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Ion chromatographic investigation of brown algae (*Fucus vesiculosus*) of the German Environmental Specimen Bank

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ABSTRACT

An investigation of the inorganic anions and cations Cl^-, HPO_4^{2-}, NO_3^-, SO_4^{2-}, NH_4^+, K^+ and Na^+ in brown algae collected from various locations in the North Sea and Baltic Sea is presented. Ion chromatography was effectively used for the simultaneous determination of anions and cations after extraction of the homogenized algae samples with deionized water following the standard preparation technique of the German Environmental Specimen Bank (ESB). Acidic and enzymatic digestion was applied to investigate the completeness of cell disintegration in the homogenized algae samples. Results obtained with brown algae clearly indicate the influence of environmental pollution in the North Sea and the Baltic Sea.

INTRODUCTION

With increased application in a variety of areas, ion chromatography (IC) is today a well-established technique in analytical chemistry. The number of articles published in various scientific journals indicates the reliability of this technique [1]. Ion chromatography is not only a versatile multicomponent analytical technique, it also possesses a relatively high sensitivity, enabling the determination of specific trace elements at the sub-part-per-billion level (w/w) [2,3]. A wide variety of environmental problems, such as climatic change, forest decline and increasing enviromental pollution of the North and Baltic Sea, can be addressed, and consequently an intensive effort is devoted to the determination of anions and cations by ion chromatography.

Nitrite can cause serious health problems in humans, such as methaemoglobinaemia in infants, and under certain conditions carcinogenic nitrosamines can be formed in the human body. Fluorine is an essential substance for humans and animals, but increased fluorine uptake can lead to serious health problems such as fluorosis of bones and teeth [4,5].

In various fields of environmental research, ion chromatography plays a key role, mainly in investigations of aqueous samples. A review of the environmental applications of ion chromatography is given elsewhere [6]. The IC analysis of anionic and cationic species in plant materials has already been reported [7–10] using different sample preparation techniques and detection methodologies. Expanding the field of application for IC is particularly attractive for the environmental sciences, since the enormous potential of the technique can help to elucidate the uptake rates, accumulation capacities or transfer routes of heavy metals and anionic species in biological materials. The German Environmental Specimen Bank (ESB) is devoted to the real-time monitoring and archiving of authentic material for retrospective analysis and is greatly interested in additional information on sample constituents less readily obtainable by other techniques. In the course of a screening programme within the ESB for the monitoring of environmental pollutants in the North Sea and Baltic Sea, investigations of the inorganic anions and cations in brown algae were performed using IC.

The purpose of this work was to establish the suitability of brown algae as possible indicator or-

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ganisms for coastal pollution by phosphate, nitrate, ammonium and other components.

EXPERIMENTAL

Materials and chemicals

Brown algae were collected in October 1991 from the Baltic and North Seas. Sampling and preparation techniques following a standard protocol laid down by the ESB were applied for the collection and preparation of the algae samples and are described elsewhere [11,12]. After cleaning and removal of adhering materials at the sampling site using sea water, the brown algal samples were deep frozen in stainless-steel containers immersed in liquid nitrogen (123K) for transport to the ESB. In the ESB laboratories, the samples were precrushed by a crusher at a temperature of 273–280 K and freeze-dried in appropriate batches, finely ground in zirconium dioxide ball mills and passed through 200-μm sieves.

Standard solutions of 1000 mg/l chloride, phosphate, nitrate, sulphate, ammonium, potassium and sodium were prepared with analytical-grade chemicals (Merck, Germany). Bakerbond octadecyl (C_{18}) columns, (Bakerbond, USA) were used to remove aromates, fatty acids, hydrocarbons and tensides from the supernatant solution. For the disintegration of the cell wall material of brown algae, a mixture of three enzymes was used: cellulase Onozuka R-10 from *Trichoderma viride* (EC 3.2.1.4), macerozyme R-10 from *Rhizopus* sp. and pectinase from *Aspergillus niger* (EC 3.2.1.15) (Merck, Germany). The blank values of Cl^-, HPO_4^{2-}, NO_3^-, SO_4^{2-}, NH_4^+, K^+ and Na^+ contained in 600 mg enzyme mix were 0.15 mg, 17.9 mg, 3.30 mg, 0.73 mg, 0.62 mg and 7.29 mg, respectively.

Methods and instrumentation

A modified Dionex ion chromatograph, system 12 (Dionex, USA), with HPIC-AG3 and HPIC-CG3 guard columns, HPIC-AS3 and HPIC-CS3 separation columns and AMMS-1 and CMMS-1 suppressor columns was used for the determination of anions and cations. Algal sap pH values were measured with a WTW pH-meter, Model pH 192, with a WTW gelatin-type E50 electrode (Weilheim, Germany). Samples were mixed with a Vortex Genie 2 TM mixer (Bender & Hobein, Zürich, Switzerland) and kept at 333 K in a water bath (a glass container with water and an MGW Lauda RC6 Thermostat, Germany). Algal suspensions were separated with a Megafuge 1.0 centrifuge (Heraeus Sepatech, Germany). The Baker spe-10 Column Processing System (J.T. Baker, USA) was used for solid phase extraction with Bakerbond octadecyl (C_{18}) columns.

Digestion technique

For the investigation of the complete removal of ions from the materials, two digestion techniques were used.

Digestion with hydrochloric acid. Samples of 200 mg of homogenized algae were weighed into 14-ml polypropylene centrifuge tubes. After the addition of 1.5 ml of 5% hydrochloric acid, the samples were kept at room temperature and shaken for 1 h. A further 3.5 ml of deionized water were added to the polypropylene tubes. The samples were shaken in a water bath at 333 K for 2 h. After 10 min centrifugation at 6000 rpm (6240 *g*), the supernatant solution was diluted with 250 ml of deionized water. The cations and anions released from the algal solid phase into the supernatant solution were measured by ion chromatography.

Enzymatic digestion. A mixture of three enzymes (200 mg of pectinase, 200 mg macerozyme and 200 mg of cellulase) was used for the enzymatic digestion of algal samples [13–15]. After the addition of 5 ml of deionized water into the 14-ml polypropylene centrifuge tube containing 200 mg of algae and enzyme, the samples were shaken at room temperature (293 K) for 1 h and separated with a centrifuge at 6000 rpm (6240 *g*). The liquid phase was diluted with 250 ml of deionized water for the determination of ions. Because of a high blank value of anions and cations, the ion levels of the enzymes were checked several times.

Determination of cations and anions released from algae samples from the Baltic Sea and North Sea

Samples of 200 mg of homogenized algae samples were weighed into 14-ml polypropylene centrifuge tubes. After the addition of 5 ml of deionized water, the samples were kept in a water bath at 333 K for 3 h and then separated by centrifugation at 6000 rpm (6240 *g*) for 10 min. Solid-phase extraction with Bakerbond octadecyl (C_{18}) columns was used for to

remove aromates, fatty acids, hydrocarbons and tensides from the clear supernatant solution. After dilution with 250 ml of deionized water, the cations and anions were determined using a modified ion chromatography Dionex system 12 [16]. The standard solutions were processed in appropriate concentrations to identify the retention time and detector response. Owing to lower blank values, the detection limits are considerably lower (1 μg/l for chloride, 2 μg/l for nitrate, phosphate and sodium, 3 μg/l for sulphate and 5 μg/l for ammonium and potassium) when no digestion at all is used and the samples are extracted with deionized water only.

RESULTS AND DISCUSSIONS

Methodological aspects

In contrast to animal cells, plant cells have a rather robust cell wall containing cellulose, polysaccharides and protopectin, which can be taken to influence the investigation of inorganic anions and cations of plant materials. Therefore, the complete removal of the ions from the homogenized algae samples using deionized water was checked using an acidic digestion technique and an enzymatic digestion technique. A comparison of the released Cl^-, HPO_4^{2-}, SO_4^{2-}, NH_4^+, K^+ and Na^+ from the algae samples after extraction with deionized water and after digestion with 5% hydrochloric acid is presented in Table I. The results show that only HPO_4^{2-}, SO_4^{2-}, NH_4^+ and Na^+ could be detected, and errors introduced by interference from the high Cl^- concentration were observed. The injection of stronger acidic solutions into the ion chromatography system resulted in a large Cl^- peak hampering the measurement of other components. It is obvious that the inorganic anions and cations were completely released from the homogenized algae by extraction with deionized water alone. This can be additionally confirmed by the data given in Table II. An enzyme mix of pectinase, macerozyme and cellulase was used to check the completeness of cell disintegration in comparison with the ESB preparation technique and the extraction of the algae samples with deionized water. Following the enzymatic attack, the rest of the robust cell structure and macromolecules in the samples are destroyed. Additional investigations of the total protein content of enzymatically treated and untreated samples show no evidence of additional protein release from the cell structure in the treated samples, confirming the finding that homogenized algae samples can be readily extracted by deionized water and analysed without additional digestion steps. The results clearly show that the anion and cation levels obtained by both techniques are similar and indicate complete cell disintegration as well as complete extraction with deionized water from the homogenized algae. It can be deduced that the cellular structure of brown algae is less stable than that of other plants and is easily decomposed by the application of the ESB preparation technique. It has still to be proved whether the ESB preparation is as complete for other plant materials as it has been

TABLE I

ION CHROMATOGRAPHIC DETERMINATION OF IONS IN HOMOGENIZED BROWN ALGAE SAMPLES AFTER EXTRACTION WITH DEIONIZED WATER (A) AND 5% HYDROCHLORIC ACID (B)

Average values (in g per kg dry mass) were calculated from means of three brown algae samples. The mass of each sample was 200 mg.

Ion	North Sea samples		Baltic Sea samples	
	A	B	A	B
Cl^-	55.8 ± 2.25	–	27.5 ± 0.70	–
HPO_4^{2-}	6.14 ± 0.55	4.34 ± 0.37	5.46 ± 0.21	5.02 ± 0.35
SO_4^{2-}	7.07 ± 0.47	6.55 ± 0.71	4.54 ± 0.11	4.96 ± 0.13
NH_4^+	18.1 ± 0.23	23.7 ± 0.32	37.7 ± 0.44	39.2 ± 1.53
K^+	0.13 ± 0.05	–	0.45 ± 0.01	–
Na^+	22.0 ± 0.39	25.2 ± 0.17	31.6 ± 0.33	27.1 ± 0.29

TABLE II

COMPARISON OF ION LEVELS IN HOMOGENIZED BROWN ALGAE SAMPLES AFTER EXTRACTION WITH DEIONIZED WATER (A) AND AFTER ENZYMATIC DIGESTION WITH A MIXTURE OF ENZYMES (B)

Average values (in g per kg dry mass) were calculated from means of three brown algae samples. The mass of each sample was 200 mg.

Ion	North Sea samples		Baltic Sea samples	
	A	B	A	B
Cl^-	55.8 ± 2.25	54.7 ± 2.33	27.5 ± 0.70	27.5 ± 0.26
HPO_4^{2-}	6.14 ± 0.55	6.60 ± 0.28	5.46 ± 0.21	5.54 ± 0.10
SO_4^{2-}	7.07 ± 0.47	6.87 ± 0.51	4.54 ± 0.11	3.40 ± 0.09
NH_4^+	18.1 ± 0.23	20.9 ± 0.14	37.7 ± 0.44	34.3 ± 2.05
K^+	0.13 ± 0.05	0.10 ± 0.06	0.45 ± 0.01	0.32 ± 0.04
Na^+	22.0 ± 0.39	20.1 ± 0.09	31.6 ± 0.33	26.1 ± 0.09

shown to be for algae or whether additional enzymatic digestion is necessary before IC analysis of anions and cations can be carried out. The distribution of cation and anion levels in the homogenized brown algae samples after three successive extractions with 5 ml of deionized water is illustrated in Table III. The results show that 75–90% of K^+, NH_4^+, Na^+, Cl^-, HPO_4^{2-} and SO_4^{2-} could be removed from the algae at the first extraction step with deionzed water (200 mg in 5 ml of deionized water). A further extraction step resulted in an additional total removal of 1–17.3%.

Investigation of the inorganic anions and cations in brown algae collected from the North Sea and Baltic Sea

The data on the average concentrations of chloride, phosphate, sulphate, ammonium, sodium and potassium for the brown algae samples from the North Sea and the Baltic Sea are summarized in Tables IV and V. The average values were calculated from means of three measurements and are given in *g* per kg dry weight of algae. A comparison of chloride and sodium levels in Tables IV and V shows that higher chloride levels were observed in the North Sea algae. The average concentration of chloride ranged from 40.0 g/kg to 72.0 g/kg for the North Sea algae and from 29.2 g/kg to 53.9 g/kg for the Baltic Sea algae. It indicated a difference in the salinity of sea water between North and Baltic Seas. The results obtained in Tables IV and V show that chloride accumulation in brown algae decreases from the North Sea to the Baltic Sea. This observation is generally in good agreement with the salinity measurements of sea water in the North and Baltic Seas [17]. Only a slight decrease in sodium levels in brown algae from the North Sea to the Baltic Sea was observed. The average sodium concentration varied from 25.7 g/kg to 39.1 g/kg in the North Sea and from 19.3 g/kg to 35.9 g/kg in the Baltic Sea. Environmental pollution due to anthropogenic influences on ecosystem species in the North and Baltic Sea areas is clearly confirmed in comparison with phosphate levels. Two significant results can be drawn from the data on phosphate in Tables IV and V: (a) the average phosphate values of the Baltic Sea algae were generally higher than those of the North Sea algae; and (b) a significant increase in phosphate levels in algae samples was found from the sampling locations 11 (Reddewitz), 12 (Zudar

TABLE III

DISTRIBUTION OF ION LEVELS IN g PER kg DRY MASS AND AS A PERCENTAGE (%) OF THE HOMOGENIZED BROWN ALGAE SAMPLES AFTER THREE SUCCESSIVE EXTRACTIONS WITH DEIONIZED WATER

Ion	First extraction	Second extraction	Third extraction
Cl^-	51.8 (86.4%)	7.55 (12.6%)	0.59 (1.00%)
HPO_4^{2-}	9.23 (85.5%)	1.43 (13.2%)	0.11 (1.30%)
SO_4^{2-}	8.83 (85.7%)	1.22 (11.8%)	0.26 (2.50%)
NH_4^+	29.5 (86.3%)	3.55 (10.4%)	1.13 (3.30%)
K^+	0.34 (75.0%)	0.08 (17.3%)	0.03 (7.70%)
Na^+	32.7 (90.0%)	2.99 (8.24%)	0.61 (1.76%)

TABLE IV

AVERAGE CONCENTRATION OF CHLORIDE, SODIUM, PHOSPHATE, AMMONIUM, SULPHATE AND POTASSIUM IN g PER kg DRY MASS OF THE HOMOGENIZED BROWN ALGAE SAMPLES COLLECTED FROM THE NORTH SEA

Ion	Sampling location[a]											
	1	2	3	4	5	6	7	8	9	10	11	12
Cl^-	59.9	40.0	60.8	58.4	72.0	68.7	56.2	53.2	59.5	52.5	62.2	58.0
Na^+	36.3	25.7	32.8	32.9	27.4	32.4	35.3	30.9	39.1	28.1	37.9	31.4
HPO_4^{2-}	10.3	10.8	8.15	6.56	4.80	7.70	7.11	7.10	7.33	6.09	9.20	9.52
NH_4^+	34.2	35.0	31.1	33.1	40.6	28.5	26.5	37.7	29.8	27.0	29.3	29.3
SO_4^{2-}	10.3	6.66	10.8	10.6	11.8	11.2	9.93	9.59	12.4	8.42	12.7	10.4
K^+	0.45	0.17	0.21	0.09	0.32	0.15	0.10	0.08	0.18	0.32	0.10	0.15

[a] Sampling locations in the North Sea: 1 = Eckwarderhörne 07302; 2 = Cuxhaven Kugelbake 10402; 3 = Altenbruch 10401; 4 = Trischendamm 07270; 5 = Meldorfer Bucht 07271; 6 = Sylt/List 07102; 7 = Sylt/Königshafen 07151; 8 = Sylt/Königshafen 07152; 9 = Sylt/Königshafen 07161; 10 = Sylt/Königshafen 07163; 11 = Sylt/Königshafen 07181; 12 = Sylt/Königshafen 07182.

gelbes Ufer), 8 (Boiensdorf) and 13 (Drigge) in the Baltic Sea. At the Baltic coast near to Zudar, gelbes Ufer and Drigge, the concentration of phosphate was measured to be 21 g per kg dry mass of brown algae. This corresponds to a 2.9-fold increase compared with the average phosphate level of 7.36 g per kg dry mass in algae from Sylt in the North Sea. This effect can be explained by the influence of higher environmental pollution in the Baltic Sea [18]. A high input of nutrients via the rivers from fertilizers and via municipal and industrial waste water generally causes a very large increase in phosphate and nitrogen in the sea water. This can be confirmed by the data given in Tables IV, V and VI. The highest concentration of 21 g/kg for phosphate (Table V) and 2.13 g/kg for nitrate (Table VI) in these experiments was measured in brown algae from Zudar gelbes Ufer in the Baltic Sea. Phosphorus is an essential nutrient for the cell structure composition of plants. But excessive quantities of nutrients in sea and lake water cause a massive growth of algae and change the life areas and life conditions of several animals in the aquatic system. Moreover, the algae samples collected in the Cuxhaven estuary contained a higher level of phosphate and nitrate. In this aquatic system, a mixing of highly polluted

TABLE V

AVERAGE CONCENTRATION OF CHLORIDE, SODIUM, PHOSPHATE, AMMONIUM, SULPHATE AND POTASSIUM IN g PER kg DRY MASS OF THE HOMOGENIZED BROWN ALGAE SAMPLES COLLECTED FROM THE BALTIC SEA

Ion	Sampling location[a]												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Cl^-	53.0	48.1	46.5	53.5	53.9	46.4	38.3	50.5	30.4	35.4	44.3	29.2	42.0
Na^+	29.9	35.9	35.6	30.7	19.3	25.7	25.3	28.6	23.7	26.6	27.5	26.0	30.7
HPO_4^{2-}	9.44	6.68	5.12	8.06	8.81	7.34	5.88	13.8	8.63	6.33	12.4	20.5	21.0
NH_4^+	26.3	30.8	39.5	27.1	20.4	23.3	22.9	24.1	18.6	21.0	23.1	19.6	21.2
SO_4^{2-}	8.16	7.33	5.89	8.47	9.39	7.03	5.81	8.11	4.71	5.17	7.71	6.09	6.49
K^+	0.10	0.33	0.64	0.26	0.21	0.15	0.16	0.16	0.17	0.27	0.36	0.35	0.10

[a] Sampling locations in the Baltic Sea: 1 = Glücksburg 08069; 2 = Gut-Öhe 08071; 3 = Langholz 08072; 4 = Kiel Strande 08073; 5 = Strukkamphuk 08074; 6 = Katharinenhof 08075; 7 = Dahme 08076; 8 = Boiensdorf 08078; 9 = Kap Arcona 08084; 10 = Lome 08085; 11 = Reddewitz 08087; 12 = Zudar gelbes Ufer 08088; 13 = Drigge 08083.

TABLE VI

AVERAGE NITRATE CONCENTRATION IN HOMOGENIZED BROWN ALGAE SAMPLES COLLECTED FROM THE NORTH AND BALTIC SEAS

Levels in other samples were below the determination limits ($\leqslant$0.20 g/kg).

Sampling location	Location	Level (g per kg dry mass)
No. 2 (North Sea)	Cuxhaven–Kugelbake	1.16 ± 0.10
No. 3 (North Sea)	Cuxhaven–Altenbruch	2.02 ± 0.18
No. 3 (Baltic Sea)	Langholz	0.28 ± 0.06
No. 4 (Baltic Sea)	Kiel Strande	1.35 ± 0.11
No. 9 (Baltic Sea)	Kap Arcona	1.38 ± 0.08
No. 12 (Baltic Sea)	Zudar gelbes Ufer	2.13 ± 0.10

fresh water and sea water occurs. The data shown in Table IV and VI show that the brown algae collected from Cuxhaven contained more phosphate (sampling location 2, Cuxhaven Kugelbake) and nitrate (sampling location 3, Cuxhaven Altenbruch) than in brown algae from other sampling sites in the North Sea. Furthermore, in the estuary areas Cuxhaven–Kugelbake (the Elbe and the North Sea), a lower chloride and sodium level (Table IV) was observed. With regard to the ammonium and sulphate levels in brown algae from the North and Baltic Seas, the trend is similar to that of chloride. The average concentration of ammonium and sulphate in the North Sea algae is higher than in the Baltic Sea algae. Ammonium ranged from 26.5 g/kg to 40.6 g/kg in North Sea algae and from 18.6 g/kg to 39.5 g/kg in Baltic Sea algae, and the average levels of sulphate ranged from 6.66 g/kg to 12.7 g/kg in the North Sea algae and from 4.71 g/kg to 9.39 g/kg in the Baltic Sea algae. Comparing sulphate data from the algae samples from the North and Baltic Seas, one can see an increase in the average concentration by a factor of 1.5. The concentration of ammonium also varied by a factor of 1.3 between the algae samples from North and Baltic Seas. Moreover, the results presented in Tables IV and V show a significant increase in ammonium levels in algae collected from Langholz in the Baltic Sea (sampling location 3) and Meldorfer Bucht in the North Sea (sampling location 5). Active agricultural production in these areas may influence this effect. The values for potassium in Tables IV and V show no significant trend except a high fluctuation of low potassium amounts. Potassium is an essential and mobile nutritive substance for plants. The simultaneous determination of low potassium concentrations and a high amount of ammonium and sodium by this measuring method may cause this fluctuation.

CONCLUSIONS

The ESB preparation technique and extraction with deionized water can be effectively used for routine ion chromatographic analysis of inorganic anions and cations in algae samples. Between 75 and 90% of K^+, NH_4^+, Na^+, Cl^-, HPO_4^{2-} and SO_4^{2-} could be removed from the algae by the first extraction with deionized water. Complete removal of the inorganic anions and cations from brown algae is guaranteed by use of the ESB standard preparation technique and extraction with deionized water. Enzymatic digestion with a mixture of pectinase, macerozyme and cellulase can be used to investigate the completeness of the cell disintegration of other plants following the ESB preparation technique and extraction with deionized water for ion chromatographic analysis. Because of a possible high blank value of anions and cations in the enzymes, their ion levels should be monitored. The results of the investigation of the inorganic anions and cations in brown algae samples from the North Sea and Baltic Sea have futher confirmed the use of brown algae as environmental monitors of local pollution in marine areas.

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