Evaluation of the metal content of farm grown *Gracilaria tikvahiae* and *Saccharina latissima* from Long Island Sound and New York Estuaries

Jang K. Kim[a,b,*], George Kraemer[c,**], Charles Yarish[b]

[a] Department of Marine Science, School of Natural Sciences, Incheon National University, 119 Academy-ro, Yeonsu-gu, Incheon 22012, Republic of Korea
[b] Departments of Ecology & Evolutionary Biology and Marine Sciences, University of Connecticut-Stamford, 1 University Place, Stamford, CT 06901, USA
[c] Department of Environmental Studies, Purchase College, 735 Anderson Hill Road, Purchase, NY 10577, USA

ARTICLE INFO

ABSTRACT

In the US, seaweed aquaculture is still a developing industry. Since a significant number of seaweed farms are located in urbanized estuaries, cultured seaweeds could accumulate contaminants such as heavy metals. A warm temperate red algal species, *Gracilaria tikvahiae* and a cold-water brown algal species, *Saccharina latissima* were cultivated at three sites in Long Island Sound (LIS) and New York (NY) estuaries to biomonitor potentially toxic metals. Metal concentrations were below almost all national and international regulatory limits for human consumption. For example, the highest measured concentrations of Hg in tissue samples of *G. tikvahiae* and *S. latissima* are 88–93% less than the limit set by the Food and Drug Administration of the USA. Concentration maxima of Cd in *G. tikvahiae* and *S. latissima* were 20% and 32%, respectively, lower than stringent French limits. Maximum levels of Pb in tissue samples of *G. tikvahiae* (but not *S. latissima*) were close to the French limits at the NY estuary site. These results indicate generally that heavy metal contents of seaweeds cultivated urbanized estuaries should be monitored since accumulation appears to be site-dependent. The great capacity for the accumulation of nitrogen and other nutrients in seaweed tissues, improving water quality, is also accompanied by the uptake of other, less desirable materials.

1. Introduction

Globally, China, Indonesia, Philippines, Korea and Japan produced over 99% of seaweed for the aquaculture market [1,2]. Seaweeds have been an important part of Asian cuisine, as well as a source of biomaterial for use in the pharmaceutical, cosmetics and hydrocolloid industries [3,4]. Outside of Asia, direct consumption of seaweeds is restricted to scattered coastal areas [5,6]. In the US, seaweed aquaculture is still developing, with commercial seaweed aquaculture (mostly the sugar kelp *Saccharina latissima*) having begun less than a decade ago [7–9]. Seaweed aquaculture is now considered one of the fastest growing marine industries in the Northeast US [1]. Demand from US markets is expected to increase [10]. Most production of seaweeds from aquaculture farms in the US has been used for direct human consumption. Since a significant number of seaweed farms are located in or near urbanized estuaries, the composition of cultured seaweed tissue is a concern [11].

Over 45% of the world's population lives near the coast, and nearly half of the coasts are under threat from human infrastructure and development-related activities [12]. Nearly 80% of the pollution load to the oceans has a terrestrial source, including sewage and industrial effluents, agricultural run-off, and atmospheric deposition of nutrients [12]. Industrial and agricultural activities have also led to metal contamination of the environment [13,14], with potential risk to human and ecosystem health [15–18]. Metals have been discharged into coastal waters by mining, electro-plating, metal processing, fertilizer, pesticide, textile, appliance manufacturing, aerospace and atomic energy installations, tanneries, and printing industries [19,20]. While pollution, as a general problem, is best addressed via source reduction, regulatory protections may lag behind the need for protection, and ecosystems often possess legacies of an industrial past [21,22].

Some seaweed species show a high affinity for metals and have been used as bioindicators [23,24] of metal pollution in estuarine and coastal waters [25–27]. Metal contamination is an important determinant for the safety of edible seaweeds [28–33], but few published studies exist for seaweeds grown in aquaculture in the US [19,34].

* Correspondence to: J.K. Kim, Department of Marine Science, School of Natural Sciences, Incheon National University, 119 Academy-ro, Yeonsu-gu, Incheon 22012, Republic of Korea.
** Corresponding author.
E-mail addresses: jang.kim@inu.ac.kr (J.K. Kim), george.kraemer@purchase.edu (G. Kraemer).

Fig. 1. Seaweed farms in New York estuary and Long Island Sound (LIS). B: Bronx River estuary farm, W: Western LIS farm and C: Central LIS farm.

In the present study, we evaluated metal accumulation by two seaweeds for which the process of biomass production and bioremediation of water quality has been demonstrated in large-scale, field experiments [7–9,35]. Our objectives were to measure the accumulation of five heavy metals in summer-grown (*Gracilaria*) and winter-grown (*Saccharina*) species cultured in Long Island Sound and New York estuaries, and to estimate total metal removal capacity during seasonal growth of these two seaweeds.

2. Materials and methods

2.1. Seaweed cultivation and sample collection

Gracilaria tikvahiae McLachlan (strain G-RI-ST$_1$) was cultivated on two 50 m long-lines at two near-shore sites in western Long Island Sound (WLIS; Fairfield, CT; 41°06.882′ N/73°15.277′ W) and at the mouth of the Bronx River (New York) Estuary (BRE; Bronx, NY; 40° 48.047′N/73° 52.164′W) (Fig. 1). Twenty gram bundles of *Gracilaria* strain were inserted every 20 cm into partially untwisted nylon line. A 50 m long-line was deployed at each of two depths (0.5, 1.0 m) in 2011 and 2012 summer to fall growing seasons (July–Oct). To develop seedstring of native *Saccharina latissima*, meiospores of wild-harvested specimens were collected in November from LIS to obtain a wide variety of genotypes. *Saccharina latissima* seed-string was out-planted on two, 50 m long-line culture units, at three near shore sites, including: WLIS, BRE and central LIS (CLIS; Branford, CT; 41°12.772′ N/73° 57.070′ W), in the 2011–2014 fall to spring growing seasons (Dec.–-June; for more information about the culture conditions, see Kim et al. [8,35]).

To analyze the metal contents of the cultivated seaweed, tissue was obtained from 20 (*Gracilaria tikvahiae*) or 10 (*Saccharine latissima*) thalli collected haphazardly during the final harvest (early October for the former and late May to early June for the latter). Only the *G. tikvahiae* samples cultured at 1.0 m depth were analyzed. After collection, algal tissue samples were washed using 0.45 μm-filtered seawater and dried in an oven at 55 °C. Tissue from two-four thalli was pooled to create a mixed replicate. Between 3 and 5 mixed replicates were analyzed. The metal concentrations of total arsenic (As), cadmium (Cd), chromium (Cr), mercury (Hg) and lead (Pb) were analyzed at the Center for Environmental Sciences and Engineering of the University of Connecticut (Storrs, CT).

2.2. Metal analysis

Tissue samples were analyzed for As, Cd, Cr, and Pb using a Perkin Elmer (Norwalk, CT) DRC-e inductively coupled plasma mass spectrometry (ICP/MS). Samples were analyzed for Hg using a Perkin Elmer (Norwalk, CT) FIMS cold vapor atomic absorption spectrometer (CVAAS). All samples were analyzed using standard protocols [36,37]. Standard quality assurance procedures were employed, including

analysis of duplicate samples, method blanks (blank), post-digestion spiked samples, and laboratory control samples (LCS) [35]. LCS was cultivated in 1000 L tanks with local seawater collected from central Long Island Sound, near Bridgeport, CT. Water and sediment samples were not analyzed because the study focused primarily on whether seaweed tissue cultured in an urban estuary would accumulate dangerous levels of metals.

2.3. Metal accumulation calculation

Simple metal accumulation rates were estimated for the final 8–11 d growth period (October 2012) for *Gracilaria tikvahiae*. Laboratory culture in 1000 L tanks provided tissue with very low metal concentrations against which the metal concentrations in tissue harvested after the final growth period were compared. The latter tissue samples reflect the incorporation of metals from local seawater collected from central Long Island Sound, near Bridgeport, CT. Total metal load at the outset of growth (biomass$_{initial}$ × metal concentration$_{initial}$) in the out-planted bundles was subtracted from the total load after biomass growth (biomass$_{final}$ × metal concentration$_{final}$). Accumulation rates were calculated as:

$$\text{Rate (μg g}^{-1}\,\text{DW d}^{-1}) = ((\text{conc}_{\text{final,field}} * \text{biomass}_{\text{final}})$$
$$- (\text{conc}_{\text{initial,lab}} * \text{biomass}_{\text{initial}}))$$
$$/(\text{biomass increase (g)} * \text{Elapsed time})$$

Accumulation rate was not estimated for *S. latissima*, since no short term samples were obtained; the only harvest was made after four to five months of growth. The decision to harvest *S. latissima* was make when we determined that the growth of the plants had decreased, evidenced by erosion of the blades.

2.4. Metal removal estimate

Total removal of metals from the water column by each species was modeled for a hypothetical aquaculture operation of 1 ha. Production (DW ha^{-1}) over the growing season, estimated from prior work [8,35], was multiplied by average tissue metal concentrations (g metal g^{-1} DW) from this study. Since estimates of removal rates and seasonal accumulation of each metal were derived from average values (i.e., no replication), these metrics are presented without statistical analysis.

2.5. Statistical analysis

Since funding and logistic complications in the field operations prevented a full factorial design, sub-sets of the data were analyzed separately. The influence of year and site on metal concentrations in *Gracilaria tikvahiae* tissue was determined for 2011 and 2012. Time-zero samples (laboratory grown, sampled prior to out-planting) were only obtained in 2012. Consequently, accumulation rates and total seasonal removal of metals by *G. tikvahiae* were estimated only for the 2012 data.

The influence of year and site on final tissue metal concentrations in *Saccharina latissima* tissue was also examined separately. *Saccharina latissima* tissue samples were obtained from all three sites only in 2013. The influence of year (2012–2014) was examined for the WLIS tissue samples, the only site with multiple years of seaweed culture.

Data analysis was conducted using Sigmaplot (v. 12.3, SigmaStat, San Jose, CA). Data were checked for homogeneity of variance and normality prior to analysis of variance. Transformation (ln) was required only of the metal concentrations in *Gracilaria tikvahiae* at the BRE and WLIS sites in 2011 and 2012. All means and standard deviations presented in figures are un-transformed values. Where ANOVA indicated significant effects of factors, Fisher LSD post-hoc tests distinguished among the treatments.
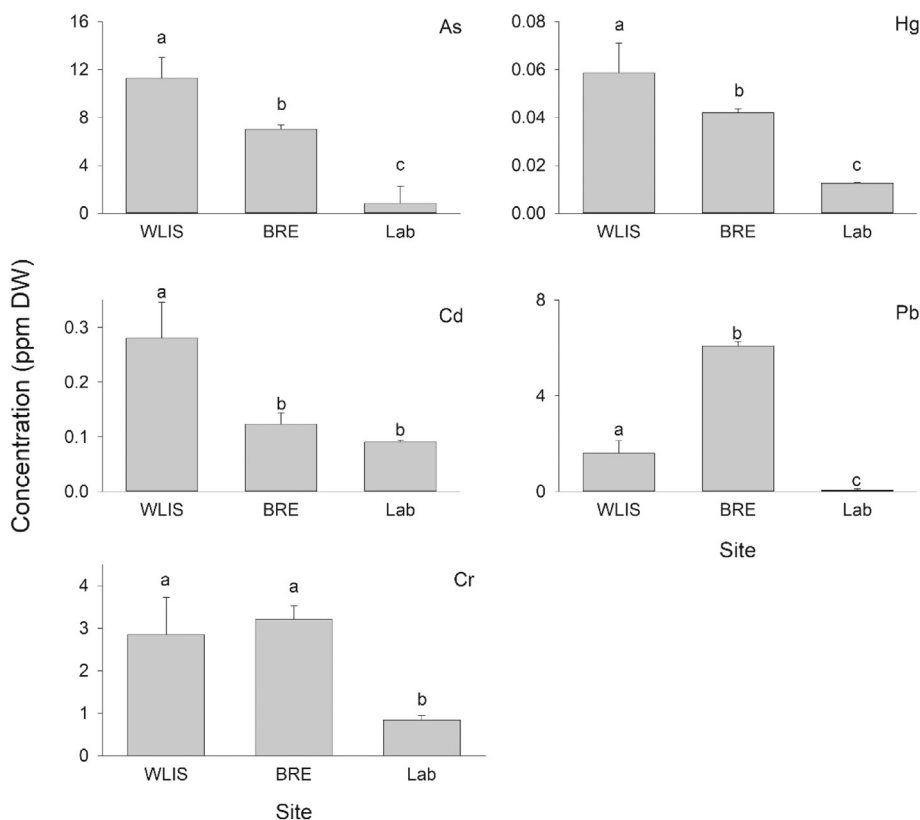
Fig. 2. Metal concentrations in tissue of *Gracilaria tikvahiae* collected during October 2012. Laboratory concentrations reflect metal accumulation prior to out-planting. Different letters indicate statistically different concentrations for each metal. Note that the Y-axis for Pb is a log scale. Data shown are the mean ± SD, n = 3. WLIS: western Long Island Sound, BRE: Bronx River Estuary, Lab: laboratory.

3. Results

3.1. Metal concentrations in Gracilaria tikvahiae in 2012

In 2012, the year for which *G. tikvahiae* samples were obtained from lab-grown tissue prior to outplanting, the accumulation of metals demonstrated different inter-site patterns (Fig. 2). In all cases, except for Cd, the metal concentrations in the lab-grown tissues were significantly lower than those at either WLIS or the BRE sites. Concentrations of As, Cd, and Hg were all significantly greater at WLIS than at the BRE site. Lead concentrations differed from the other metals; BRE tissue possessed Pb concentrations that were significantly higher than the WLIS (4-fold) and lab-grown tissues (115-fold) (Table 1).

3.2. Inter-annual variability in concentration of metals in Gracilaria tikvahiae

Inter-annual variability in metal concentrations in *G. tikvahiae* tissue also existed (Fig. 3), with the two sites behaving differently. Tissue concentrations increased from 2011 to 2012 at the WLIS site for As and Cd, decreased for Cr and Pb, and remained unchanged for Hg. Metal concentrations in *G. tikvahiae* tissue growth at the BRE site did not vary significantly from 2011 to 2012, though the power of the tests was low (0.29–0.44) (Table 2).

3.3. Metal accumulation rates for Gracilaria tikvahiae in 2012

Simple metal accumulation rates, calculated from the final harvest (October) of the 2012 season, differed widely among those metals analyzed (Fig. 4). Rates averaged across the WLIS and BRE sites were

Table 1
Results of ANOVA of 2012 data for metal concentration in *Gracilaria tikvahiae* at Bronx River estuary (BRE), Fairfield (WLIS) sites, and laboratory-cultured tissue. WLIS: western Long Island Sound. **Boldface** text indicates significant difference between sites.

Metal	Source of variation	df	MS	F value	P value	Tukey HSD test result
Arsenic	Site	2	44.45	40.46	**< 0.001**	WLIS > BRE > lab
	Error	6	1.10			
	Total	8				
Cadmium	Site	2	0.0308	19.44	**0.002**	WLIS > BRE = lab
	Error	6	0.00158			
	Total	8				
Chromium	Site	2	4.85	16.46	**0.004**	WLIS = BRE > lab
	Error	6	0.295			
	Total	8				
Mercury	Site	2	0.00163	30.3	**< 0.001**	WLIS > BRE > lab
	Error	6	0.0000538			
	Total	8				
Lead	Site	2	27.87	212.90	**< 0.001**	BRE > WLIS > lab
	Error	6	0.131			
	Total	8				
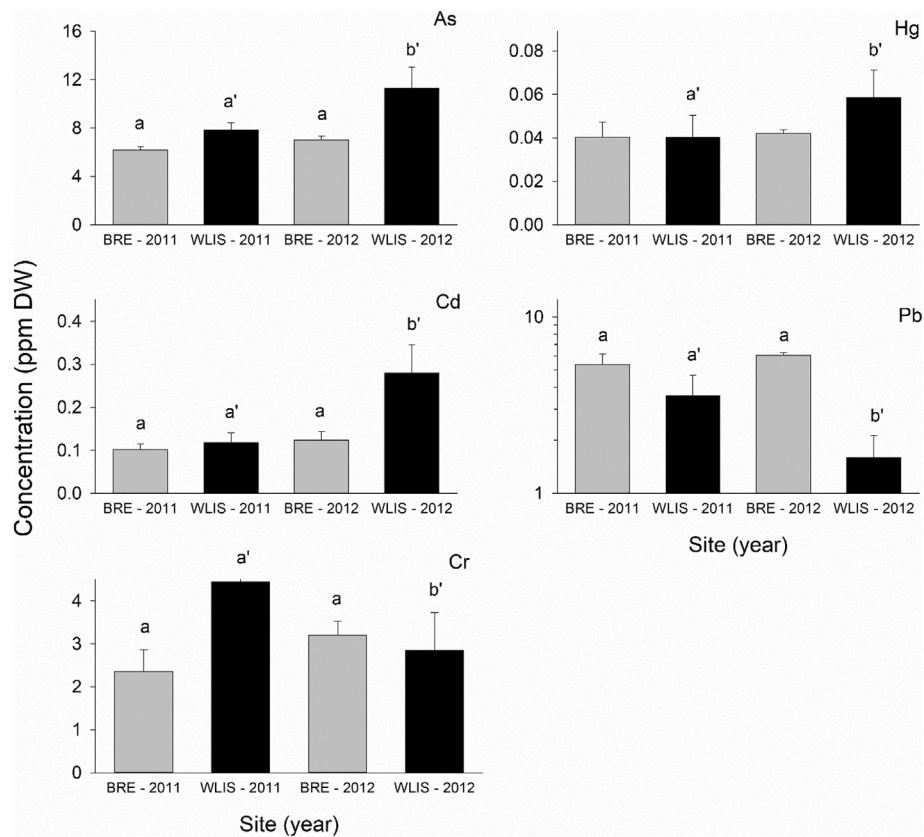
Fig. 3. Metal concentrations in tissue of *Gracilaria tikvahiae* collected during October 2011 and 2012 from two study sites. Different letters indicate within-site differences in metal concentration between 2011 and 2012. Data shown are the mean ± SD, n = 3 or 5. BRE: Bronx River Estuary, WLIS: western Long Island Sound.

the highest for arsenic and lead, and lowest for cadmium and mercury. Rates of accumulation of all heavy metals by *G. tikvahiae* were higher at the WLIS site than at the BRE site.

3.4. Metal concentrations in Saccharina latissima

Measurements for *Saccharina latissima* samples collected in 2013, revealed differences among the BRE, WLIS, and CLIS sites in the level of tissue metal concentration (Fig. 5; Table 3). Tissue concentrations of As

Table 2
Results of ANOVA of 2011 and 2012 data for metal concentration in *Gracilaria tikvahiae* cultured at the Bronx River estuary (BRE) and Fairfield (WLIS) sites. WLIS: western Long Island Sound.

| Metal	Source of variation	df	MS	F value	P value	Tukey HSD test result
Arsenic	Site	1	0.463	62.2	< 0.001	**WLIS: 2012 > 2011**
	Year	1	0.222	29.8	< 0.001	BRE: 2012 = 2011
	Site × year	1	0.0493	6.64	**0.024**	**2011: WLIS > BRE**
	Error	12	0.0744			**2012: WLIS > BRE**
	Total	15				
Cadmium	Site	1	0.841	24.9	< 0.001	**WLIS: 2012 > 2011**
	Year	1	1.026	30.4	< 0.001	BRE: 2012 = 2011
	Site × year	1	0.426	12.6	**0.004**	**2011: WLIS = BRE**
	Error	12	0.405			**2012: WLIS > BRE**
	Total	15				
Chromium	Site	1	2.78	4.82	**0.049**	**WLIS: 2011 > 2012**
	Year	1	0.525	0.91	0.36	BRE: 2012 = 2011
	Site × year	1	5.59	9.63	**0.009**	**2011: WLIS > BRE**
	Error	12	6.93			**2012: WLIS = BRE**
	Total	15				
Mercury	Site	1	0.00026	3.46	0.088	2012 > 2011
	Year	1	0.00037	4.92	**0.047**	
	Site × year	1	0.00026	3.46	0.088	
	Error	12	0.0000753			
	Total	15				
Lead	Site	1	36.9	55.7	< 0.001	**WLIS: 2011 > 2012**
	Year	1	1.49	2.25	0.16	BRE: 2012 = 2011
	Site × year	1	6.49	10.3	0.007	**2011: BRE > WLIS**
	Error	12	0.662			**2011: BRE > WLIS**
	Total	15				
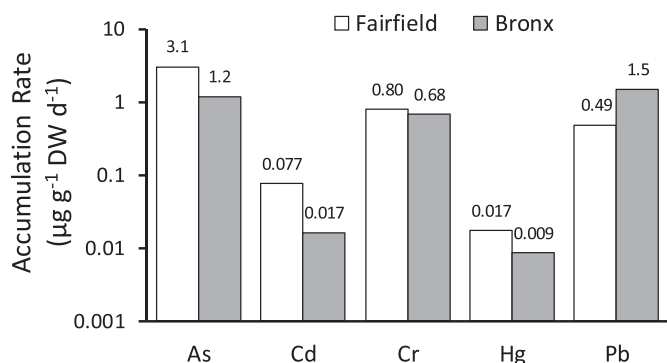
Fig. 4. Metal accumulation rate for *Gracilaria tikvahiae* tissue during 8–11 days' growth during Oct 2012 prior to harvest.

and Cr did not differ significantly among the three sites. Cadmium and Pb concentrations were higher at the BRE site than at either WLIS or CLIS. Mercury, on the other hand, occurred at different tissue concentrations at all three sites. Inter-annual differences in tissue concentrations were recorded for all metals (Fig. 6; Table 4), though the temporal patterns were dissimilar.

3.5. Total metal removal by Saccharina latissima and Gracilaria tikvahiae

We can estimate the metal removal using scaled-up data for a one-hectare farm, from prior studies [8,35]. Maximum production was observed at the BRE site, with estimated dry yields of 3.5 MT DW ha^{-1} and 12 MT DW ha^{-1} for *Saccharina latissima* and *Gracilaria tikvahiae*, respectively. Similarly, relative bioremediation of As and Cd were estimated for *G. tikvahiae* and *S. latissima* (i.e., the metals responsible for ca. 50% of the total metal removal; Fig. 7), with the smaller biomass

yield of the latter compensated for by higher tissue concentration in the former. The greater bioremoval of Cr, Hg, and Pb by *G. tikvahiae*, relative to *S. latissima*, was mostly a function of greater biomass production by the former. However, the low tissue concentrations resulted in the removal of < 100 g ha^{-1} for individual metals, and was generally much less.

4. Discussion

The increasing human consumption of seaweed is accompanied by safety concerns [38]. In fact, legal standards exist only for a handful of marine products and for a few metals. The U.S. Food and Drug Administration (FDA), whose mission is to protect the public health by ensuring foods and other biological products (e.g., cosmetics) are safe for human use, provides scant guidance. Limits for metals in marine products are currently set by the FDA only for Hg, and only for marine animal tissues (Table 5). This latter limitation also applies to international agencies with mandates similar to that of the FDA; the Canadian Food Inspection Agency, European Union Law, and the country of France have few metal limits set only for animal marine products.

With the exception of Pb, the metal concentrations recorded in tissues of both species all fell below limits set by US and international agencies (Table 5). Highest measured concentrations of Hg in tissue of *Gracilaria tikvahiae* and *Saccharina latissima* were 88–93% less than the limit set by the FDA. Concentration maxima of Cd in *G. tikvahiae* and *S. latissima* were 20% and 32%, respectively, lower than the stringent French limits. Maximum levels of Pb in tissues of *G. tikvahiae* (but not *S. latissima*) were close to the French limits only at the BRE site. It is tempting to attribute this to the nearby scrap metal yard and general industrial history of the area [21].

In this study, total As was also analyzed. However, the toxicity of As is mainly determined by inorganic form [33,39,40]. Inorganic arsenic is
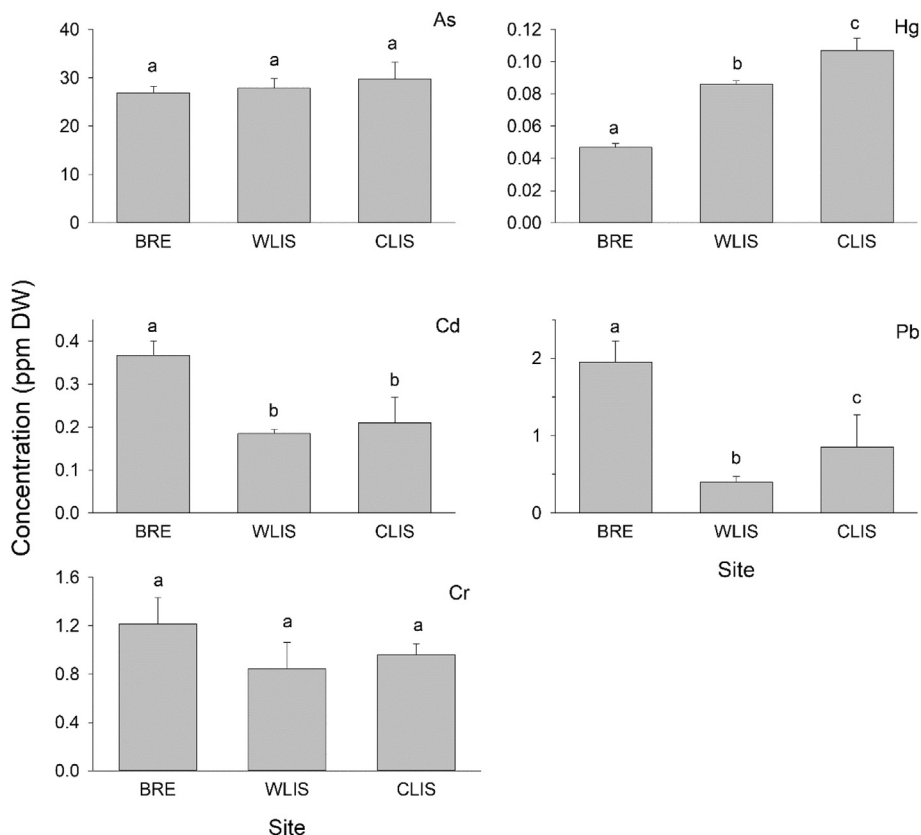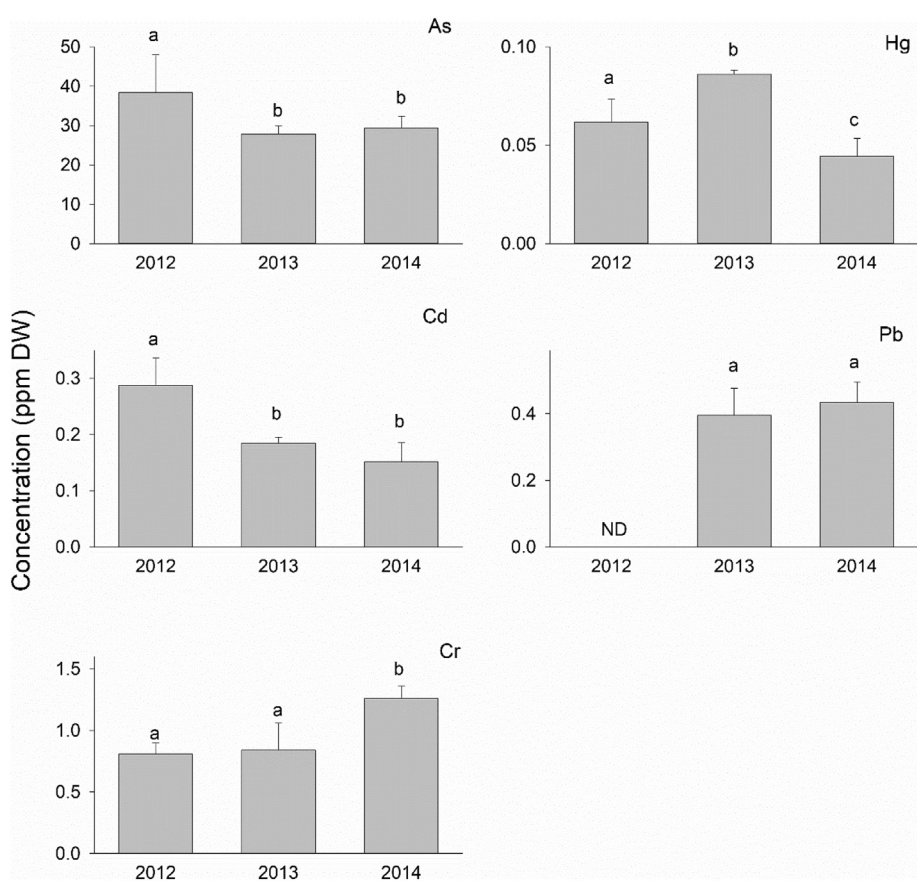

Fig. 5. Metal concentrations in tissue of *Saccharina latissima* grown during November through May 2013. Different letters indicate statistically different concentrations for each metal. Data shown are the mean ± SD, n = 4. BRE: Bronx River Estuary, WLIS: western Long Island Sound, CLIS: central Long Island Sound.

Table 3
Results of ANOVA of 2013 data for metal concentration in *Saccharina latissima* cultured at Bronx River estuary (BRE), Fairfield (WLIS), and Branford (CLIS) sites. WLIS: western Long Island Sound, CLIS: central Long Island Sound. **Boldface** text indicates significant difference between sites.

Metal	Source of variation	df	MS	F value	P value	Tukey HSD test result
Arsenic	Site	2	8.47	1.39	0.29	BRE = WLIS = CLIS
	Error	9	6.08			
	Total	11				
Cadmium	Site	2	0.0392	24.9	**< 0.001**	**BRE > (WLIS = CLIS)**
	Error	9	0.00157			
	Total	11				
Chromium	Site	2	0.145	4.16	0.053	BRE = WLIS = CLIS
	Error	9	0.0345			
	Total	11				
Mercury	Site	2	0.00748	156.8	**< 0.001**	**CLIS > WLIS > BRE**
	Error	9	0.000215			
	Total	11	0.0070			
Lead	Site	2	2.56	30.3	**< 0.001**	**BRE > (CLIS = WLIS)**
	Error	9	0.0846			
	Total	11				

Fig. 6. Metal concentrations in tissue of *Saccharina latissima* grown during November through May over three years of the study at the WLIS site (the only site for which all three years of culture were obtained). Different letters indicate statistically different concentrations for each metal. ND = not detected. Data shown are the mean ± SD, n = 4 or 5.

categorized as a Group A human carcinogen by the US EPA, and a Class 1 carcinogen by the International Agency for Research on Cancer (IARC) [41]. The limits set by US and international agencies for vegetables are lower than those of marine products. Mac Monagail et al. [33] reported little accumulation of As in beef and poultry when raised on feed containing As-containing seaweed.

From our data, we view Pb with concern. Rate of uptake of this heavy metal by *Gracilaria tikvahiae* was second only to the uptake by As (Fig. 4). The FDA's provisional tolerable daily Pb intake is only 6 μg. This translates into limited consumption limits of 1–4 g (DW) per day of *G. tikvahiae*, and 3–15 g of *Saccharina latissima*. Therefore, the metal concentrations of cultivated seaweeds near urban areas should be carefully and continually monitored when sites are located near locations characterized by current and past industrial activities involving

heavy metals. Nutrient bioextraction (removal of carbon and nitrogen) by seaweed aquaculture in the same urbanized estuaries is significant [1,8,35,42]. In absolute terms, the metal concentrations in field-cultured seaweed tissue in this study were low, indicating that aquaculture in the LIS and New York estuary could not be considered as having a metal bioremediatory function (Fig. 7).

The two macroalgal species differed in the accumulation of the metals examined in this work. The concentration of As was greater in *Saccharina latissima* tissue than in that of *Gracilaria tikvahiae*, which indicates the higher affinity of the alginate molecule in the kelp cell wall compared with the affinity of agar in *Gracilaria* [43]. Concentrations of Cr and Pb were greater in *G. tikvahiae* tissue, while levels of Cd and Hg were similar in tissue of each. The differences could be large; accumulation of Cr was 3–5 times greater than Cd in *S. latissima* tissue,

Table 4
Results of ANOVA of 2012–2014 data for metal concentrations in *Saccharina latissima* cultured at the Fairfield (WLIS) site. WLIS: western Long Island Sound.

Metal	Source of variation	df	MS	F value	P value	Tukey HSD test result
Arsenic	Site	2	154.6	4.09	**0.047**	**2012 > (2013 = 2014)**
	Error	11	37.8			
	Total	13				
Cadmium	Site	2	0.0249	19.2	**< 0.001**	**2012 > (2013 = 2014)**
	Error	11	0.00130			
	Total	13				
Chromium	Site	2	0.308	15.4	**< 0.001**	**2014 > (2012 = 2013)**
	Error	11	0.0201			
	Total	13				
Mercury	Site	2	0.00192	23.8	**< 0.001**	**2014 > 2012 > 2014**
	Error	11	0.0000808			
	Total	13				
Lead*	Site	1	0.00338	0.61	0.462	2013 = 2014
	Error	7	0.00557			
	Total	8				

* Years 2013, 2014 only (no data for 2012).
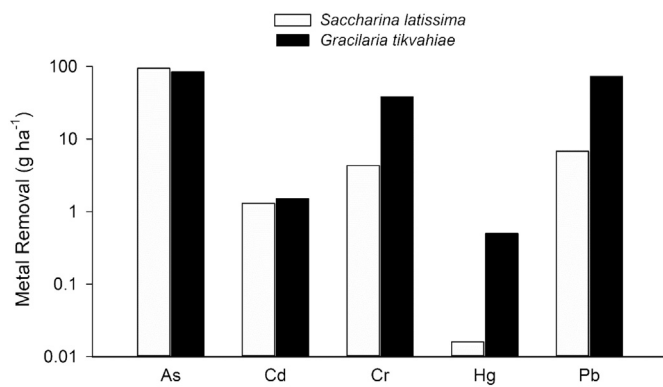
Fig. 7. Total metal removal by *Saccharina latissima* and *Gracilaria tikvahiae* in a hypothetical seaweed farm in Long Island Sound.

and 10–26 times greater in *G. tikvahiae*. The Pb concentrations measured in *S. latissima*, in this study, were similar to levels recorded for the related phaeophyte *Laminaria longicruris* (now known as *S. longicruris*) in Long Island Sound at the mouth of the Thames River [44], for a group phaeophytes sold for human consumption [31], and for *Ascophyllum nodosum*, even in anthropogenically impacted harbors [45]. Levels of Cd were similar to those of Morrison et al. [45] and Almela et al. [31], but only about half of those for *S. longicruris* in eastern Long Island Sound [44]. No published records of metal levels exist for *G. tikvahiae* in Long Island Sound. *Gracilaria corticata* from the heavily-trafficked Straits of Hormuz [46] was reported to have accumulated Pb and Cd to levels that were 3–15 and 20–60 times greater, respectively, than *G. tikvahiae* in this study, highlighting the intra-generic variability

and/or location effect in potential accumulation.

The tissue metal concentrations reported here are marked by geographic and temporal variability, the former generally greater than the latter. Past studies have shown that metal concentrations in the water column and surface sediments of LIS increase from east to west in Long Island Sound [21,47]. Overall, our data only partially reflect this pattern. Tissue concentrations of Pb increased from east to west for *Gracilaria tikvahiae*, similarly concentrations of Cd, Cr, and Pb in *Saccharina latissima* tissue followed this trend, too. The east-west gradient in tissue Cd concentration may derive from a combination of differing metal input (e.g., differing industrial legacy, wastewater effluent inputs) [21] and possibly also an inverse relationship between salinity and Cd accumulation [48]. Tissue Hg concentrations increase from west to east for both species, suggesting local scale variability in input and/or sediment characteristics [21]. The mouth of the Housatonic River, a known Hg source [21], lies < 10 km from the WLIS site, while the Connecticut River empties into LIS about 20 km from the CLIS site.

In summary, this demonstration-scale aquaculture project showed accumulation of metals during culture of *Gracilaria tikvahiae* and *Saccharina latissima* in LIS. With the exception of Pb, tissue metal concentrations were below most existing regulatory limits set for human consumption. Taken together, with the variability due to site and species, it would be prudent to monitor the heavy metal content of cultivated seaweeds destined for consumption by humans.

Conflicts of interest

The authors declare no conflict of interest.

Table 5
Regulatory limits for human consumption (ppm DW, except where noted). Blank cells indicate the absence of regulatory limits for that metal. Values for *Gracilaria tikvahiae* and *Saccharina latissima* are the maximum values determined during the length of the study. DW: dry weight; FW: fresh weight.

Regulatory Agency	Metal				
	As	Cd	Cr	Hg	Pb
US Food and Drug Administration[a]				1.0	
Canadian Food Inspection Agency[b]				0.5	
EC Regulation 2006R1881[c]		(0.05–0.1 FW)		(0.5–1.0 FW)	(0.3–1.5 FW)
France	< 3.0 (inorganic)	0.5		0.1	5.0
G. tikvahiae (this study; max)		0.34		0.072	6.6
S. latissima (this study; max)		0.40		0.118	2.2

[a] Fish, shellfish, crustaceans other aquatic animals.
[b] All fish products, except swordfish, shark, tuna, escolar, orange roughy, marlin.
[c] Muscle meat of fish (0.3 mg kg^{-1} FW), bivalve mollusks (1.5 mg kg^{-1} FW).

Statement of informed consent, human/animal rights

No conflicts, informed consent, human or animal rights applicable.

Declaration of authors' agreement

All authors agreed to the authorship and submission of the manuscript to Algal research for peer review.

Author contributions

JKK, GPK and CY conceived the research and designed the experiments. All authors performed the experiments, analyzed and interpreted the data. JKK and GPK wrote the original draft. CY supervised and edited the manuscript. All authors edited and approved the final manuscript.

Acknowledgements

References

[1] J.K. Kim, C. Yarish, E.K. Hwang, M. Park, Y. Kim, Seaweed aquaculture: cultivation technologies, challenges and its ecosystem services, Algae 32 (2017) 1–13.

[2] FAO, The State of World Fisheries and Aquaculture, http://www.fao.org/3/i9540en/I9540EN.pdf, (2018).

[3] S. Mabeau, J. Fleurence, Seaweed in food products: biochemical and nutritional aspects, Trends Food Sci. Technol. 4 (1993) 103–107.

[4] M. Caliceti, E. Argese, A. Sfriso, B. Pavoni, Heavy metals contamination in the seaweeds of the Venice lagoon, Chemosphere 47 (2002) 443–454.

[5] C.J. Hunter, Edible seaweeds – a survey of the industry and prospects for farming the Pacific Northwest, Mar. Fish. Rev. 37 (1975) 19–26.

[6] O. Mouritsen, C. Dawczynski, L. Duelund, G. Jahreis, W. Vetter, M. Schröder, On the human consumption of the red seaweed dulse (*Palmaria palmata* (L.) Weber & Mohr), J. Appl. Phycol. 25 (2013) 1777–1791.

[7] G.P. Kraemer, J.K. Kim, C. Yarish, Seaweed aquaculture: bioextraction of nutrients to reduce eutrophication, Association of Massachusetts Wetland Scientists Newsletter 89 (2014) 16–17.

[8] J.K. Kim, G.P. Kraemer, C. Yarish, Use of sugar kelp aquaculture in Long Island Sound and the Bronx River Estuary for nutrient extraction, Mar. Ecol. Prog. Ser. 531 (2015) 155–166.

[9] J.M. Rose, S.B. Bricker, S. Deonarine, J.G. Ferreira, T. Getchis, J. Grant, J.K. Kim, J.S. Krumholz, G.P. Kraemer, K. Stephenson, G.H. Wikfors, C. Yarish, Nutrient bioextraction, in: R.A. Meyers (Ed.), Encyclopedia of Sustainability Science and Technology, Springer Press, New York, 2015, pp. 1–33.

[10] E.J. Cottier-Cook, N. Nagabhatla, Y. Badis, M.L. Campbell, T. Chopin, W. Dai, J. Fang, P. He, C.L. Hewitt, G.H. Kim, Y. Huo, Z. Jiang, G. Kema, X. Li, F. Liu, H. Liu, Y. Liu, Q. Lu, Q. Luo, Y. Mao, F.E. Msuya, C. Rebours, H. Shen, G.D. Stentiford, C. Yarish, H. Wu, X. Yang, J. Zhang, Y. Zhou, C.M.M. Gachon, Safeguarding the Future of the Global Seaweed Aquaculture Industry, United Nations University (INWEH) and Scottish Association for Marine Science Policy Brief, 2016 978-92-808-6080-1(12 pp.).

[11] T.L. Getchis, Northeastern U.S. Aquaculture Management Guide: A Manual for the Identification and Management of Aquaculture Production Hazards, United States Department of Agriculture National Institute of Food and Agriculture, 2014 (285p).

[12] UNEP, The UNEP Annual Report, http://staging.unep.org/annualreport/2010/pdfs/UNEP-AR-2010-FULL-REPORT.pdf, (2011).

[13] Y. Lee, H. Kim, S. Kim, A. Park, Y.J. Kim, T. Han, E.M. Choi, The effects of silver and arsenic on antioxidant system in *Lemna paucicostata*: different effects on glutathione system, Toxicol. Environ. Health Sci. 8 (2016) 332–340.

[14] D.H. Lee, Y.J. Rhee, K.S. Choi, S.E. Nam, H.J. Eom, J.S. Rhee, Sublethal concentrations of atrazine promote molecular and biochemical changes in the digestive gland of the Pacific oyster *Crassostrea gigas*, Toxicol. Environ. Health Sci. 9 (2017) 50–58.

[15] J. Park, M.T. Brown, S. Depuydt, J.K. Kim, D.S. Won, T. Han, Comparing the acute sensitivity of growth and photosynthetic endpoints in three *Lemna* species exposed to four herbicides, Environ. Pollut. 220 (2017) 818–827.

[16] H.Y. Kim, B. Yim, C.H. Bae, Y.-M. Lee, Acute toxicity and antioxidant responses in

[17] the water flea *Daphnia magna* to xenobiotics (cadmium, lead, mercury, bisphenol A, and 4-nonylphenol), Toxicol. Environ. Health. Sci. 9 (2017) 41–49.

[18] Y.J. Kim, M.G. Choi, H.J. Kim, H.S. Kim, J.H. Yang, Y.R. Seo, Safety assessment of red-colored iron oxide as heavy metal Lead pigment, Toxicol. Environ. Health Sci. 9 (2017) 36–40.

[19] M.L. Asare, S.J. Cobbina, F.J. Akpabey, A.B. Duwiejuah, Z.N. Abuntori, Heavy metal concentration in water, sediment and fish species in the Bontanga Reservoir, Ghana, Toxicol. Environ. Health Sci. 10 (2018) 49–58.

[19] J. Wang, C. Chen, Biosorbents for heavy metals removal and their future, Biotechnol. Adv. 27 (2009) 195–226.

[20] J.H. Yang, H.S. Kim, B.K. Koo, C.M. Lee, J.-H. Jung, Y.R. Seo, Considerations of human health risk assessment in chemical accident: suggestions from a toxicogenomic approach, Toxicol. Environ. Health Sci. 10 (2018) 79–89.

[21] J.C. Varekamp, A.E. McElroy, J.R. Mullaney, V.T. Breslin, Metals, organic compounds, and nutrients in Long Island Sound: sources, magnitudes, trends, and impacts, in: J.S. Latimer, M. Tedesco, R.L. Swanson, C. Yarish, P. Stacey, C. Garza (Eds.), Long Island Sound: Prospects for the Urban Sea, Springer Press, New York, 2014, pp. 203–283.

[22] K.J. Van Meter, P. Van Cappellen, N.B. Basu, Legacy nitrogen may prevent achievement of water quality goals in the Gulf of Mexico, Science 360 (2018) 427–430.

[23] G.W. Bryan, L.G. Hummerstone, Brown seaweed as an indicator of heavy metals in estuaries in south-west England, J. Mar. Biol. Assoc. U. K. 53 (1973) 705–720.

[24] H. Ali, E. Khan, M.A. Sajad, Phytoremediation of heavy metals-concepts and applications, Chemosphere 91 (2013) 869–881.

[25] V. Besada, J.M. Andrade, F. Schultze, J.J. González, Heavy metals in edible seaweeds commercialised for human consumption, J. Mar. Syst. 75 (2009) 305–313.

[26] E.J. Olguín, G. Sánchez-Galván, Heavy metal removal in phytofiltration and phycoremediation: the need to differentiate between bioadsorption and bioaccumulation, New Biotechnol. 30 (2012) 3–8.

[27] J. Park, G.S. Jin, M.S. Hwang, M.T. Brown, T. Han, Toxicity tests using the kelp *Undaria pinnatifida* for heavy metal risk assessment, Toxicol. Environ. Health Sci. 8 (2016) 86–95.

[28] J.A. Norman, C.J. Pickford, T.W. Sanders, W. Waller, Human intake of arsenic and iodine from seawater–based food supplements and health foods available in the UK, Food Add. Contam. 5 (1987) 103–109.

[29] C. Van Netten, S.A. Hoption Cann, D.R. Morley, J.P. Van Netten, Elemental and radioactive analysis of commercially available seaweed, Sci. Total Environ. 255 (2000) 169–175.

[30] Y.M. Hsu, I.M. Hwang, T.R. Yeh, Inorganic elements determination for algae/*spirulina* food marketed in Taiwan, J. Food Drug Anal. 9 (2001) 178–182.

[31] C. Almela, S. Algora, V. Benito, M.J. Clemente, V. Devesa, M.A. Súñer, D. Vélez, R. Montoro, Heavy metals, total arsenic and inorganic arsenic contents of algae food products, J. Agric. Food Chem. 50 (2002) 918–923.

[32] C. Almela, M.J. Clemente, D. Velez, R. Montoro, Total arsenic, inorganic arsenic, lead and cadmium contents in edible seaweed sold in Spain, Food Chem. Toxicol. 44 (2006) 1901–1908.

[33] M. Mac Monagail, E. Cummins, R. Bermejo, E. Daly, D. Costello, L. Morrison, Quantification and feed to food transfer of total and inorganic arsenic from a commercial seaweed feed, Environ. Int. 118 (2018) 314–324.

[34] R.J. Saunders, N.A. Paul, Y. Hu, R. de Nys, Sustainable sources of biomass for bioremediation of heavy metals in wastewater derived from coal-fired power generation, PLoS One 7 (5) (2012) e36470.

[35] J.K. Kim, G.P. Kraemer, C. Yarish, Field scale evaluation of seaweed aquaculture as a nutrient bioextraction strategy in Long Island Sound and the Bronx River Estuary, Aquaculture 433 (2014) 148–156.

[36] D.A. Cristol, L. Savoy, D.C. Evers, C. Perkins, R. Taylor, C.W. Varian-Ramos, Mercury in waterfowl from a contaminated river in Virginia, J. Wildl. Manag. 76 (2012) 1617–1624.

[37] L. Savoy, P. Flint, D. Zwiefelhofer, H. Brant, C. Perkins, R. Taylor, O. Lane, J. Hall, D. Evers, J. Schamber, Geographic and temporal patterns of variation in total mercury concentrations in blood of harlequin ducks and blue mussels from Alaska, Mar. Poll. Bull. 117 (2017) 178–183.

[38] R. Pereira, C. Yarish, A.T. Critchley, Seaweed aquaculture for human foods in land based and IMTA systems, in: R.A. Meyers (Ed.), Encyclopedia of Sustainability Science and Technology, Springer Press, New York, 2013, pp. 9109–9128.

[39] E.F. Brandon, P.J.C.M. Janssen, L. de Wit-Bos, Arsenic: bioaccessibility from seaweed and rice, dietary exposure calculations and risk assessment, Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 31 (2014) 1993–2003.

[40] J.M. Ronan, D.B. Stengel, A. Raab, J. Feldmann, L. O'Hea, E. Bralatei, E. McGovern, High proportions of inorganic arsenic in *Laminaria digitata* but not in *Ascophyllum nodosum* samples from Ireland, Chemosphere 186 (2017) 17–23.

[41] K. Straif, L. Benbrahim-Tallaa, R. Baan, Y. Grosse, B. Secretan, F. El Ghissassi, V. Bouvard, N. Guha, C. Freeman, L. Galichet, V. Cogliano, A review of human carcinogens–part C: metals, arsenic, dusts, and fibres, Lancet Oncol 10 (2009) 453–454.

[42] H. Wu, J.K. Kim, Y. Huo, J. Zhang, P. He, Nutrient removal ability of seaweeds on *Pyropia yezoensis* aquaculture rafts in China's radial sandbanks, Aquat. Bot. 137 (2017) 72–79.

[43] K.C. Guven, T. Akyuz, T. Yurdun, Selectivity of heavy metal binding by algal polysaccharides, Toxicol. Environ. Chem. 47 (1995) 65–70, https://doi.org/10.1080/02772249509358127.

[44] N. Shimshock, G. Sennefelder, M. Dueker, F. Thurberg, C. Yarish, Patterns of metal accumulation in *Laminaria longicruris* from Long Island Sound (Connecticut), Arch. Environ. Contam. Toxicol. 22 (1992) 305–312.

[45] L. Morrison, H.A. Baumann, D.B. Stengel, An assessment of metal contamination

along the Irish coast using the seaweed *Ascophyllum nodosum* (Fucales, Phaeophyceae), Environ. Pollut. 152 (2008) 293–303.

[46] A. Dadolahi-Sohrab, M. Garavand-Karimi, H. Riahi, H. Pashazanoosi, Seasonal variations in biomass and species composition of seaweeds along the northern coasts of Persian Gulf (Bushehr Province), Journal of Earth System Science 121 (2012) 241–250.

[47] A. Sweeney, S.A. Sañudo-Wilhelmy, Dissolved metal contamination in the East River–Long Island sound system: potential biological effects, Mar. Poll. Bull. 48 (2004) 663–670.

[48] W.X. Wang, R.C. Dei, Kinetic measurements of metal accumulation in two marine macroalgae, Mar. Biol. 135 (1999) 11–23.