Patterns of Elemental Concentrations and Heavy Metal Accumulation in Edible Seaweed, *Gracilaria fisheri* (Xia and Abbott) Abbott, Zhang and Xia (Gracilariales, Rhodophyta) Cultivated in Southern Thailand

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ABSTRACT

Patterns of elemental concentration and heavy metal accumulation were examined in the edible seaweed, *Gracilaria fisheri*, cultivated in ponds located in three provinces of Southern Thailand. Seaweed, water, and sediment were collected in the dry season during the cultivation period. The amounts of macro-elements, micro-elements and heavy metals in seaweed were found in the following ranges: calcium (Ca) 9.22–10.01, magnesium (Mg) 11.40–13.40, potassium (K) 1.85–23.35, sodium (Na) 1.60–4.96, copper (Cu) 1.65–2.91, manganese (Mn) 557.05–746.75, zinc (Zn) 30.15–36.60, iron (Fe) 172.95–841.23, nickel (Ni) 6.03–8.26, chromium (Cr) 1.43–2.80, cadmium (Cd) 0.08–0.13, and lead (Pb) 4.79–6.60 $\mu g \cdot g^{-1}$ dry weight. None of the elements showed a relationship between levels in seaweed and in either water or sediment, but we did find relationships with other elements. Macro-elements Mg, K and Na in seaweed showed a relationship with Mg, Na and Zn concentrations in the water, and with Cu, Mn, Fe and Ni concentrations in the sediment. Apparently, micro-elements Mn and Fe in seaweed were related to Cu, Mn and Cr concentrations in the sediment. Our study indicated that the patterns of element and heavy metal concentration in seaweed, *G. fisheri*, were inconsistent with the patterns of those elements in its surrounding environment.

Keywords: agarophyte, cultivation, food, macroalgae, safety

INTRODUCTION

Seaweeds have long been consumed as a natural source of food and medicine in Asian countries (Besada *et al*., 2009) and western countries (Almela *et al*., 2006) due to their high fiber and mineral concentrations and low fat content (Rodenas de la Rocha *et al*., 2009). Seaweeds are popularly used as food products, adhesives, pharmaceutical products and others (Murty and Banerjee, 2012). Tiwari and Troy (2015) mentioned that marine seaweed contains higher amounts of macro-elements, including calcium (Ca), magnesium (Mg), potassium (K) and sodium (Na), and micro-elements, including copper (Cu), manganese (Mn), zinc (Zn) and iron (Fe), than those reported for land vegetables. Unfortunately, seaweeds also exhibit a high metal accumulation capacity (Almela *et al*., 2006). Heavy metals such as nickel (Ni), chromium (Cr), cadmium (Cd) and lead (Pb) are considered toxic elements when present in excessive quantities in water (Aziz *et al*., 2008). Also, it is well known that metals in water are directly absorbed by seaweed (Muse *et al*., 1999). Thus, concern about heavy metals in seaweed and the surrounding environment has been expressed (Hashim and Chu, 2004). There are

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Received 24 July 2018 / Accepted 7 September 2018

strong relationships between heavy metal content in seaweeds and environmental parameters (Rodenas de la Rocha *et al*., 2009). Therefore, seaweeds have recently become known as a bio-indicator for marine environmental contamination (Besada *et al*., 2009). For instance, *Ulva lactuca* was used as a bio-indicator of Cu, Mn, Zn, Fe and Pb pollution in tropical and sub-tropical marine waters (Kozhenkova *et al*., 2006). Chernova *et al*. (2002) also mentioned that brown algae and seagrasses accumulate pollutants proportionally to their environment and they are used as indicator organisms for integral assessment of heavy metal pollution levels of coastal waters.

Gracilaria fisheri (Xia and Abbott) Abbott, Zhang and Xia is a commercial red algal species that is commonly used as raw material for agar production (Chirapart *et al*., 2006). In Thailand, this alga is also eaten fresh and used in dried products (Benjama and Masniyom, 2012). This alga has been commonly cultured in natural earthen ponds (Chirapart and Lewmanomont, 2004) and abandoned shrimp ponds, and it requires approximately two months to produce a crop (Ruangchuay *et al*., 2010). However, knowledge regarding nutrient content and environmental characteristics of this species is still limited. Therefore, the study aimed (1) to evaluate the concentrations of macro-elements (Ca, Mg, K and Na), micro-elements (Cu, Mn, Zn and Fe) and heavy metals (Ni, Cr, Cd and Pb) in cultivated *G. fisheri*, and (2) to investigate the relationship between the concentrations of these elements in *G. fisheri* and concentrations in water and sediment.

MATERIALS AND METHODS

Algal sampling

Samples of *Gracilaria fisheri* were cultivated in earthen ponds situated in southern Thailand provinces, including Pattani (P1), Songkhla (P2), and Surat Thani (P3) (Figure 1) in early 2014. In each province, the samples were randomly collected from four different ponds, all of which were located in coastal areas. The ponds were filled with natural saline water for algal cultivation, without any water exchange until harvest. The pond sizes were in the ranges of 0.16–0.48 ha for P1; 0.16–0.64 ha for P2; 0.16–0.48 ha for P3. The distances among ponds in each site were between 0.5–4.0 km for P1; 0.1–5.0 km for P2 and 0.5–5.0 km for P3. Average water depth and temperature were recorded in every pond with overall ranges of 28–72 cm and 30–33 ºC, respectively, while salinity was in the range of 15–24 ‰. Samples of seaweed

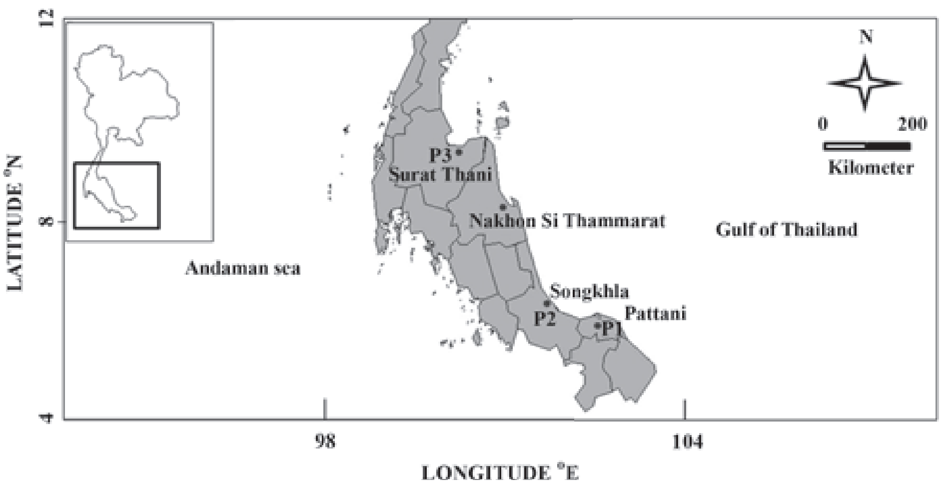

Figure 1. Map of experimental ponds in Pattani (P1), Songkhla (P2) and Surat Thani (P3) provinces, Thailand.

at the harvesting stage, water, and sediment were randomly collected by hand from three different points of each pond. Approximately 1 kg of both seaweed and sediment were collected, along with 1 L of water, from near the pond bottom at each sampling point. The samples were kept in an insulated cooler and transferred back to the laboratory. The fresh algal samples were then cleaned, and contaminants such as mollusks and epiphytes were removed. Before analysis, the samples were dried at 60ºC in a vacuum oven for 24 hours, and then kept in plastic bags. Sediment samples were dried, ground, sieved with 200 mesh sieves, and then stored in plastic containers before analysis.

Determination of element contents (AOAC, 2000)

The analysis of macro- and micro-element and heavy metal contents was conducted with three replications for each type of sample collected from each pond. Atomic Absorption Spectrophotometry (AAS) of Perkin ElmerAnalyst 100 model was used to determine the concentrations of Ca, Mg, Cu, Mn, Zn, Fe, Ni, Cr, Cd and Pb in seaweed (method 975.03) and in sediment (method 965.09). For the concentrations of K and Na in the seaweed (method 956.01) and in sediment (method 983.02), the analysis was performed using the Flame Photometric method. Element contents in the seaweed and sediment samples were extracted using 1 g of dried and ground sample in a 150 mL Pyrex beaker. Then, 10 mL HNO_3 was added to the dried sample and let soak thoroughly. Next, 3 mL 60% $HClO_4$ was added, and the beaker was heated slowly on a hot plate until frothing ceased and HNO_3 was almost evaporated. After cooling, 10 mL HCl (1+1) was added to the solution and transferred to a 50 mL volumetric flask while filtering with microfiber filters before the analysis.

The AAS was also used to determine the element concentrations in water of Ca, Mg, Cu, Mn, Zn, Fe, Ni, Cr, Cd and Pb (method 974.27), K (method 973.53) and Na (method 973.54). Element concentrations in the water samples were measured by placing 100 mL of sample in a 250 mL beaker. The pH of the sample and standard solution was adjusted to 2.5 with HCl. Then, 2.5 mL ammonium pyrrolidine dithiocarbamate was added to the sample and mixed, followed by 10 mL methyl iso-butyl ketone. The beaker was vigorously shaken for 1 minute before extraction. The AAS instrument was calibrated with standard solution and the calibration curve was established from the average of each standard before and after reading samples against a blank. Analytical blanks were run in a procedure similar to the specimens.

Data analysis

One-way ANOVA was used to compare the means of element amounts and heavy metal accumulations in seaweed, water and sediment. The differences among means were compared at the significance level of $P<0.05$ by using Tukey HDS test. Pearson's correlation coefficient test was used to determine the relationships between element concentrations in seaweed and water, and between seaweed and sediment. The recommended daily intake (g DW) of *G. fisheri* from different sites was calculated based on the permissible daily doses (µg) of heavy metals reported in Subba Rao *et al*. (2007).

RESULTS

The study demonstrated the relationships between some elements and heavy metals in seaweed and water and between seaweed and sediment. The element and heavy metal concentrations showed different patterns among sampling sites (provinces).

Element concentrations and heavy metal accumulations in Gracilaria fisheri

The concentrations of macro-elements in *G. fisheri* were in the pattern of Mg>K>Ca>Na (Figure 2a). The macro-element amounts of Ca, Mg, K and Na in the seaweed at P1 (Pattani Province) were 9.22, 11.59, 23.35 and 4.96 $mg{\bullet}g^{-1}$ DW, respectively. The amounts of K and Na in seaweed at P1 were significantly higher than those at the other two sites. There was no significant difference ($P>0.05$) in Ca or Mg concentration in the seaweed at the three sites. The micro-element contents in *G. fisheri* showed no significant differences (P>

0.05) among the three sites and were found in the decreasing order of Mn>Fe>Zn>Cu (Figure 2b). The amounts of micro-elements in the seaweed from all samples were in the following ranges: Cu 1.65–2.91, Mn 557.05–746.75, Zn 30.15–36.60, Fe 172.95–841.23 $\mu g \cdot g^{-1}$ DW. The concentrations of heavy metals in seaweed showed no significant differences (P>0.05) among the three sites except Cd, which was higher at P2 (Songkhla Province) than those of the other two sites (Figure 2c). Heavy metals in *G. fisheri* showed the pattern of Ni>Pb>Cr>Cd, with the following ranges of concentrations: Ni 6.03–8.26, Pb 4.79–6.60, Cr 1.43–2.80, Cd 0.08–0.13 $\mu g \cdot g^{-1}$ DW.

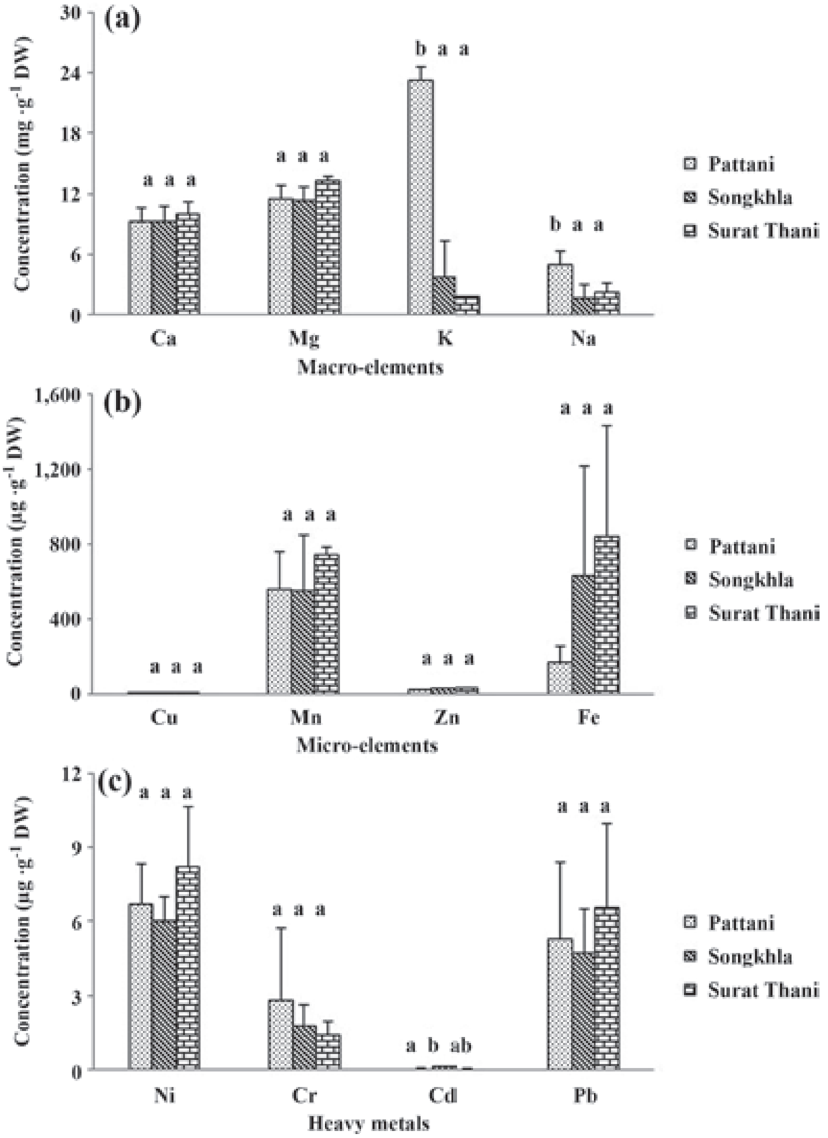

Figure 2. Element concentrations and heavy metal accumulations in *Gracilaria fisheri* collected from ponds in three Thai provinces. (a) Macro-elements; (b) Micro-elements; (c) Heavy metals. Different letters above the bars indicate statistically significant difference (p<0.05).

Element concentrations and heavy metal accumulations in water

In the water used for seaweed cultivation, the micro-element amounts were ranked as Ca>Na>K>Mg, and were higher than those in sediment and seaweed. The micro-element concentrations in ambient water were in the following ranges: Ca 4,971–5,723, Na 1,408–1,846, K 1,018–1,261, Mg 625–820 mg•L^{-1}. The Mg concentration at P1 (625 mg•L^{-1}) was significantly lower ($P<0.05$) than those at the other two sites (Figure 3a). However, no significant difference was found in the amounts of Ca, K or Na in water among the three sites. In this study, the concentrations of micro-elements and heavy metals in the water followed the pattern of Pb>Ni>Fe>Mn>Cr>Cu>Zn>Cd. The average concentrations of Pb, Ni, Fe, Mn, Cr, Cu, Zn and Cd in the water were 547.53, 506.92, 377.92, 150.28, 105.75, 60.78, 42.44, 38.12 µg•L^{-1}, respectively. The pattern of Fe>Zn>Cu was the same in the seaweed and water. The Fe concentration in water at P3 (Surat Thani Province) was significantly higher ($P<0.05$) than those at the other two provinces (Figure 3b). There was no difference in the amounts of the other three micro-elements (Cu, Mn and Zn) among the three provinces. The heavy metal concentrations in the water showed the pattern of Pb>Ni>Cr>Cd, and and differed from the pattern in seaweed. There were no significant differences ($P>0.05$) in heavy metal concentrations in water among the three sites (Figure 3c).

Element concentrations and heavy metal accumulations in sediment

The general sediment characteristics of the ponds were sandy silt in P1, and silt in P2 and P3. The amounts of macro-elements in the sediment showed the decreasing order of Ca>Mg>K>Na with the following ranges of concentrations: Ca 82–278, Mg 134–162, K 75–87 and Na 34–48 mg•g^{-1} DW. The amount of Ca in the sediment at P2 was significantly higher ($P<0.05$) than those of the other two provinces (Figure 4a). The concentrations of Mg, K and Na in the sediments of the three provinces were not significantly different ($p>0.05$). In addition, micro-element and heavy metal amounts in the sediments showed the pattern of Fe>Mn>Zn>Ni>Cu>Pb>Cr>Cd, with the following ranges: Fe 11,193–12,446, Mn 5,462–10,042, Zn 840.50–989.58, Ni 212–297, Cu 67.92–213.23, Pb 24.29–34.03, Cr 12.72–16.17, Cd 0.99–1.23 µg•g^{-1} DW. The amounts of Cu and Mn in sediment were significantly different ($P<0.05$) among the three sites (Figure 4b). The heavy metal accumulations in sediment in the three provinces showed no significant differences ($P>0.05$) (Figure 4c).

Correlation of elements and heavy metals in different habitats

Pearson correlation tests showed relationships among element concentrations in *G. fisheri* (Table 1). There were positive correlations among some elements in the seaweed: K and Na (r=0.85), Mn and Mg (r=0.77), Ni and Mg (r=0.77), Ni and Mn (r=0.70). In addition, Cd in the seaweed was inversely related with K (r=-0.61) and Na (r=-0.62), whereas Pb concentration showed a positive relationship only with Mn (r=0.65). Also, there were relationships between concentrations in seaweed and water (Table 2) and between seaweed and sediment (Table 3) for some macro-elements (Mg, K and Na), micro-elements (Mn and Fe), and heavy metals (Ni, Cr and Pb). Magnesium in the seaweed showed a negative relationship with Na in the water (r=-0.63) and a positive relationship with Cu in the sediment (r=0.80). Of the micro-elements, Mn in the seaweed showed a positive relationship with Cu (r=0.60) and Cr (r=0.63) in the sediment. The Fe concentration in the seaweed showed a positive relationship with Cu (r=0.67) and Mn (r=0.60) in the sediment. There was no relationship between Cu or Zn in the seaweed and elements in the water and sediment. The heavy metals Ni and Cr in the seaweed only showed relationships to some elements in the water, whereas Pb in the seaweed was only related to Cr in the sediment. Ni in the seaweed showed a negative relationship with Na (r=-0.87) and a positive relationship with Mn (r=0.63) in the water. Chromium in the seaweed showed a negative relationship with Mg (r=-0.61) in the water. Lead in the seaweed showed a positive relationship with Cr (r=0.62) in the sediment. However, there was no relationship between Cd in the seaweed and elements in the water or sediment.

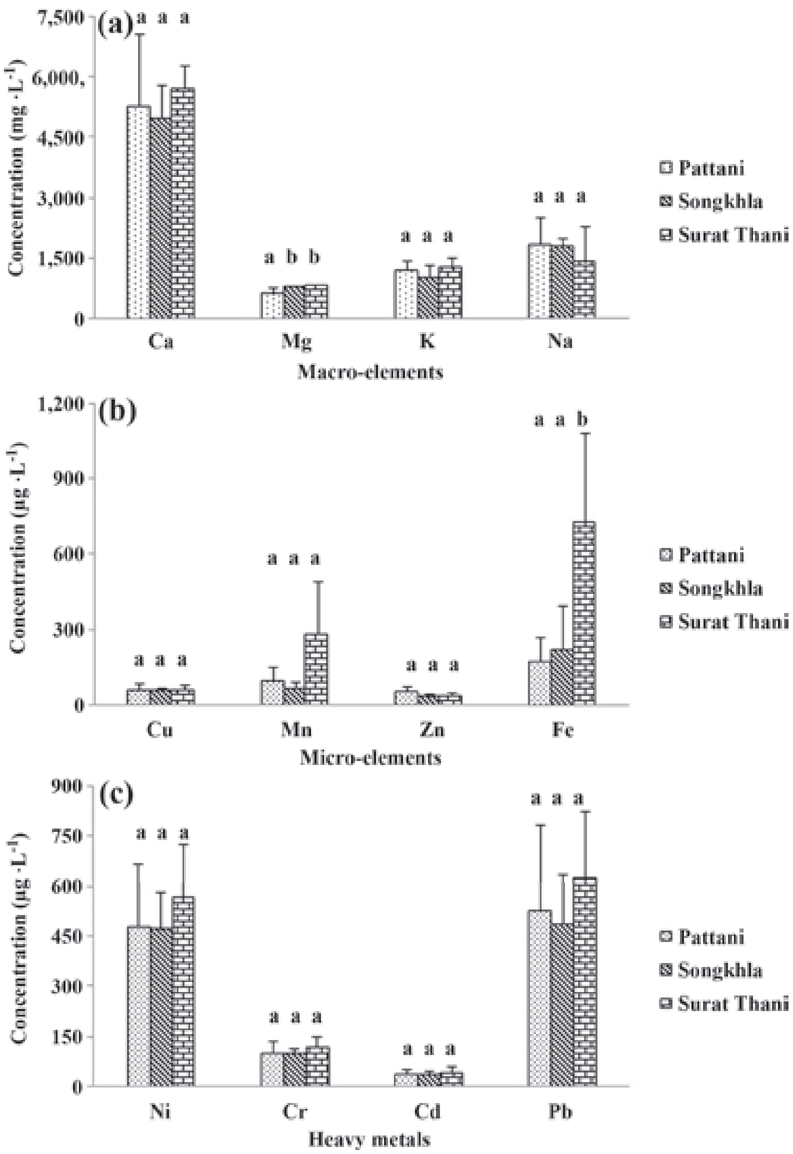

Figure 3. Element concentrations and heavy metal accumulations in water collected from ponds in three Thai provinces. (a) Macro-elements; (b) Micro-elements; (c) Heavy metals. Different letters above the bars indicate statistically significant difference ($p<0.05$).

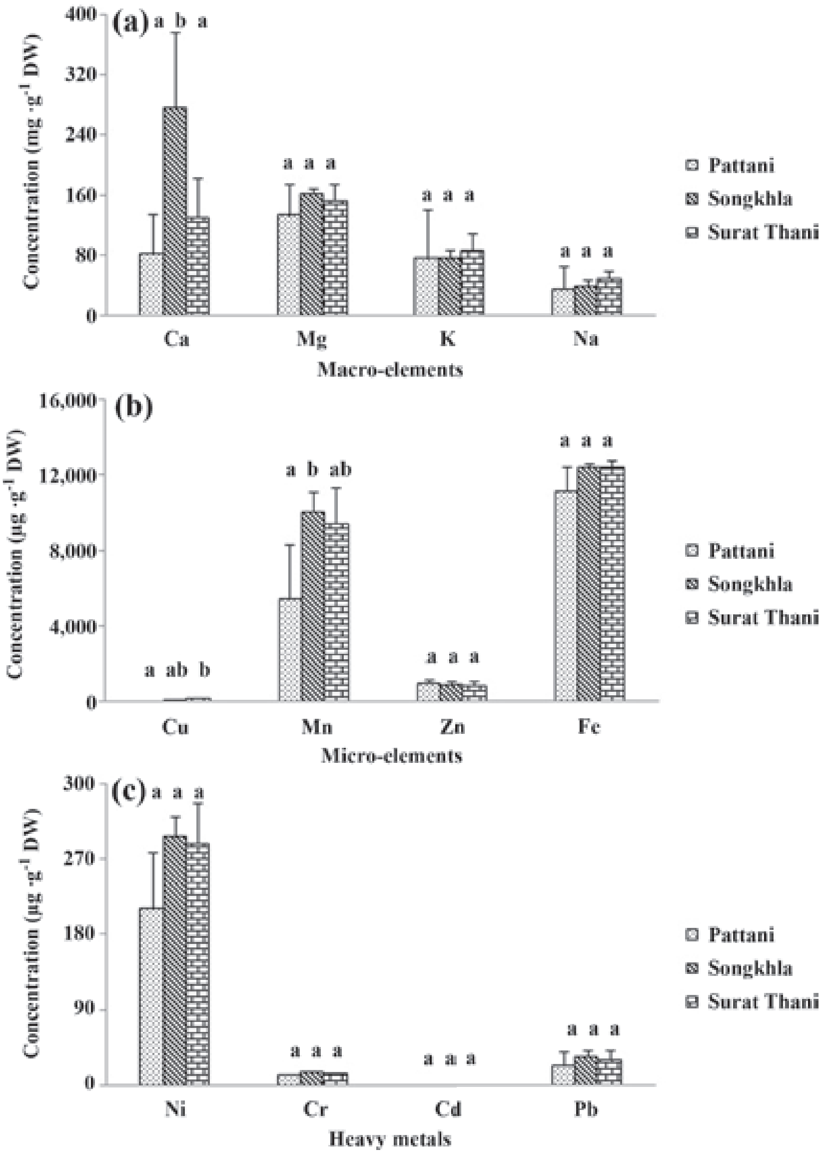

Figure 4. Element concentrations and heavy metal accumulations in sediment collected from ponds in three Thai provinces. (a) Macro-elements; (b) Micro-elements; (c) Heavy metals. Different letters above the bars indicate statistically significant difference ($p<0.05$).

Table 1. Correlations among element and heavy metal concentrations in *Gracilaria fisheri* (n=12).

	Ca	Mg	K	Na	Cu	Mn	Zn	Fe	Ni	Cr	Cd	Pb
Ca	1											
Mg	0.27	1										
K	-0.22	-0.34	1									
Na	-0.22	-0.03	0.85**	1								
Cu	0.17	0.25	-0.19	-0.30	1							
Mn	0.23	0.77**	-0.15	0.16	0.24	1						
Zn	0.06	0.17	-0.32	-0.21	-0.17	0.48	1					
Fe	0.18	0.43	-0.51	-0.45	0.56	0.40	-0.04	1				
Ni	-0.07	0.77**	-0.11	0.17	0.03	0.70*	0.40	0.03	1			
Cr	-0.40	0.31	0.33	0.47	0.11	0.23	0.00	-0.18	0.31	1		
Cd	-0.11	-0.18	-0.61*	-0.62*	0.36	-0.30	0.10	0.28	-0.28	0.00	1	
Pb	0.28	0.56	-0.04	0.33	0.15	0.65*	0.06	0.33	0.30	0.35	-0.07	1

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

Table 2. Correlations between element and heavy metal concentrations in *Gracilaria fisheri* and the ambient water (n=12).

	Seaweed											
	Ca	Mg	K	Na	Cu	Mn	Zn	Fe	Ni	Cr	Cd	Pb
Water												
Ca	0.53	0.12	-0.06	-0.25	0.24	0.10	0.02	0.17	0.17	-0.48	-0.09	-0.17
Mg	0.08	0.06	-0.83**	-0.83**	0.19	-0.03	0.07	0.45	-0.08	-0.61*	0.41	-0.22
K	0.36	0.42	0.08	0.03	0.35	0.29	-0.16	0.30	0.44	-0.20	-0.24	0.24
Na	0.20	-0.63*	0.20	-0.05	-0.01	-0.38	-0.34	0.06	-0.87**	-0.38	-0.01	-0.13
Cu	0.31	0.08	0.02	-0.23	0.18	0.17	0.17	0.03	0.28	-0.39	-0.21	-0.27
Mn	-0.14	0.49	-0.29	-0.26	-0.10	0.38	0.32	0.35	0.63*	-0.12	-0.16	-0.14
Zn	-0.29	0.14	0.66*	0.58*	0.14	0.02	-0.29	-0.28	0.28	0.54	-0.48	-0.16
Fe	0.34	0.45	-0.44	-0.06	-0.17	0.41	-0.05	0.26	0.20	-0.19	-0.06	0.47
Ni	0.37	0.25	-0.18	-0.35	0.24	0.23	0.20	0.07	0.41	-0.40	-0.13	-0.25
Cr	0.38	0.26	-0.20	-0.34	0.16	0.20	0.17	0.05	0.43	-0.42	-0.14	-0.28
Cd	0.41	0.37	-0.12	-0.28	0.31	0.29	0.18	0.01	0.47	-0.17	-0.17	-0.15
Pb	0.35	0.25	-0.11	-0.28	0.24	0.24	0.16	0.08	0.41	-0.37	-0.18	-0.23

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

Table 3. Correlations between element and heavy metal concentrations in *Gracilaria fisheri* and the ambient sediment (n=12).

	Seaweed											
	Ca	**Mg**	**K**	**Na**	**Cu**	**Mn**	**Zn**	**Fe**	**Ni**	**Cr**	**Cd**	**Pb**
Sediment												
Ca	-0.21	-0.35	-0.52	-0.54	-0.08	-0.25	0.46	-0.07	-0.23	-0.25	0.53	-0.26
Mg	-0.15	0.04	-0.45	-0.27	0.05	-0.14	-0.19	0.05	-0.18	0.01	0.25	0.00
K	-0.33	0.22	-0.12	0.12	-0.17	0.01	-0.26	-0.07	0.07	0.28	-0.13	0.16
Na	-0.12	0.42	-0.29	0.03	-0.18	0.16	-0.07	0.04	0.14	0.37	-0.04	0.23
Cu	0.37	0.80**	-0.53	-0.26	0.52	0.60*	-0.09	0.67*	0.49	-0.01	0.14	0.55
Mn	-0.06	0.11	-0.77**	-0.69*	0.29	-0.10	-0.16	0.60*	-0.28	-0.20	0.57	0.01
Zn	-0.46	-0.41	0.29	0.25	-0.01	-0.18	-0.32	-0.11	-0.43	0.27	0.03	-0.05
Fe	-0.21	0.20	-0.67*	-0.50	0.18	0.03	-0.11	0.32	0.00	-0.11	0.36	0.03
Ni	-0.10	0.24	-0.63*	-0.33	0.20	0.23	0.03	0.30	0.05	-0.01	0.39	0.34
Cr	-0.06	0.44	-0.49	-0.14	0.37	0.63*	0.42	0.52	0.27	0.31	0.44	0.62*
Cd	-0.44	0.16	-0.27	-0.05	0.08	0.07	-0.13	0.03	0.07	0.31	0.16	0.21
Pb	-0.24	-0.03	-0.30	-0.03	-0.02	-0.05	-0.21	0.02	-0.17	0.03	0.19	0.20

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

DISCUSSION

Our results showed higher amounts of Ca, Mg and Na and lower amounts of K in *G. fisheri* than those previously reported from Pattani Bay, Thailand (Benjama and Masniyom 2012). Concentrations of Ca and Mg in *G. fisheri* (9.52± 1.41 and 12.13 ±1.03 mg•g^{-1} DW) in the present study were found at similar levels to those in *Gracilaria confervoides* (16.20 and 16.57 mg•g^{-1} DW) and *Gracilaria corticata* (11.73 and 4.58 mg•g^{-1} DW). Meanwhile, amounts of K and Na in *G. fisheri* (9.64±1.72 and 2.96±1.17 mg•g^{-1} DW) were much lower than those in *G. confervoides* (11.20 and 27.80 mg•g^{-1} DW) and *G. corticata* (114.75 and 26.29 mg•g^{-1} DW) (Modera-Pineiro *et al*., 2012). The variation of Ca, Mg, K and Na concentrations in seaweed depends on the species (Baghel *et al*., 2014; Syad *et al*., 2013), season (Kumar *et al*., 2015) and sampling site (Subba Rao *et al*., 2007). The pattern of Fe>Zn>Cu in *G. fisheri* was similar to the pattern in *Gracilaria verrucosa* (Khaled *et al*., 2014). The Fe in the seaweed (0.55± 0.42 mg•g^{-1} DW) was at a higher concentration than the other micro-elements and higher than in *Gracilaria changii* (0.09 mg•g^{-1} DW) (Norzian and Ching, 2000) but lower than in *Gracilaria pachidermatica* (4.50 mg•g^{-1} DW) (Sanchez-Rodriguez *et al*., 2001), both which were cultured in open water areas. Chernova and Sergeeva (2008) reported that the variation in Fe concentration in the brown seaweed *Sargassum* was due to differences in specific surface area among algal species. The accumulation of heavy metals from sediment by plants was found to be affected by chemical characteristics of sediment including pH, chlorine concentration, redox conditions and cation exchange (Nazli and Hashim, 2010). The Pb concentration was higher than Cd in *G. fisheri*; however, the amounts of Pb and Cd (4.79–6.60, 0.08–0.13 µg•g^{-1} DW) in this study were higher than those previously reported (Benjama and Masniyom, 2012). However, the provisional tolerable weekly intake (WHO, 2000) for an adult is 420 µg for Cd and 300 µg for Pb (assuming a 60 kg body weight). The pattern of Pb>Cr>Cd found in *G. fisheri* in this study is similar to *G. verrucosa* from a natural source in Egyptian mediterranean sea (Khaled *et al*., 2014) and *Gracilaria gracilis* from a Venice lagoon, Italy (Caliceti *et al*., 2002), but different from that in red alga, *Tichocarpus crinitus* from Risovaya and Sivuchya bays in Russia, with the order of

Cr>Pb>Cd (Barabanova *et al*., 2010). Element concentrations may vary with different biological factors such as surface habit of algal thallus, the age of tissues, or by geological factors such as chemical composition of seawater, hydrodynamics, currents and geographical latitude of the habitat which controls the day duration, illumination and water temperature (Kozhenkova *et al*., 2006).

In the present study, high concentrations of macroelements (Ca, Mg, K and Na) in the water were likely due to the condition of the brackish waters sampled, as has been reported by Gilles (2013). A previous study indicated that the amount of microelements and heavy metals in water varied among the seasons (Salem *et al*., 2014). For example, the pattern of Cu>Ni>Zn>Cr>Fe>Mn>Pb>Cd was found during summer while the pattern of Ni>Cu>Zn>Cr> Fe>Mn>Pb>Cd was recorded in autumn. The concentrations of the micro-elements in water in this study were higher than those reported in ambient water of *G. verrucosa* and *G. gracilis* (Chakraborty *et al*., 2014; Malea *et al*. 2015). The average concentrations of Cu, Zn, Cd and Pb in the water of the present study were higher than those found in Gulf San Jorge, Southern Atlantic (Muse *et al*., 1999). The high amounts could have been caused by drainage, which can lead to increases of some elements by many orders of magnitude in coastal areas (Steele *et al*. 2009). High concentration of Pb in water has been found to be toxic to aquatic plants (Wang *et al*., 2014). However, the heavy metal amounts in water sampled from this study were lower than those in the ambient water of *G. verrucosa* (Chakraborty *et al*., 2014) and *G. gracilis* (Malea *et al*., 2015). Additionally, the concentrations of Cu, Zn, Ni, Cr, Cd and Pb in the water was lower than the maximum limit standards of 1,000 µg•L^{-1} for Cu, Zn, Ni and Cr that were reported by Aziz *et al*. (2008).

Our study also detected high concentrations of the macro-elements Ca and Mg in the sediment. This could be explained by the admixture of fine shell debris in bottom sediments. Shell debris is mainly composed of calcium carbonate, and we noticed that mollusk shells were abundant in the bottom sediments of each culture pond. Mollusk shells are commonly used as hard substrata for vegetative propagation (Meneses, 1996). The shells fall to the bottom and eventually are decomposed into fine fragments (Krebs, 2003). Additionally, the concentrations of micro-elements and heavy metals in the sediment varied among the seasons. The pattern of Fe>Mn>Zn>Cu>Cr>Ni>Pb>Cd in sediment has been reported from a coastal lagoon in summer compared to Fe>Mn>Zn>Cu>Cr>Pb>Ni>Cd in autumn (Salem *et al*., 2014). Our study indicated the amounts of micro-elements and heavy metals in the sediment were higher than those reported in sediments of *G. verrucosa* beds (Chakraborty *et al*., 2014) and *G. gracilis* beds (Malea *et al*., 2015). We assume that the excessive concentrations of Cu, Zn, Ni, Cd and Pb found in the sediments could be due to the geochemical characteristics at our sampling sites, which were located in coastal zones. Vashchenko *et al*. (2010) reported that surface sediments of coastal areas are most contaminated by anthropogenic pressure because they are located at the boundary between land and ocean. The concentrations of Cu, Zn and Ni in the sediments were significantly higher than those in unpolluted marine sediment, which generally consists of less than 20 µg•g^{-1} Cu, 50 µg•g^{-1} Zn, and 100 µg•g^{-1} Ni (Giusti, 2001). On the other hand, the concentrations of Cd and Pb in the sediments were similar to that in unpolluted sediment, usually less than 1 µg•g^{-1} Cd, and 2–50 µg•g^{-1} Pb (Giusti, 2001). The concentrations of Cr, Pb and Cd showed the same pattern of Pb>Cr>Cd in seaweed, water and sediment.

In the current study no correlation was observed between Fe and other elements in *G. fisheri*. However, a high correlation has been reported between Fe and other metals in *Sargassum* due to metal runoff from urban areas (Chernova and Sergeeva, 2008). Our study showed that the amount of Pb in the seaweed was only correlated with Mn in the seaweed; however, positive relationships between Pb concentration and Cu, Zn, Fe, Ni and Cr concentrations have been observed in submerged macrophytes (Wang *et al*., 2014). The average amount of Cd in the water at P2 was lowest, at 34.50 µg•L^{-1}, while the salinity in this site (15 ‰) was lower than those of P1 (24 ‰) and P3 (21 ‰). This finding is contrary to a previous report that indicated a decrease of Cd concentration with increasing salinity (Neff, 2002). In addition, there was a positive relationship between Ni and Mg in *G. fisheri*, which was similar to findings reported for the green alga *Ulva* (Rybak *et al*., 2012). On the other hand, there was no relationship between Mg in *G. fisheri* and Ni in water and sediment, while such relationship was observed in *Ulva* (Rybak *et al*., 2012), and we attribute this difference due to the different situations. Among the micro-elements, Mn in the seaweed showed a positive relationship with Cu (r=0.60) and Cr (r=0.63) in the sediment, whereas Wang *et al*. (2014) reported that Mn in submerged macrophytes did not show a relationship with elements in sediment. The average concentration of Cu in the water at P3 was highest, at 63.58 $\mu g \cdot L^{-1}$, and this location had the highest water depth (72 cm). This finding conforms to a previous study indicating that Cu concentrations and water depth have a positive relationship (Lobban and Harrison, 1994).

CONCLUSION

The element concentrations and heavy metal accumulations in *Gracilaria fisheri* were in the order of Mg>K>Ca>Na>Mn>Fe>Zn>Cu>Ni>Pb>Cr>Cd. The concentrations of macro-elements, including Ca, Mg, K and Na, were higher in water than in sediment and seaweed. In this study, Ca and Mg were dominant macro-elements in *G. fisheri* and sediment. Mn and Fe were the main micro-elements in *G. fisheri*, water and sediment, while Ni and Pb were dominant heavy metals. Macro-elements, including Mg, K and Na in *G. fisheri* showed relationships with some elements from both the water and sediment. Micro-elements in the seaweed only were correlated with some elements in the sediment. Heavy metals Ni, Cr and Pb in the seaweed had stronger relationships with some elements in the water than elements in the sediment. According to our study, we suggest that the daily consumption of *G. fisheri* should be in the range of 38–52 g DW.

ACKNOWLEDGEMENTS

This study was financially supported by the Faculty of Science and Technology, Prince of Songkla University, Pattani, Thailand.

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