***Highlights (for review)**

- Arsenic content in dominant seaweed from the Thermaikos Gulf was determined
- Total arsenic values are lower than those found in other Mediterranean seaweeds
- As speciation of water extracts from the algae are performed by LC-ICP-MS
- Arsenosugars are measured in all samples

LC-ICP-MS analysis of arsenic compounds in dominant seaweeds from the Thermaikos Gulf (Northern Aegean Sea, Greece)

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Abstract

The content of total arsenic and arsenic compounds in the dominant seaweed species in the Thermaikos Gulf, Northern Aegean Sea, was determined in samples collected in different seasons. Total arsenic was determined by acid digestion followed by ICP-MS. Arsenic speciation was analyzed by water extraction followed by LC-ICP-MS. Total arsenic concentrations in the seaweeds ranged from 1.39 to 55.0 mg kg^{-1}. *Cystoseira* species and *Codium fragile* showed the highest total As contents, while *Ulva* species (*U. intestinalis, U. rigida* and *U. fasciata*) had the lowest. Arsenosugars, the most common arsenic species in seaweeds, were found in all samples and glycerol-arsenosugar was the most common form. However, phosphate-arsenosugar and sulfate-arsenosugar were also present. Inorganic arsenic was measured in seven algae species and detected in another. Arsenate was the most abundant species in *Cystoseira barbata* (27.0 mg kg^{-1}). Arsenobetaine was measured in only one sample. Methylated arsenic species were measured at very low concentrations. The information should contribute to further understanding the presence of arsenic compounds in dominant seaweeds from the Thermaikos Gulf.

Keywords: LC-ICP-MS, arsenic speciation, seaweeds, Thermaikos Gulf

1. Introduction

Arsenic in the marine ecosystem may derive from natural sources or anthropogenic activities. Inorganic arsenic predominates in seawater. In living organisms, organoarsenic compounds were detected years ago, but more recently a large number and variety of organic forms have been identified (Francesconi and Edmonds, 1998; Le, et al., 2004; Taleshi, et al., 2010).

Marine algae contain most of their arsenic in the form of arsenosugars. In recent decades, a number of studies have described the mechanisms of the transformation and accumulation of arsenicals, as well as correlations among arsenosugars and algal orders (Thomson, et al., 2007). Furthermore, there have been several reports of the potentially toxic character of such organoarsenic compounds (Andrewes, et al., 2004; Feldmann and Krupp, 2011). The presence and behavior of arsenic compounds in algae is of interest not only to assess the pollution level in a particular zone, but to evaluate the health risk posed by the consumption of seaweed. The presence of arsenic compounds has been reported in marine algae from several zones around the world (Tukai, et al., 2002; Yang, et al., 2012). However, studies on arsenic speciation in algae or aquatic plants in the Mediterranean zone are scarce (Nischwitz and Pergantis, 2005a; Šlejkovec et al., 2006; Llorente-Mirandes et al., 2010; Ruíz Chancho et al., 2010).

In the present study, total arsenic content and arsenic species are determined in 10 seaweed species collected in three sampling sites in the Thermaikos Gulf (Greece). We selected this area since it is affected by urban and industrial sources of pollution. The only research on metal accumulation by seaweeds in the Thermaikos Gulf have been done by Sawidis and Voulgaropoulos (1986), Djingova et al. (1987), Haritonidis and Malea (1995; 1999) and Malea and Haritonidis (1999a; 1999b; 2000). These studies concerned three species (*Ulva linza* formerly *Enteromorpha linza*, *U. rigida* and *Gracilaria gracilis*) and only the metals Cr, Co, Ni, Fe, Pb, Cu, Cd and Zn were studied. Moreover, there are some studies on metal accumulation (Fe, Cu, Zn, Cd, Pb, Na, K, Ca and Mg) by the former seaweeds or seaweeds of the same genus as in the present study (*Padina pavonica, Cystoseira zosteroides, Codium bursa, Gracilaria bursa-pastoris* and *Ulva rigida*) in other areas of Greece (Malea et al., 1995; Boubonari et al., 2008a,b). There is no measurement of As accumulation by seaweeds in the Thermaikos Gulf, but this issue has been sparsely reported in other areas (Burger, et al., 2007; Pell, et al., 2013; Sales, 2010).

The aim of this study is to provide data on arsenic speciation in the Thermaikos Gulf, to improve the understanding of arsenic-alga interactions in both this and other marine environments.

2. Materials and Methods

2.1. Study area

The Gulf of Thermaikos is a water mass located in the northwestern Aegean Sea (Figure 1). The area, volume and maximum depth of this gulf are 518 km^2, 11.33 km^3 and 36 m, respectively. The rivers Axios, Aliakmon, Loudias and Galikos flow into the Gulf. The total annual discharge from the rivers is $10.2x10^6$ m^3 and the total sediment supply 3-4 x 10^6 m^3 (Collins, 1981). To the north, the Thermaikos Gulf becomes narrow and continues into the Gulf of Thessaloniki, on the northern coast of which the city of Thessaloniki is located. In the Gulf of Thessaloniki, the currents have a cyclic direction (Ganoulis and Krestenitis, 1982). The Thessaloniki Gulf receives industrial, partially treated domestic and agricultural effluents (Christophoridis et al., 2009; Nikolaidis et al., 2006a; Nikolaidis et al., 2006b). In particular, effluents from food industries, leather tanning, chrome painting, petrochemical distilleries and electrolytic manganese dioxide factories are discharged into the northwestern part of the Gulf of Thessaloniki (Marcantonatos, 1990). The harbor of Thessaloniki is widely used by cargo ships, which release quantities of oil, lubricants, etc. into the sea.

The Kalochori area (K) is situated near the industrial zone, the harbor and the pump room of the main wastewater treatment plant (WWTP) of Thessaloniki. The coastal area Viamyl (V) receives local effluents from a small WWTP and a small factory that treats starch, direct urban discharges and freshwater inputs from a stream (Anthemoundas River). This area is considered to be polluted (Orfanidis et al., 2010). Agia Triada (AT) is a tourist resort located relatively far from the harbor facilities, industrial activities and domestic effluents (Christophoridis et al., 2009) (Figure 1). At sites K, V and AT during June, September and December 2007 the water temperature, salinity and pH near the bottom varied between 10.4 and 26.6^{o}C, 33.4 and 36.9 psu and 7.4 and 9.4, respectively.

2.2. Sampling and sample pretreatment

Seasonal samplings (June, September and December 2007) of the dominant seaweed species were carried out at three sampling stations in the Gulf of Thessaloniki: K (Kalochori, 40^o 36' N, 22^o 51' E), V (Viamyl, 40^o 33' N, 22^o 58' E) and AT (Agia Triada, 40^o 30' N, 22 ^{o} 52' E) (Figure 1). The seaweed samples were clipped off at the sediment level, washed in seawater at the sampling site and carried to the laboratory in plastic bags containing seawater. In the laboratory, the seaweeds were identified. Then, samples were frozen at -20°C until they were transported by plane to Barcelona in a fridge with CO_2(s). There, the samples were defrosted. A stereomicroscope (Zeiss) was used to remove any remaining impurities, such as sand, epiphytes and other attached material. Then, samples were dried at 40°C, pulverized by hand in a glass mortar and stored in PET bottles until analysis.

2.3. Instrumentation

Digestions were performed in a medium-pressure closed microwave system from Milestone (Ethos ONE Touch, Milestone S.R.L., Italy). An Agilent 7500ce ICP-MS (Agilent Technologies, Germany) with an Ari Mist HP nebulizer (Burgener, Canada) was used to measure total arsenic content and arsenic species. For arsenic speciation, a coupled LC-ICP-MS system was used, with an Agilent 1200 quaternary pump that had a vacuum degasification module and autosampler and the analytical columns Hamilton PRP-X100 (250 × 4.1 mm, 10 µm, Hamilton, USA) and Zorbax-SCX300 (250 × 4.6 mm, 5 µm, Agilent), which were protected by guard columns filled with the corresponding stationary phases. The outlet of the HPLC column was directly connected via PEEK tubing to the nebulizer of the ICP-MS, which was the arsenic-selective detector. Ion intensity at m/z 75 (^{75}As) was monitored using time-resolved analysis software. In addition, the ion intensities at m/z 77 ($^{40}Ar^{37}Cl$ and ^{77}Se) were monitored to detect potential argon chloride ($^{40}Ar^{35}Cl$) interference at m/z 75.

2.4. Standards and reagents

All solutions were prepared with doubly deionised water obtained from a Millipore water purification system (Elix&Rios) (18.2 MΩ cm^{-1} and TOC < 30 µg L^{-1}). Nitric acid (69%) (Panreac, Hiperpur) and 31% hydrogen peroxide (Merck, Selectipur) were used in the digestions. Mobile phases were prepared with 98% formic acid (Panreac, P.A.), ammonium dihydrogen phosphate (Panreac, P.A.), 25% aqueous ammonia solution (Panreac, P.A.) and pyridine (Scharlau, P.A.). Stock standard solutions (1000 mg As l^{-1}) were prepared as follows: arsenite (AsIII) from As_2O_3 (NIST, USA, Oxidimetric Primary Standard 83d, 99.99%) dissolved in 4 g L^{-1} NaOH (Merck, Suprapure); arsenate (AsV) from $Na_2HAsO_4 \cdot 7H_2O$ (Carlo Erba) dissolved in water; methylarsenate (MA) prepared from $(CH_3)AsO(ONa)_2 \cdot 6H_2O$ (Carlo Erba) dissolved in water; and dimethylarsenate (DMA) prepared from $(CH_3)_2AsNaO_2 \cdot 3H_2O$ (Fluka) dissolved in water. Arsenocholine (AC) from $(CH3)_3As^+CH_2CH_2OHBr^-$ was supplied by the "Service Central d'Analyse" (CNRS Vernaison, France); a certified reference material of arsenobetaine (AB) solution, CRM 626, from $(CH_3)_3As^+CH_2COO^-$ was provided by BCR, and trimethylarsine oxide (TMAO) was prepared from $(CH_3)_3AsO$ (Argus Chemicals, S.R.L) dissolved in water. All stock solutions were kept at 4°C and were further diluted daily for the analyses. Arsenic standard solution from NIST High-Purity Standards with a certified concentration of 1000 ± 2 mg As L^{-1} was used as a calibrant in the determination of total arsenic content by ICP-MS. AsV, DMA, MA, AC, TMAO and AB were standardized against an arsenic standard solution from NIST High-Purity Standards for our internal quality control. NIES CRM 09 Sargasso (*Sargassum fulvellum*) seaweed, supplied by NIES (Japan), had a certified total arsenic content of 115 ± 9.2 mg As kg^{-1}. An aliquot of freeze-dried extract of *Fucus serratus*, containing the four common arsenosugars (phosphate (PO_4-sug), sulfate (SO_4-sug), sulphonate (SO_3-sug) and glycerol (gly-sug)) was used to assign the peaks in the chromatograms (Madsen et al., 2000).

2.5. Total arsenic analyses

Digestion was performed in triplicate (samples, blanks and CRM). Digestion was only performed in samples for which a sufficient amount was available. Dried samples (40°C, 0.1 g) were mineralized with 8 ml of nitric acid and 2 ml of hydrogen peroxide. Mixtures were digested according to the following program: 10 min from room temperature to 90°C, then maintained for 5 min at 90°C, 10 min from 90°C to 120°C, 10 min from 120°C to 190°C and then 10 min maintained at 190°C. Solutions were passed through ash-free filter papers (Whatman 40), diluted up to 20 ml with water and stored at 4°C in PET bottles. A 1:20 dilution was performed before measuring the total arsenic by ICP-MS (external calibration, ^{103}Rh as internal standard, measurements in triplicate). He was used as the collision gas.

2.6. Arsenic speciation

Sample water extracts were obtained in an end-over-end system (16 h, 35 rpm) in a sample: water ratio of 1:100. Extracts were centrifuged (2800 rpm, 10 min) and passed through PET syringe filters (Chromafil PET, Macherey–Nagel, 0.45 µm) before analysis. Extraction pellets were washed three times with water and digested. Thus, arsenic content was measured in pellet digests and in extracts (as described in the preceding paragraph), and total arsenic in the original sample was evaluated as the sum of these contents. Arsenic speciation was carried out in extracts by LC-ICP-MS using the method developed for marine algae (Ruíz Chancho et al., 2010). Anionic exchange chromatography was performed just after the extraction. Extracts were stored at 4°C until cationic exchange chromatography, which was performed one or two days after the extraction. The performance of the two chromatographic systems is described in Table 1. Arsenic species in extracts were identified by a retention time comparison with standards. MA, DMA, arsenite, arsenate, AB, TMAO and AC were quantified from external curves obtained with the corresponding standards. Extraction blanks were analyzed for each extraction batch. We quantified PO_4-sug with the MA calibration curve, SO_3-sug and SO_4-sug with the As(V) calibration curve, and gly-sug with the calibration curve of the AC standard, as other authors suggested (Francesconi and Sperling, 2005). An unknown cation was quantified with the calibration curve of AC. As(III) was quantified by subtracting the species quantified in cationic exchange chromatography from the front peak of anionic exchange chromatography.

3. Results

3.1. Total arsenic concentrations

Total arsenic concentrations in seaweed tissue ranged from 1.4 mg As kg^{-1} (*Ulva rigida, AT, Sept.*) to 55.0 mg As kg^{-1} (*Cystoseira barbata, AT, Dec.*) (Table 2). The winter *Cystoseira barbata* samples from the AT station accumulated the highest total As concentration (55.0 mg kg^{-1}), followed by the samples of *Codium fragile* (23.0; 39.0 mg kg^{-1}), *Cystoseira spinosa* (16.8 mg kg^{-1}), *Padina pavonica* (11.3; 11.5 mg kg^{-1}), *Gracilaria gracilis* (5.7; 12.2 mg kg^{-1}) and *Gracilaria* sp. (7.1 mg kg^{-1}). Finally, *Ulva intestinalis, U. rigida* and *U. fasciata* accumulated lower values (1.9-4.3; 1.4-2.7 and 2.2 mg kg^{-1}, respectively). Total arsenic ranged from 1.9 to 23.0 mg kg^{-1} in June, from 1.4 to 16.8 mg kg^{-1} in September and from 2.2 to 55.0 mg kg^{-1} in December.

3.2. Arsenic species determination

The extraction efficiency (Table 3) for arsenic compounds ranged widely, from 9% (*Codium fragile,* station AT, summer sampling) to 69% (*Cystoseira barbata*, station AT, winter sampling). The main compounds quantified were arsenosugars. Gly-sug was present in all the samples and SO_4-sug in none. The major arsenosugar in green seaweeds was gly-sug. SO_3-sug was not found in any samples and PO_4-sug was quantified in *C. fragile* (station V, summer sampling). All brown algal samples contained quantified gly-sug and SO_3-sug, and PO_4-sug was detected in a *P. pavonica* sample (station AT, autumn sampling). Red algal samples contained a considerable number of arsenosugars, except for *Hypnea musciformis* samples, in which only gly-sug was detected and quantified. In some samples, other arsenic compounds were the most common species: TMAO was the major quantified arsenic species in *Enteromorpha intestinalis* (station V, winter sampling) and As(V) in *Cystoseira* spp. samples. AB was quantified in one sample (*C. barbata*) and DMA, MA and As(III) in several. As examples, the anionic and cationic exchange chromatograms obtained for *P. pavonica* (station AT, summer sampling) are shown in Figure 2.

3.3. Quality Assessment of the obtained data

3.3.1 Column recovery

Column recovery was calculated as the ratio of the sum of the species eluted from the chromatographic columns to the total arsenic in the extract injected into the column. Column recoveries ranged between 11% and 103% (Table 3). Low column recovery values were obtained in some samples. These values could be attributable to the presence of several arsenic compounds that could not be quantified with the separation method used.

3.3.2. Certified reference material (CRM)

In order to check the accuracy of the technique, the total arsenic concentration was determined in the CRM NIES nº9 (Sargasso). A total of 99 ± 9 mg kg^{-1} was found, which is in agreement with the certified value (115 ± 9.2 mg kg^{-1}), if we consider the associated uncertainties.

3.3.3. Analysis of Fucus serratus *extract*

We used an extract from the brown seaweed *Fucus serratus* (Madsen, et al., 2000) to identify the arsenosugars present in our seaweed samples. For quality control purposes, we quantified As species in *F. serratus* extracts. Our results[1] (DMA: 0.01 ± 0.01 µg; gly-sug: 0.07 ± 0.01 µg; PO_4-sug: 0.07 ± 0.01 µg; SO_3-sug: 0.56 ± 0.04 µg; SO_4-sug: 0.37 ± 0.02 µg) confirm those reported by Madsen *et al.* (2000) and other values in the literature on the same extract (Kohlmeyer et al., 2003; Llorente-Mirandes et al., 2010; Ruíz Chancho et al., 2008; Šlejkovec et al., 2006).

3.3.5. Limit of Detection (LOD) and Limit of Quantification (LOQ)

LODs and LOQs were estimated (Table 3). The LOD is the lowest concentration of an analyte that can be reliably differentiated from background noise (signal-to-noise ratio greater than 3). The LOQ is the lowest concentration that can be quantified (signal-to-noise ratio greater than 10). To calculate the LOD and the LOQ, the standard deviations of the base line and the peak base of each analyte multiplied by 3 or 10 (LOD and LOQ respectively) were calculated in the peak height calibration curve. The arsenosugar LODs and LOQs were estimated by means of a correction factor, which is the ratio between the concentration of arsenosugar in *F. serratus* and the peak height.

4. Discussion

The values reported in the results were compared with those reported in the literature for the same alga species or genus from other geographical areas (see Table 4). The arsenic content in the samples was either in the range of the bibliography (Cystoseira spp.), at a higher concentration (*Codium*

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[1] Values for the *F. serratus* extract are given as the absolute amount (µg) in the extract.

fragile) or at a lower concentration (*P. pavonica, Gracilaria* spp. and *Ulva* spp). These differences can be attributed to local environmental arsenic levels, to distinct arsenic accumulation capacity and to the growth cycle stage of the alga.

Several authors studied the relationship between the phylum and the arsenic content and proposed different sequences (Morita and Shibata, 1990; Almela et al., 2002; Tukai et al., 2002; Almela et al., 2006). We did not find a defined sequence among the phyla, since the green alga *C. fragile* had a relativity high arsenic content and the arsenic contents in samples of *P. pavonica, Gracilaria* spp. and *Ulva* spp. were at the same level.

The data available in the literature suggest that algae naturally accumulate more arsenic in winter than in warmer seasons (Lai et al., 1998). Our results for *C. fragile* in station V and *G. gracilis* in stations V and K are consistent with this suggestion. Nonetheless, not enough data were obtained in the present study to draw further conclusions on this subject.

4.2. Arsenic species determination

Most samples had low arsenic content with low concentrations of arsenicals. In addition, extraction efficiencies and column recoveries varied widely. A wide range of extraction efficiencies is also reported in the literature (Francesconi and Kuehnelt, 2004; Rubio, et al., 2010). Usually, the most abundant arsenic compounds in the samples are arsenosugars with a strong polar character, thus water is a suitable extractant, since it can penetrate the sample matrix (Francesconi and Kuehnelt, 2004). However, samples also contained a large fraction of arsenic compounds that was not extracted with water. This fraction could be associated with lipids and might account for up to 50% of total arsenic in algae (Francesconi, 2003), and also with arsenic bound to cell components or proteins that are not extracted with water (Koch et al., 2000a, b).

Even though arsenosugars are the most common arsenical in most samples, the main arsenic compound in *Cystoseira* spp. was As(V). In addition, AB was quantified in *C. barbata* (0.020 ± 0.003 mg As kg-1). We postulated in previous studies that this may be attributable to the presence of microorganisms (Llorente-Mirandes et al., 2010), while other authors attributed the occurrence of arsenobetaine to non-removed epiphytes (Šlejkovec et al., 2006).

In some cases, unknown peaks were observed. For instance, in the chromatogram of Figure 3 (*Ulva rigida*, Agia Triada, 30/Sept/2007) an unknown peak was obtained from the cationic exchange column, eluted between AB and gly-sug, and the species might be identified using an LC-ES-MS/MS system (Nischwitz and Pergantis, 2005a, b).

4. Conclusions

This is the first study of arsenic species occurrence in the Thermaikos Gulf and its contribution may be useful to further understanding of the behavior and fate of arsenic in the marine environment. Total As concentrations in the dominant seaweeds in the Gulf of Thessaloniki generally varied between seaweed species. *Cystoseira* species and *Codium fragile* showed the highest total As concentrations, while *Ulva* species *(U. intestinalis, U. rigida* and *U. fasciata)* the lowest. Total arsenic contents in seaweed tissues in this survey were generally comparable to those previously reported for these species or species of the same genus from other coastal locations. Arsenosugars were the main arsenic compounds determined in the study, except for *Cystoseira* spp., which presented inorganic arsenic as the predominant form.

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Table 1: Chromatographic conditions used for the separation of arsenic species

	Anion exchange	Cation exchange
Column	PRP-X100 (250 x 1.4 mm) 10 µm (Hamilton, USA)	Zorbax 300-SCX (150 x 4.1 mm), 5 mm (Agilent, Germany)
Mobile phase	20 mM $NH_4H_2PO_4$ pH 5.8	20 mM pyridine pH 2.6
Injection volume	20 µl	20 µl
Flow	1.5 ml min^{-1}	1.5 ml min^{-1}
Arsenic species	As (III), DMA, MA, As (V) PO_4-sug, SO_3-sug, SO_4-sug	AB, AC, TMAO Gly-sug

Table 2: Mean ± standard deviation of the total arsenic concentrations (mg kg^{-1}) in the dominant seaweeds of Thermaikos Gulf, n=3.

Species	Division	Phylum	Family	Month	Station AT	Station K	Station V
Cystoseira barbata	Brown	Ochrophyta	Sargassaceae	Dec	55 ± 2	-	-
Cystoseira spinosa	Brown	Ochrophyta	Sargassaceae	Sept	16.8 ± 0.8	-	-
Padina pavonica	Brown	Ochrophyta	Dictyotaceae	Jun	11.5 ± 0.8	-	-
				Sept	11.3 ± 0.5	-	-
Gracilaria sp.	Red	Rhodophyta	Gracilariaceae	Dec	-	-	7.1 ± 0.4
Gracilaria gracilis	Red	Rhodophyta	Gracilariaceae	Dec	-	9.0 ± 0.2	12.2 ± 0.5
				Jun	-	-	5.7 ± 0.2
				Sept	-	7.2 ± 0.5	6.2 ± 0.2
Hypnea musciformis	Red	Rhodophyta	Cystocloniaceae	Jun	4.6 ± 0.4	-	5.0 ± 0.2
Codium fragile	Green	Chlorophyta	Codiaceae	Jun	23 ± 1	-	7.4 ± 0.3
				Dec	-	-	39.0 ± 0.4
Ulva intestinalis	Green	Chlorophyta	Ulvaceae	Jun	1.9 ± 0.1	-	-
				Dec	-	-	4.3 ± 0.2
Ulva rigida	Green	Chlorophyta	Ulvaceae	Sept	1.4 ± 0.1	2.7 ± 0.3	-
Ulva fasciata	Green	Chlorophyta	Ulvaceae	Dec	-	-	2.2 ± 0.2

Table

Table 3: Arsenic speciation, extraction efficiency and column recovery values (mean ± standard deviation, mg As kg^{-1}, n=3).

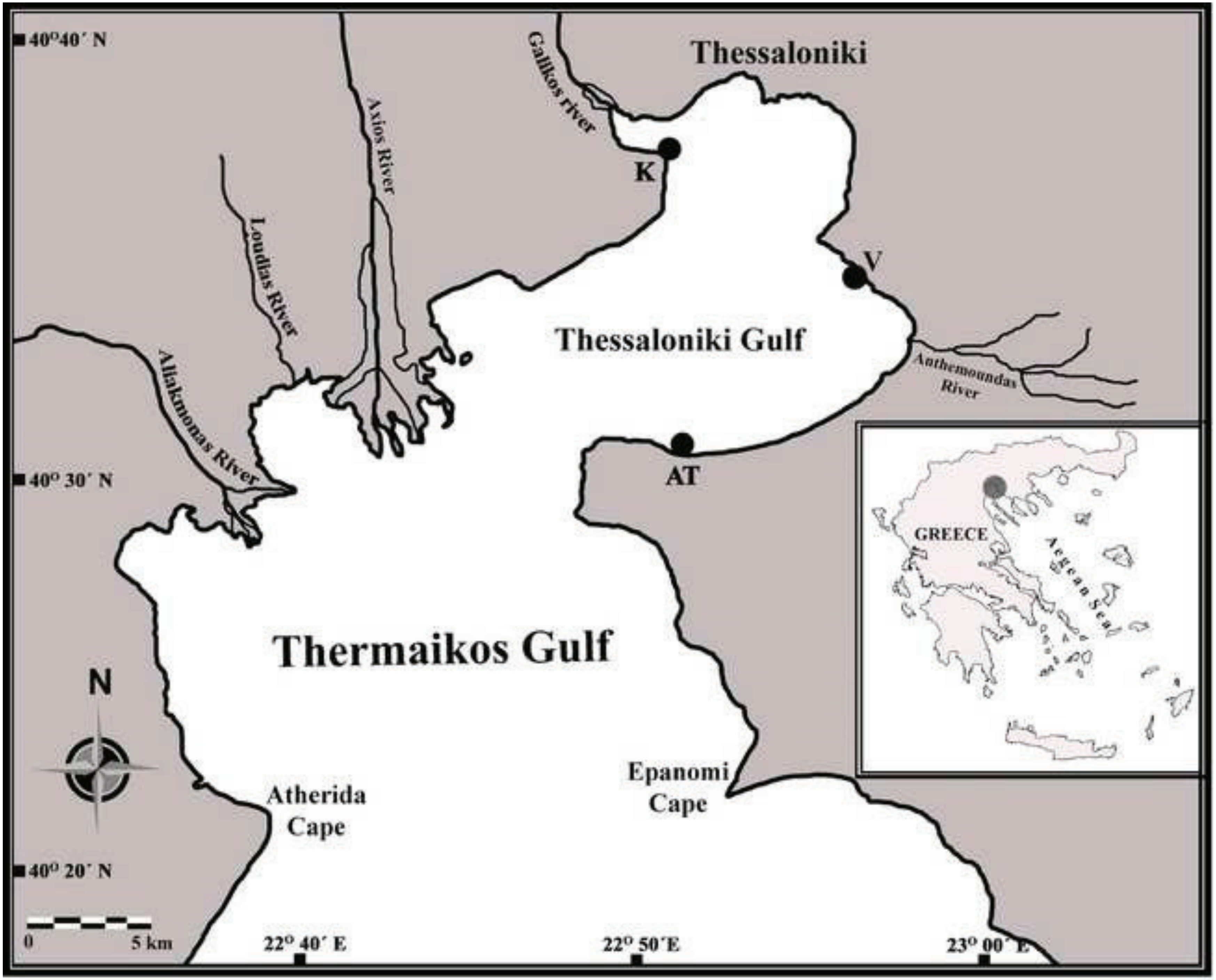
Species	Site - Date	As (III)	DMA	MMA	As (V)	AB	TMAO	PO_4-sug	SO_3-sug	Gly-sug	Unknown cation	Extraction efficiency	Column recovery
Cystoseira barbata	Ag. Triada - 22/Dec/2007	-	-	-	27.0 ± 0.8	0.020 ± 0.003	-	-	1.43 ± 0.08	0.40 ± 0.06	-	69%	76%
Cystoseira compressa	Ag. Triada - 28/Sep/2007	-	0.072 ± 0.004	-	3.27 ± 0.07	-	-	-	1.3 ± 0.1	0.35 ± 0.01	-	30%	66%
Padina pavonica	Ag. Triada - 30/Jun/2007	-	Detected	0.07 ± 0.01	0.164 ± 0.003	-	-	-	0.62 ± 0.01	0.388 ± 0.006	-	30%	38%
	Ag. Triada - 28/Sept/2007	-	Detected	Detected	0.38 ± 0.02	-	-	Detected	0.52 ± 0.04	0.197 ± 0.005	-	48%	22%
Gracilaria sp.	Kalohori - 22/Dec/2007	0.05 ± 0.01	-	-	0.50 ± 0.02	-	-	-	1.21 ± 0.05	1.27 ± 0.07	-	45%	68%
Gracilaria gracilis	Viamyl - 30/Jun/2007	-	-	-	Detected	-	-	0.12 ± 0.01	-	0.253 ± 0.002	-	17%	36%
	Viamyl- 22/Dec/2007	Detected	-	Detected	Detected	-	-	Detected	1.26 ± 0.04	1.10 ± 0.02	-	48%	75%
	Kalohori - 28/Sept/2007	-	0.052 ± 0.006	-	Detected	-	-	0.28 ± 0.05	Detected	0.164 ± 0.001	-	9%	70%
	Viamyl- 22/Dec/2007	-	-	-	0.68 ± 0.007	-	-	0.53 ± 0.01	Detected	0.232 ± 0.005	-	41%	17%
	Viamyl - 28/Sept/2007	-	-	-	-	-	-	-	-	0.754 ± 0.009	-	20%	40%
Hypnea musciformis	Ag. Triada - 30/Jun/2007	0.048 ± 0.009	-	-	-	-	-	-	-	0.155 ± 0.006	0.046 ± 0.008	25%	16%
	Viamyl- 30/Jun/2007	-	-	Detected	-	-	-	-	-	0.103 ± 0.005	-	18%	11%
Codium fragile	Ag. Triada - 30/Jun/2007	-	0.062 ± 0.002	-	-	-	-	-	-	0.70 ± 0.02	-	14%	19%
	Viamyl - 30/Jun/2007	-	0.059 ± 0.01	-	-	-	-	0.26 ± 0.04	-	1.20 ± 0.04	-	47%	44%
	Viamyl- 22/Dec/2007	-	-	-	-	-	-	-	-	5.0 ± 0.9	0.104 ± 0.045	30%	46%
Enteromorpha intestinalis	Ag. Triada - 30/Jun/2007	0.049 ± 0.02	-	-	-	-	-	-	-	0.044 ± 0.001	0.038 ± 0.004	30%	27%
	Viamyl- 22/Dec/2007	0.041 ± 0.004	-	-	-	-	0.081 ± 0.004	-	-	0.068 ± 0.001	-	21%	103%
Ulva rigida	Ag. Triada - 28/Sept/2007	-	-	-	-	-	-	-	-	0.075 ± 0.002	0.031 ± 0.006	26%	24%
	Kalohori - 28/Sept/2007	-	-	-	-	-	-	-	-	0.433 ± 0.005	-	65%	28%
	Viamyl - 22/Dec/2007	-	-	-	Detected	-	-	-	-	0.179 ± 0.007	-	21%	35%
Limit of Detection		0.01	0.02	0.02	0.03	0.003	0.009	0.03	0.01	0.008			
Limit of Quantification		0.04	0.05	0.06	0.1	0.01	0.03	0.1	0.3	0.03			

Table 4: Literature data on total arsenic concentrations (mg As kg^{-1}) in seaweed species of the genus *Cystoseira, Padina, Gracilaria, Hypnea, Codium* and *Ulva.*

Species	Geographical locations	As concentrations[A]	References
Cystoseira barbata	Tartous (Syria)	131 ± 1	Al-Masri et al., 2003
	Venice Lagoon (Italy)	360 ± 50	Caliceti et al., 2002
	Thermaikos Gulf	55.0	present study
Cystoseira compressa	Piran (Slovenia)	17.6 ± 0.9	Šlejkovec et al., 2006
	Black sea	28.0-48.4	Güven et al., 1992
Cystoseira mediterranea	Lloret de Mar (Spain)	39.0	Llorente-Mirandes et al., 2010
Cystoseira spinosa	Thermaikos Gulf	16.8	present study
Cystoseira sp.	Lattakia (Syria)	4.20 ± 0.14	Al-Masri et al., 2003
	Tartous (Syria)	5.95 ± 0.20	Al-Masri et al., 2003
Padina pavonica	Piran (Slovenia)	1.89 ± 0.09	Šlejkovec et al., 2006
	Tartous (Syria) May 1999	18.3 ± 0.2	Al-Masri et al., 2003
	Tartous (Syria) June 2000	20.9 ± 0.7	Al-Masri et al., 2003
	Thermaikos Gulf	1.3-1.5	present study
Padina fraseri	Sydney (Australia)	13	Tukai et al., 2002
Gracilaria gracilis	Venice Lagoon (Italy)	32 ± 1	Caliceti et al., 2002
	Black sea	12.3-17.7	Güven et al., 1992
	Thermaikos Gulf	5.7-12.2	present study
Gracilaria sp.	Thermaikos Gulf	7.1	present study
Hypnea musciformis	Thermaikos Gulf	4.6 – 5.0	Present study
Hypnea pannosa	Baja California (Mexico)	0.28	Sánchez-Rodríguez et al
Codium vermilara	Lloret de Mar (Spain)	27	Llorente-Mirandes et al., 2010
Codium fragile	Thermaikos Gulf	7.4-39	present study
Ulva fasciata	Thermaikos Gulf	2.2	present study
Ulva intestinalis	Thermaikos Gulf	1.9-4.3	present study
Ulva lactuca	Sydney (Australia)	2.9	Tukai et al., 2002
Ulva rigida	Tartous (Syria)	5.03 ± 0.04	Al-Masri et al., 2003
	Lloret de Mar (Spain)	5.3	Llorente-Mirandes et al., 2010
	Venice Lagoon (Italy)	12 ± 2	Caliceti et al., 2002
	Piran (Slovenia)	1.35 ± 0.07	Šlejkovec et al., 2006
	Black sea	3.2-6.4	Güven et al., 1992
	Thermaikos Gulf	1.39-2.7	Present study

[A] Values given as in original source

Figure 1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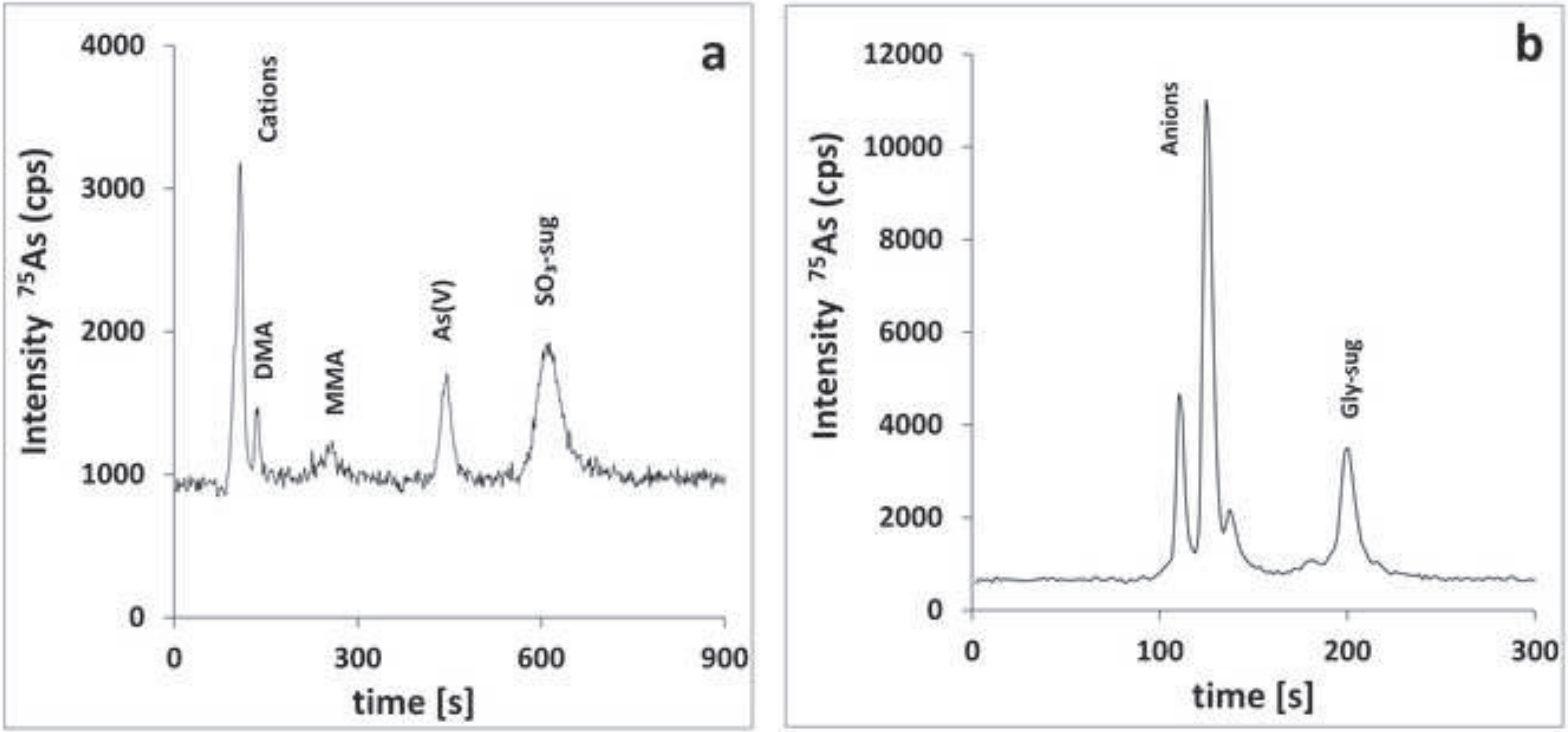
Anthemoundas River
Thermaikos Gulf
Atherida Cape
Epanomi Cape
GREECE
Aegean Sea

Figure 2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Cations
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b
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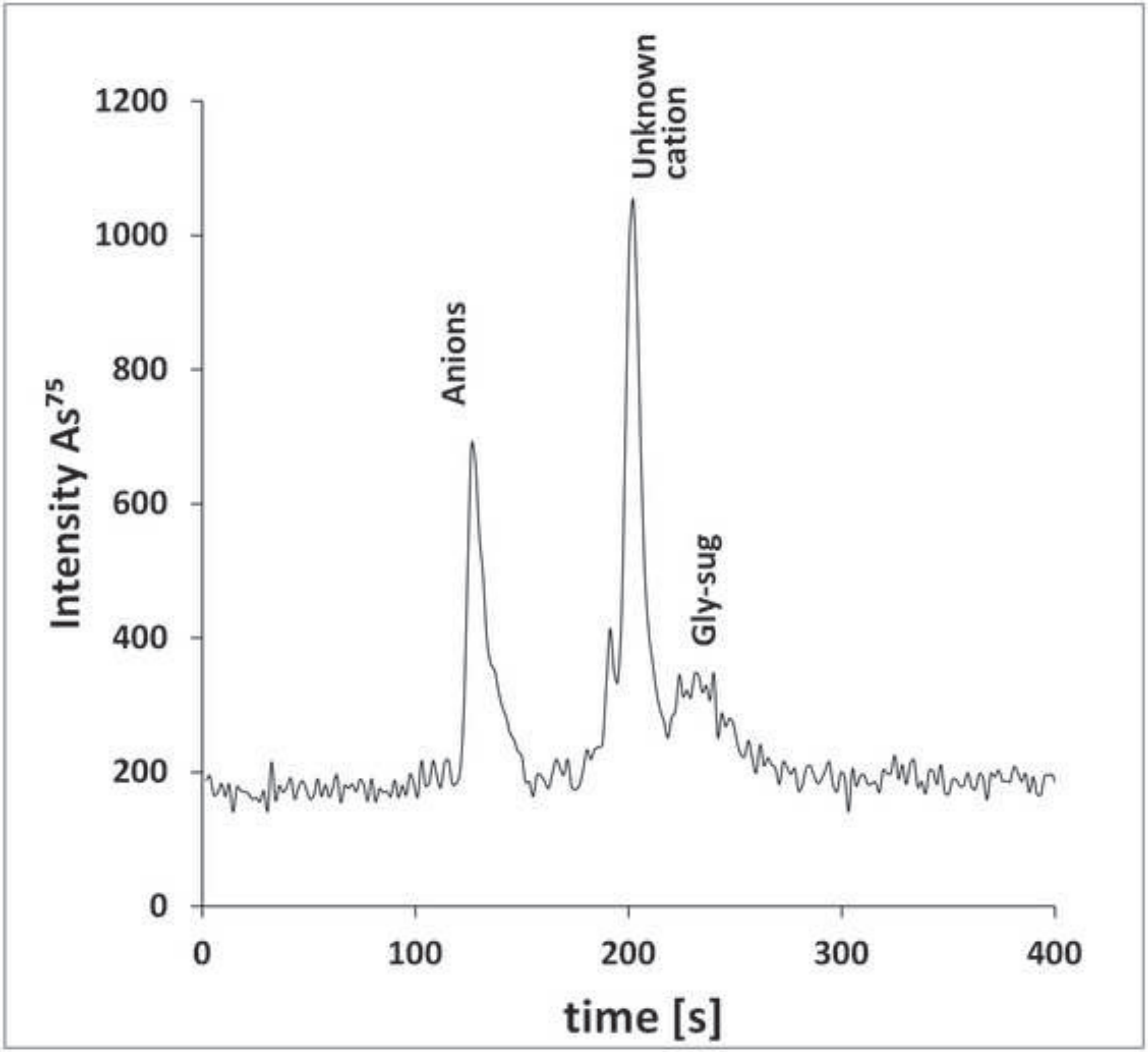
Gly-sug
0
100
200
300
time [s]

Figure 3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time [s]
Anions
Unknown cation
Gly-sug

Figure captions

Fig. 1: Geographical location of the study site and map of Thermaikos Gulf indicating the sampling stations: Kalochori (K), Viamyl (V) and Agia triada (AT).

Fig. 2: Anionic (a) and cationic (b) chromatograms for *Padina pavonica* from station Agia Triada sampling on June 2007.

Fig. 3: Cationic exchange chromatogram for *Ulva rigida* from station Agia Triada, sampling on September 2007. Labelled chromatographic peaks: anions, unknown cation and gly-sug.