Recovery %
As	38.3±1.3	37.32±2.6	97.4
Cu	3.875±0.087	3.57±1.8	92.1
Cr	5.92±0.52	5.68±1.4	95.9
Cd	0.4259±0.0084	0.38±0.1	89.2
Pb	1.032±0.039	0.98±0.2	94.9
Hg	0.1129±0.0032	0.097±0.01	85.9

(FAO, 2020) in mainland of China. However, seaweeds contain a moisture content of 15–20% (Makawita et al., 2018) and therefore the average daily per capita consumption of dried seaweed will be 20.3 g/day.

2.4 Risk assessment

Assessment of the probable health risks is done via Target Hazard Quotient (THQ) and Hazard Index (HI) values (USEPA Data base 2007) (Eq. 1, 2, 3). As per the guidance of WHO/FAO joint research, 1997, for average exposure, mean concentration is used.

$$\text{Exposure Dosage} = \frac{Ci \times Di \times Ed}{Bw \times At} \quad (1)$$

$$\text{Target Hazard Quotient(THQ)} = \frac{\text{Exposure Dosage}}{\text{RfD}} \quad (2)$$

$$\text{Hazard Index (HI)} = \sum_{k=1}^{n=k} \text{Target Hazard Quotient (THQ)} \quad (3)$$

Average consumption (mg/kg) is denoted by Ci, Di is the daily seaweed intake, Ed is the exposure duration, Bw is the average weight of the person, At is the life time of a person, RfD is the recommended dosage. As per USEPA guidelines for the assessment of risk, when HI < 1, there will be no health risk. If HI ≥ 1, there is a high risk of having adverse effect on human health.

2.5 Statistical analysis

Statistical analysis was done through MINITAB software using one-way ANOVA for mean comparisons.

3 Results

In validating the analytical method used, elemental levels of the standard reference materials were taken into consideration. Table 1 illustrates the comparison of results obtained by analysis of the SRM through ICP-OES, as well as the certified values and recovery percentages. Recovery percentage refers to the percentage mean of the measured values to the certified values.

The obtained recovery values were ranging from 85.9 to 97.4, and therefore can be concluded that the analytical values

obtained by analyzing the local samples through the same method, are accurate.

Results obtained from the ICP-OES analysis are depicted in Table 2. As per the analytical data, Pb and Hg were not present in any of the seaweed samples analyzed from any of the locations. Even the seasonality has not impacted this result. Additionally, Arsenic metal was detected in only few locations including southern coast (Matara & Hikkaduwa) from Chlorophytes and Phaeophytes. In Rhodophytes, Arsenic was identified in all 04 locations. However, there is a reduction in Arsenic level in north east monsoon season and the levels observed in different classes of seaweeds were significantly different ($p < 0.05$) across the season.

Though Ni and Cd levels have not shown a significant difference in the south western monsoon season, the difference is significant for Ni and Cd in chlorophytes in site 02 and 04 and phaeophytes in site 02. Cr is detected in highest concentration out of the analyzed heavy metals and is significantly lower in site 01 for chlorophytes. But, when in comparison with the previously done studies on the Cr levels (Dominguez et al., 2010) detected Cr levels in this study at all sites, are significantly higher. The reason for these higher concentrations maybe due to the increased levels of industrialization taking place around the country, during the past decade. In Jaffna site, a steel manufacturing facility is located nearby and operates with metal finishing and electroplating as well. Hikkaduwa and Matara areas are highly industrialized resulting with considerable release of industrial disposals from dyeing industries. Therefore, extensively high levels of Cr present in the samples will be the outcome of such human interventions.

For Cu, no significant differences are observed for different sites and seaweed varieties in ($p < 0.05$) north east monsoon season, but however is significantly different in south western monsoon season.

On basis the data of Tables 4 and 5, Table 6 depicts the HI of the three varieties of seaweeds in four different locations at two seasons. By assessing the results, it is evident that South Western Monsoon season has the lesser value of hazardous index for most of the locations. However, HI being <1, only site 01 and 02 showcase that the seaweeds grown in the area are safe for consumption. But in North East monsoon season, only chlorophytes are having a HI < 1, even from site 01 and 02.

4 Discussion

As a mean value of all the sites tested, As, Cr and Cd show the least concentration in phaeophytes (*Sargassum* sp.), which

Table 2. Concentration in mg kg⁻¹ of metals and metalloids in three varieties of seaweeds in different coastal areas of Sri Lanka – South Western Monsoon Season.

Seaweed	Site	Metal concentration (mg / kg)						
		As	Cu	Cr	Ni	Cd	Pb	Hg
Chlorophyta	01	ND	1.5 ± 1.1 ^a	47.4 ± 9.6 ^a	6.5 ± 2.1 ^a	4.5 ± 2.2 ^a	ND	ND
	02	ND	3.4 ± 1.5 ^a	51.7 ± 7.2 ^{ab}	7.4 ± 1.9 ^a	5.4 ± 1.7 ^a	ND	ND
	03	7.4 ± 1.4 ^a	2.8 ± 1.1 ^{ab}	63.8 ± 8.4 ^{ad}	8.2 ± 1.3 ^a	5.2 ± 1.6 ^a	ND	ND
	04	8.1 ± 2.1 ^b	4.1 ± 2.2 ^a	77.5 ± 9.8 ^{bcd}	6.8 ± 1.7 ^a	5.9 ± 1.9 ^a	ND	ND
Rhodophyta	01	1.0 ± 1.8 ^c	2.0 ± 0.7 ^{ad}	43.2 ± 6.7 ^{ace}	5.9 ± 2.0 ^a	3.7 ± 0.2 ^a	ND	ND
	02	1.4 ± 2.0 ^a	4.7 ± 1.0 ^a	50.9 ± 8.1 ^{af}	8.2 ± 2.2 ^a	4.1 ± 1.0 ^a	ND	ND
	03	6.1 ± 1.0 ^{ad}	6.1 ± 2.4 ^a	71.4 ± 11.8 ^{agh}	7.9 ± 2.4 ^a	3.8 ± 1.8 ^a	ND	ND
	04	7.6 ± 1.9 ^{ae}	8.4 ± 3.5 ^{bce}	68.2 ± 8.5 ^a	8.5 ± 1.6 ^a	6.2 ± 2.5 ^a	ND	ND
Phaeophyta	01	ND	1.8 ± 0.4 ^{ad}	35.8 ± 7.6 ^{aed}	9.7 ± 2.7 ^a	4.0 ± 1.8 ^a	ND	ND
	02	ND	2.7 ± 1.3 ^{ae}	49.2 ± 5.8 ^{ae}	10.7 ± 3.7 ^a	3.1 ± 1.0 ^a	ND	ND
	03	5.8 ± 1.8 ^a	4.3 ± 2.2 ^a	58.0 ± 9.9 ^a	10 ± 0.7 ^a	4.1 ± 0.7 ^a	ND	ND
	04	8.1 ± 2.5 ^{af}	5.6 ± 0.8 ^a	60.7 ± 4.9 ^a	9.8 ± 2.4 ^a	5.7 ± 2.0 ^a	ND	ND

Table 3. Concentration in mg kg⁻¹ of metals and metalloids in three varieties of seaweeds in different coastal areas of Sri Lanka – North East Monsoon Season.

Seaweed	Site	Metal concentration (mg / kg)						
		As	Cu	Cr	Ni	Cd	Pb	Hg
Chlorophyta	01	ND	3.1 ± 1.2 ^a	33.5 ± 11.1 ^a	7.5 ± 2.4 ^a	3.8 ± 0.8 ^a	ND	ND
	02	ND	3.9 ± 1.0 ^a	35.8 ± 9.9 ^{aa}	10.5 ± 1.8 ^{ab}	4.2 ± 1.2 ^{ab}	ND	ND
	03	7.5 ± 2.1 ^a	3.8 ± 1.7 ^a	66.4 ± 7.2 ^b	8.5 ± 2.0 ^a	6.4 ± 2.1 ^a	ND	ND
	04	ND	4.1 ± 2.5 ^a	61.4 ± 8.6 ^c	9.1 ± 1.3 ^{ad}	6.8 ± 0.9 ^{ad}	ND	ND
Rhodophyta	01	1.0 ± 0.5 ^{ba}	5.4 ± 1.8 ^a	68.2 ± 9.8 ^{db}	8.2 ± 1.7 ^a	5.6 ± 1.5 ^a	ND	ND
	02	ND	4.8 ± 0.6 ^a	71.9 ± 7.6 ^{ef}	7.7 ± 1.1 ^a	5.7 ± 1.7 ^a	ND	ND
	03	5.9 ± 1.0 ^{ab}	6.7 ± 2.1 ^a	75.4 ± 11.9 ^{fe}	7.4 ± 1.4 ^a	5.1 ± 2.7 ^a	ND	ND
	04	4.3 ± 2.1 ^a	7.0 ± 0.7 ^a	77.2 ± 10.6 ^{gd}	7.4 ± 1.9 ^a	4.9 ± 1.3 ^a	ND	ND
Phaeophyta	01	ND	4.7 ± 1.2 ^a	57.6 ± 10.2 ^a	6.2 ± 2.4 ^a	4.1 ± 1.6 ^a	ND	ND
	02	ND	5.2 ± 2.0 ^a	72.1 ± 11.7 ^{hc}	3.5 ± 2.0 ^{ac}	5.0 ± 2.4 ^{acc}	ND	ND
	03	6.2 ± 1.7 ^{ac}	4.9 ± 2.0 ^a	59.2 ± 10.3 ⁱ	7.3 ± 2.8 ^a	4.3 ± 2.1 ^a	ND	ND
	04	ND	5.1 ± 0.3 ^a	66.6 ± 12.4 ^j	8.2 ± 2.4 ^a	4.7 ± 1.9 ^a	ND	ND

** Mean ± Standard deviation of triplicates of samples; significant difference among columns in Tables 2 and 3 separately, were denoted by different superscripts ($p < 0.05$). Means within the same column that have no common letters denote statistically significant differences among the figures concerned.

Table 4. Estimated exposure dosage and target hazard quotient for heavy metals – South Western Monsoon Season.

Seaweed	Site	As		Cu		Cr		Ni		Cd		Pb	Hg
		Ex Dosage	THQ	Ex Dosage	THQ	Ex Dosage	THQ	Ex Dosage	THQ	Ex Dosage	THQ		
Chlorophyta	01	0.00	0.00	0.06	0.00	2.01	0.67	0.28	0.01	0.19	0.19	ND	ND
	02	0.00	0.00	0.14	0.00	2.19	0.73	0.31	0.02	0.23	0.23	ND	ND
	03	0.31	1.05	0.12	0.00	2.71	0.90	0.35	0.02	0.22	0.22	ND	ND
	04	0.34	1.15	0.17	0.00	3.29	1.10	0.29	0.01	0.25	0.25	ND	ND
Rhodophyta	01	0.04	0.14	0.08	0.00	1.83	0.61	0.25	0.01	0.16	0.16	ND	ND
	02	0.06	0.20	0.20	0.00	2.16	0.72	0.35	0.02	0.17	0.17	ND	ND
	03	0.26	0.86	0.26	0.01	3.03	1.01	0.34	0.02	0.16	0.16	ND	ND
	04	0.32	1.08	0.36	0.01	2.90	0.97	0.36	0.02	0.26	0.26	ND	ND
Phaeophyta	01	0.00	0.00	0.08	0.00	1.52	0.51	0.41	0.02	0.17	0.17	ND	ND
	02	0.00	0.00	0.11	0.00	2.09	0.70	0.45	0.02	0.13	0.13	ND	ND
	03	0.25	0.82	0.18	0.00	2.46	0.82	0.42	0.02	0.17	0.17	ND	ND
	04	0.34	1.15	0.24	0.01	2.58	0.86	0.42	0.02	0.24	0.24	ND	ND

Table 5. Estimated exposure dosage and target hazard quotient for heavy metals – North East Monsoon Season.

Seaweed	Site	Ex Dosage	THQ	Ex Dosage	THQ	Ex Dosage	THQ	Ex Dosage	THQ	Ex Dosage	THQ	Pb	Hg
		As		Cu		Cr		Ni		Cd			
Chlorophyta	01	0.00	0.00	0.13	0.00	1.42	0.47	0.32	0.02	0.16	0.16	ND	ND
	02	0.00	0.00	0.17	0.00	1.52	0.51	0.45	0.02	0.18	0.18	ND	ND
	03	0.32	1.06	0.16	0.00	2.82	0.94	0.36	0.02	0.27	0.27	ND	ND
	04	0.00	0.00	0.17	0.00	2.61	0.87	0.39	0.02	0.29	0.29	ND	ND
Rhodophyta	01	0.04	0.14	0.23	0.01	2.90	0.97	0.35	0.02	0.24	0.24	ND	ND
	02	0.00	0.00	0.20	0.01	3.05	1.02	0.33	0.02	0.24	0.24	ND	ND
	03	0.25	0.83	0.28	0.01	3.20	1.07	0.31	0.02	0.22	0.22	ND	ND
	04	0.18	0.61	0.30	0.01	3.28	1.09	0.31	0.02	0.21	0.21	ND	ND
Phaeophyta	01	0.00	0.00	0.20	0.00	2.45	0.82	0.26	0.01	0.17	0.17	ND	ND
	02	0.00	0.00	0.22	0.01	3.06	1.02	0.15	0.01	0.21	0.21	ND	ND
	03	0.26	0.88	0.21	0.01	2.51	0.84	0.31	0.02	0.18	0.18	ND	ND
	04	0.00	0.00	0.22	0.01	2.83	0.94	0.35	0.02	0.20	0.20	ND	ND

Table 6. Hazard index for sample seaweed classes from identified areas of Sri Lanka.

Seaweed	Site	South Western Monsoon	North Eastern Monsoon
		HI	
Chlorophyta	01	0.88	0.65
	02	0.98	0.71
	03	2.19	2.29
	04	2.51	1.18
Rhodophyta	01	0.92	1.37
	02	1.11	1.28
	03	2.06	2.14
	04	2.33	1.93
Phaeophyta	01	0.70	1.01
	02	0.85	1.25
	03	1.84	1.92
	04	2.27	1.16

RfD values of each element were 0.3, 40, 3, 20, and 1 µg per kg body weight per day for As, Cu, Cr, Ni and Cd respectively. Recommended RfD values are with reference to USEPA and mean levels of the metals were used for calculations.

is significantly different to that of chlorophytes and rhodophytes. However, Cu and Ni are significantly lower ($p < 0.05$) in chlorophytes. This result is in accordance with the study results of Rubio et al. (2017) where it is highlighted that the red seaweeds generally contain higher concentrations of Cd than brown seaweeds. There are significant differences in metal concentrations found at different sites tested across the country, where as site 01 (Jaffna Valaipadu) shows the least concentration of all the metals in all seaweed varieties. This could be due to the significantly low levels of industrialization in the area. Considering all the tested areas and in all the seaweed species, presence of metals could be sequenced as Cr > Ni > Cd > Cu > As > Pb = Hg. However, this result may vary in different locations inside the country as well as the globe. As per (Quing et al., 2018), in south east China, this result varies as As > Cu > Cr > Ni > Cd > Pb > Hg. In Italian

coastal regions, metal concentration varies as Al > As > Cd (Desideri et al., 2016). In a study done in Kenya coastal regions, concentrations vary from Pb > Cd > Hg (Mutia, 2018). Nevertheless, on positive note, Sri Lankan Seaweeds in neither of the locations were positive for Pb and Hg, where as in India (Rao et al., 2007) and China (Quing et al., 2018), Pb and Hg concentration was positive for all the tested locations.

Having said that, it is evident that the metal ions in higher concentrations are toxic to humans as well as to the plant itself. Metal ions enter the plant by diffusion through plasma membrane, by endocytosis and due to the activity of special metal ion transporters in the plasma membrane (Colangelo et al., 2006). Excessively entered trace metals in plant cells are leading to oxidative stress which will in return leads to destruction of membranous organelles (Glińska et al., 2007), destruction of enzyme functioning by changing the confirmation and by altering the water balance (Patra et al., 2004). These will result in plant growth inhibition and necrosis. However, to tolerate these high levels of metal concentrations, plants have developed defense mechanisms. In these mechanisms, metal ions are chelated within the rhizosphere, bound by the cell wall components and the migration is blocked by the callus layer of the plant. Another strategy of removing the metal ion from the cytoplasm is by, sequestration in cell walls and vacuole (Krzyszowska et al., 2009) and (Krzyszowska et al., 2010)

This activity of cell wall is depending upon the ability of the cell wall to bind divalent and trivalent metal ions. This ability depends on the availability of the functional groups present such as COOH, OH and SH occurring compounds in cell wall. However, the presence of these groups rely largely on the amount of polysaccharides present (Pelloux et al., 2007). Therefore, it is obvious that the seaweeds with high polysaccharide content rich with carboxyl groups will possess a higher ability to reduce the metal ion absorption to the plant cell.

Though some researches have shown significant levels of arsenic, specifically in brown seaweeds; genus *Sargassum* (Domínguez, 2004) in other global locations, it is evident that in Sri Lankan geography, arsenic levels in seaweeds are significantly low. Edirisinghe and Jinadasa (2015) have

extensively studied on the cadmium and arsenic levels of seaweeds in Sri Lanka. As per their study, 18 different species including *Sargassum* sp., *Padina* sp., and *Ulva* sp. extracted from North Eastern coast and South Western coast were tested for the presence of arsenic, by using accelerated microwave digestion followed by analysis through atomic absorption spectroscopy. According to their study, the highest levels of arsenic was found in *Chaetomorpha crassa* at 3.32 mg/kg level. However, arsenic was not detected in the four varieties obtained from the Northern coastal regions of Sri Lanka. This result is in par with the observations and results obtained in site 01 as well. In addition, parallel studies conducted with two different methodologies have resulted in Arsenic values which are not significantly different from each other. Also, analysis results of the NIST-SRM 3232 for Kelp powder (*Thallus laminariae*) signify that the results obtained through the evaluation methodologies could be justified.

Differences observed at these locations may be due to geography, presence of different industries and the nature of the seaweed along with the samples selected. A previously done study has also concluded that there are significant differences present in the levels of metals present on 16 different species of seaweeds tested (Jadeja and Batty, 2013) and suggests due to different sample origins.

At the north east monsoon season, concentrations of metals have shown a significant difference. In terms site 04 (Matarra), As is not detected in both chlorophytes and phaeophytes. Similarly, in site 02 (Kalpitiya) As is not detected in rhodophytes. This contrasts with the result obtained in south western monsoon season. However, this result is in par with the test result obtained by Mutia et al., 2018, where it is declared that the Cd concentration of the study area has varied from ND range to 1.0 mg kg⁻¹ and stresses the fact that the biological availability of heavy metals may vary significantly depending upon the ambient environment conditions. Since the two monsoon seasons depict the dry and wet seasons for the respective areas, it is clear that the result obtained by this study can also be justified using the same interpretation. In analyzing As content, total As level is tested in the study. However, it is identified that the organic As is less toxic than the inorganic As (Quing et al., 2018) and seaweeds contain 90% of inorganic arsenosugars, out of the total As content (Rose et al., 2007). As per Ronan et al., 2017, seaweeds accumulate the aforesaid inorganic arsenic from the sea, as hydrogen arsenate, replacing the phosphate anion, which then metabolizes to arsenosugars and arsenolipids. As per this study, 50% of the total arsenic is comprising of inorganic arsenic, ranging up to 87 mg kg⁻¹ in *L. digitata* while the total arsenic content ranged up to 131 mg kg⁻¹. Another aspect of assessing the bio-accessibility of arsenic is proposed by Laparra et al. (2003), where effect of cooking the seaweed is also taken into consideration. As per this study, cooking the seaweed increases the bio-accessibility by a fraction of 26 µg g⁻¹ as seen in the increment of inorganic arsenic content of *Porphyra* sp. and *H. fusiforme*, by 73% and 88% respectively. However, these differences are not captured in calculating the THQ levels and therefore, HI in the study signifies the maximum levels of toxicity in the raw form of seaweeds.

In considering the HI value, South Western Monsoon (SWM) season shows lower values than North East Monsoon (NEM). However, lowest HI is achieved for phaeophytes (*Sargassum* sp.) from site 01 (Jaffna) followed by phaeophytes in site 02 and chlorophytes in site 01. However, latter sites and seaweed types indicate HI values with proximity to 1. However, in NEM season, only chlorophytes from site 01 and 02 are at the safe levels for consumption, with having HI < 1. In addition, previously done studies show that the effects from heavy metals does not impose any high risk on human health in Italy (Rubio, 2017) and South Korea (Desideri et al., 2016).

Per capita consumption of seaweeds per day was taken as 5.2 g (FAO, 2016) This value may change depending upon the different consumption patterns of different countries and continents. Therefore, calculated values of HI can vary when considering different areas of the globe.

5 Conclusion

As per the study done on Sri Lankan coastal areas, it is evident that the least levels of metal and metalloids elements were present in site 01 (Jaffna) in phaeophytes (*Sargassum* sp.). Also, this area shows the least HI for chlorophytes as well. However, since the seaweeds are grown for commercial purposes only in the site 01 area presently, it is evident that the chlorophyte and phaeophyte varieties claim very low health risk for potential heavy metals and are suitable for consumption purposes.

However, it is also required to continuously monitor the levels of metals present and imposing of regulations on maximum levels to be sought for.

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