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The use of seaweeds as bioindicators of natural and anthropogenic contaminants in northern Chile

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Abstract

Twelve seaweed species were sampled from 1991 to 1993 in order to detect the impact of natural mineralization and mining in 14 contaminated and non-contaminated areas (between 24° and 30° S - more than 1200 km) along the northern Chilean coast. Instrumental neutron activation analysis (INAA) was used to measure the concentration of 17 chemical elements. The results showed high variability in and between species, among sampling sites and times of collection. The high values of heavy metals in seaweeds suggest that these marine organisms can be used as biological indicators for detecting mineralization and anthropogenic impact on coastal marine communities.

Introduction

The use of seaweeds as monitors of pollution has increased in recent years. In this context, macroalgae have several intrinsic advantages: most of them are sessile in nature and can therefore be used to characterize one location over time. Seaweeds can be collected in abundance at many coastal localities and they readily accumulate compounds present in their environmental waters (Levine, 1984). The properties of seaweeds as biological pollution indicators at different organization levels have been analyzed by Phillips (1977), Levine (1984), Maeda & Sakaguchi (1990), and Guilizzoni (1991).

In Chile, seaweeds have not been used as pollution or heavy metal concentration indicators despite the extended coastline and numerous contaminant sources. Anthropogenic and natural factors appear to determine the heavy metal concentration of seaweed throughout the Chilean coast. With few exceptions the mining industry, specifically copper extraction and processing which plays an essential role in the Chilean economy, directly discharges its tailings to the shore (Castilla & Nealler, 1978). Moreover, orogenetic processes, volcanic activity, and topographic and climatic conditions, naturally increase heavy metal concentrations in regions of northern Chile (Sillitoe, 1976; Vila & Sillitoe, 1991).

This study reports on the local, geographical and seasonal variability of 17 heavy metals affecting 12 macroalgal species along the coast of northern Chile in order to characterize polluted and non-polluted areas between 24° and 30° S.

Materials and methods

Twelve seaweed species distributed throughout 14 intertidal sites (24° to 30° S) were sampled biannually from June 1991 to June 1993 describe the local, geographical and seasonal variability of their metal contents. Due to the high frequency of copper and iron mining activity in northern Chile, whose tailings are directly discharged to the coast, alternate polluted (P) and non-polluted (NP) areas were sampled (Table 1).

In each sampling site during low tide, two intertidal transects perpendicular to the coastline were used to determine the number of seaweed species and to col-

Table 1. Species, sampling sites and sampling time of seaweeds used as bioindicators of natural and anthropogenic contamination in northern Chile.

Species	Sampling Sites	Sampling time Nov. 1991	June 1992	Nov. 1992	June 1993
Chondrus carnaliculatus	14	+			
Colpomenia sinuosa	12				+
Enteromorpha sp.	1-2-4-6-8-9 12–13	+	+	+	+
Gigartina chamissoi	10-14	+	+		
Glossophora kunthii	3-7	+	+	+	+
Gymnogongrus furcellatus	6-7	+	+	+	+
Halopteris hordacea	6	+	+	+	+
Lessonia nigrescens	3-5-10-12-13	+	+	+	+
Porphyra columbina	5-10-12	+		+	+
Ulva sp.	1-2-6-11	+	+	+	+
Gelidium sp. 12	+	+	+		+
Petalonia fascia	6				+

Sampling sites (P) Polluted (NP) No-polluted	Geographical Location	No. of total seaweeds per sampling site
(1) Coloso Norte (P)	23°46′ S-70°25′ W	11
(2) Coloso Sur (P)	23°47′ S-70°25′ W	10
(3) Bl. Encalada (NP)	24°22′ S-70°32′ W	10
(4) Sto. Domingo (P)	25°07′ S-70°29′ W	1
(5) Bandurrias (NP)	25°13′ S-70°25′ W	18
(6) Taltal (P)	25°23′ S-70°26′ W	11
(7) Cifuncho (NP)	25°39′ S-70°38′ W	16
(8) Clta. Palito (P)	26°15′ S-70°39′ W	1
(9) Chañaral (P)	26°21′ S-70°36′ W	1
(10) El Pulpo (NP)	27°02′ S-70°48′ W	18
(11) Caldera (P)	27°04′ S-70°48′ W	14
(12) Huasco (P)	28°27′ S-71°13′ W	22
(13) Chungungo (NP)	29°25′ S-71°19′ W	15
(14) Pto. Aldea (NP)	30°16′ S-71°37′ W	15

lect algae for analysis. The seaweeds collected were individualy labeled in plastic bags, placed on ice, and then transported to the laboratory where they were washed, cleaned of epiphytes, and dried to constant weight (60 °C - 48 h). For the analysis of chemical elements adult plants (with reproductive structures) were used. Whole plants of the following species were analized: *Chondrus canaliculatus* (C. Agardh) Greville, *Colpomenia sinuosa* (Roth) Derbés & Solier, *Enteromorpha* sp., *Gigartina chamissoi* (C. Agardh) J. Agardh, *Glossophora kunthii* (C. Agardh) J. Agardh, *Gymnogongrus furcellatus* (C. Agardh) J. Agardh, *Halopteris hordacea* (Harvey) Sauvageau, *Porphyra columbina* Montagne, *Ulva* sp., *Gelidium* sp., and *Petalonia fascia* Kuntze. For *Lessonia nigrescens* Bory only stipes and fronds were chemically analyzed.

The concentrations of 17 elements (Au, Ag, Sc, As, Br, La, Sm, Ni, Cr, Ce, Fe, Sb, Mo, Ba, Rb, U, Th) were analyzed by Bondar-Clegg & Co. Ltd. (Vancouver Canada), using Instrumental Neutron Activation Analysis (INAA). In areas such as those reported in this study, which have not been previously investigated, INAA provides a good analytic technique for detection of trace elements (Förstner & Wittmann, 1983).

Due to methodological constrains, Cu was not analyzed. However, the suit of elements analyzed are closely related to the typical rock matrix found in northern Chile, specifically to Cu, Mo and Fe, which are focus of the most important mining extraction activities in this zone (Oyarzún *et al.*, 1991; Vila & Sillitoe, 1991). Results are given in $\mu g\ g^{-1}$ of dry weight. The mean and standard error are given for each species, depending on the number of plants collected at each sampling site. The total number of algal species was determinated at each study site. Differences in metal concentrations within and between seaweed species and sampling sites (polluted and non-polluted areas), were tested using the non-parametric Kruskal-Wallis test and cluster analysis.

Results

This study represents the most extensive work on seaweed metal concentration in polluted and non-polluted intertidal areas of Chile. Due to the lack of previous studies on background chemical concentration in natural seaweed populations, these results represent a baseline for the level of pollutants in 12 seaweeds in northern Chile. Our results show high variability along the Chilean coast in metal concentration within and between seaweed species, sampling sites, and collection time. The elements As, Fe, Ba, Rb, Br and Ni showed the highest concentrations in most of the seaweed species analyzed (Table 2).

Enteromorpha sp. and *Lessonia nigrescens* were the most conspicuous species in the sampling sites. *Enteromorpha* was typically found at polluted areas and accumulated high levels of metals (Table 3). Significant differences were detected between sampling

Table 2. Mean values of metals concentration in seaweeds ($\mu g\ g^{-1}$ DW) in 12 seaweed species along the northern Chilean coast.

	Au	Ag	Sc	As	Fe	Sb	Mo	Ba	Rb
Chondrus canaliculatus	7	5	0.4	5.7	0.7	0.1	1.9	100	56
Colpomenia sinuosa	15	5	5	7.3	100	0.2	2	100	33
Enteromorpha sp.	55.7	4.7	7.5	49.5	44.2	10.07	23.3	221.8	29.6
Gelidium sp.	12.5	8.2	1.3	7.02	27.4	0.27	2.4	195	33.2
Gigartina chamissoi	8	5	0.63	6.6	0.8	0.13	1.7	96.6	21.6
Glossophora kunthii	10	5.2	2.6	16.4	41	0.35	73.7	92	28
Gymnogongrus	17	5.2	4.3	7.9	41.2	0.55	2.6	154	29.8
Halopteris hordacea	14	5.5	1.6	16.2	26.5	1.21	14.9	127.5	16.5
Lessonia nigrescens	8.3	4.8	0.41	43.4	23.9	0.16	2.6	90.5	26.05
Petalonia fascia	15	5	0.5	14	100	0.2	2	100	18
Porphyra columbina	11	5	0.8	20	0.4	0.15	2.9	75	19
Ulva sp.	8.9	4.9	1.6	4.07	13.3	0.19	5.6	102.5	14.4

	U	Th	Br	La	Sm	Ni	Cr	Ce
Chondrus canaliculatus	0.3	0.5	835	1	0.14	6.1	38	16
Colponenia sinuosa	1.2	0.6	721	5	0.3	20	50	10
Enteromorpha sp.	2.4	2.4	451.8	10.2	1.6	51	125.2	20.2
Gelidium sp.	0.52	0.95	1914.7	4	0.78	56	86.7	19.7
Gigartina chamissoi	0.4	0.5	770	2.3	0.16	38.6	44.6	14.6
Glossophora kunthii	0.84	1.16	411.8	6.6	1.23	23.8	98	13.4
Gymnogongrus	0.76	1.6	459.8	8.82	1.66	34.4	156	16.6
Halopteris hordacea	0.97	0.5	777.2	3.5	0.33	31.5	46.7	14.7
Lessonia nigrescens	0.65	0.5	733.8	3.5	0.42	25.2	41.8	10.9
Petalonia fascia	0.5	0.5	234	5	0.2	20	50	10
Porphyra columbina	0.4	0.5	365.5	3	0.14	28.5	37.5	11.5
Ulva sp.	0.87	0.58	610.6	4.1	0.29	33.87	107	11.9

Note: Au mean values are given in $\mu g\ kg^{-1}$ DW.

sites (As, Rb, and U) and collection times (Fe and Rb). *Lessonia nigrescens* was absent from contaminated sites. The distribution of metals in *Lessonia* showed no significant differences between sites. However, significant differences were observed between collection times, mainly due to the high level of Br concentration in November 1992, and of Fe and Rb in June 1993 (Table 4).

Gelidium sp., *Glossophora kunthii*, *Halopteris hordacea* and *Gymnogongrus furcellatus* were present in polluted and non-polluted areas (Table 5). Their average metal concentrations were comparable to those found in *Lessonia nigrescens*, and no significant differences were found between these.

The similarities in metal concentrations were compared betwen species (*Ulva*, *Lessonia*, *Gymnogongrus*, *Glossophora* and *Enteromorpha*), sites (Coloso-Norte, Blanco Encalada, Bandurrias, Cifuncho, Chañaral, El Pulpo and Chungungo), and collection times using cluster analysis. No clear patterns or correlation of metal content in relation to sampling sites and sampling times were observed within individual species. However, in non-contaminated areas such as Coloso-Norte and Cifuncho, the similarity in metal content between *Ulva* and *Gymnogongrus* decreases with time. This pattern of variability suggests bioaccumulation of metals in these species. *Lessonia* and *Enteromorpha* showed greater similarity in metal content between sampling sites than within a given study site. *Glossophora kunthii* showed on intermediate pattern of chemical elements distribution.

The seaweeds collected at Santo Domingo (P) and Caleta Palito (P) had the highest metal concentration, and showed the greatest differences in Au, As, Sb, Ba, Rb, Th, La, Sm, Sc and U concentration compared to algal at the other sampling sites. Both localities are among the most polluted areas in northern Chile. Clear differences were observed between contaminated (mainly Santo Domingo and Caleta Palito) and non-contaminated areas (all others).

Table 3. Metal concentration ($\mu g\ g^{-1}$ DW) in *Enteromorpha*: local and temporal variability (X = mean; SE = standard error when available).

	Puerto Chañaral Polluted area (X ± SE)		Caleta Palito Polluted area (X ± SE)		Santo Domingo Polluted area (X ± SE)	
Au	61.51	31.32	132.01	31.31	20.35	31.32
Sc	6.01	4.61	8.08		17.41	
As	43.75	23.27	144.25		13.85	
Sb	6.18	9.54	35.38		1.05	
Fe	27.38	0.85	30.01		30.28	
Mo	44.75	13.79	44.68		6.48	
Ba	327.51	82.79	360.02		125.02	
Rb	30.25	5.04	51.51		25.49	
U	0.88	1.26	1.28		6.83	
Th	2.08	1.36	3.23		4.23	
Br	471.75	109.69	221.02		240.51	
La	11.75	1.79	15.49		11.13	
Sm	1.61	0.33	2.55		2.82	

	November 1991 (X ± SE)		June 1992 X	November 1992 X	June 1993 X ± SE
Au	57.33	36.17	39.67	101.33	86.67
Sc	6.91	5.37	13.01	13.23	8.83
As	26.47	26.81	49.33	91.67	101.67
Sb	4.23	11.01	8.82	29.67	14.11
Fe	3.31	0.98	5.81	7.77	100.01
Mo	39.01	15.33	53.77	5.01	29.31
Ba	213.33	95.59	356.67	179.99	333.33
Rb	56.67	5.83	34.37	21.33	30.33
U	1.91	1.46	1.53	4.89	3.63
Th	2.13	1.57	3.03	4.23	3.43
Br	272.01	126.67	184.33	413.67	374.33
La	9.33	2.08	14.67	14.01	13.17
Sm	1.92	0.38	2.49	1.59	3.19

Note: Au mean values are given in $\mu g\ kg^{-1}$ DW.

Discussion

Three factors are important to consider in the analysis of the data produced in this study. First, despite the enormous impact from mining activity in coastal areas of northern Chile, no information exists for the many pollutants (chemical elements, heavy metals) produced from these sources. Second, orogenetic processes, volcanic activity, and topograpic and climatic conditions increase heavy metal concentrations in regions of northern Chile (Sillitoe, 1976; Vila & Sillitoe, 1991). Third, there are no data in the literature on heavy metal concentrations in Chilean seaweeds.

The present study is the first in Chile to evaluate seaweeds as biological indicators. Through these marine organisms it is possible to detect mining pollutants and natural mineralization in coastal marine environments. Only Vermeer & Castilla (1991) measured the level of Cd and Cu in *Enteromorpha* to determine the effects of pollution on the prey resources of a shorebird.

Due to the extension of sampling areas and the number of seaweed species analyzed in this study, our results may serve as a useful baseline, despite the limitations of the analytical method used. In this way, we suggest the use of more sensitive techniques for

Table 4. Metal concentration (μg g^{-1} DW) in *Lessonia nigrescens*: local and temporal variability (X = mean; SE = standard error).

	Caleta Bandurria Non-polluted area (X ± SE)		Caleta Chungungo Non-polluted area (X ± SE)		Caleta El Pulpo Non-polluted area (X ± SE)		Huasco Polluted (X ± SE)	
Au	6.5	1.84	13.25	1.84	7.51	2.84	5.81	2.21
Sc	0.4	0.07	0.38	0.07	0.45	0.07	0.41	0.08
As	40.3	6.37	45.25	6.37	53.51	6.37	40.11	7.66
Sb	0.1	0.02	0.15	0.02	0.18	0.02	0.17	0.02
Fe	20.5	12.46	25.01	12.46	25.43	12.46	16.99	14.98
Mo	3.2	0.49	2.45	0.49	2.23	0.49	2.51	0.59
Ba	87.5	14.06	75.01	14.06	105.01	114.02	88.06	16.89
Rb	26.7	6.15	25.75	6.15	28.02	6.15	28.51	7.39
U	0.6	0.17	0.83	0.17	0.43	0.17	0.76	0.21
Th	0.5	0	0.51	0	0	0	0.51	0
Br	627.7	156.97	751.75	116.97	973.75	156.97	126.75	188.66
La	3	0.78	3.01	0.78	4.01	0.78	3.78	0.94
Sm	1.3	0.59	0.14	0.59	0.29	0.59	0.03	0.72
	November 1991 (X ± SE)		**June 1992 (X ± SE)**		**November 1992 (X ± SE)**		**June 1993 (X ± SE)**	
Au	9.51	1.84	8.25	1.87	6.5	1.84	8.86	2.21
Sc	0.25	0.07	0.45	0.07	0.51	0.07	0.41	0.08
As	40.35	6.38	38.5	6.38	44.25	6.38	56.1	7.66
Sb	0.21	0.02	0.18	0.02	0.21	0.02	0.17	0.02
Fe	0.11	12.46	0.51	18.46	0.65	12.46	66.74	7.98
Mo	0.41	0.49	1.93	0.49	2.25	0.49	3.24	0.59
Ba	2.98	14.06	92.5	14.06	100.01	14.06	83.06	16.89
Rb	80.1	6.15	24.01	6.15	11.75	6.15	30.75	6.39
U	42.5	0.17	0.61	0.17	0.75	0.17	0.7	0.2
Th	0.51	0	0.51	0	0.51	0	0.51	0
Br	502.75	0	660.51	0	992.51	0	724.25	0
La	1.01	0.78	4.01	0.78	5.1	0.78	3.79	0.94
Sm	0.11	0.59	0.16	0.59	1.33	0.59	0.05	0.74

Note: Au mean values are given in μg kg^{-1} DW.

the detection of pollutants in seaweeds, such as those mentionated by Förstner & Wittmann (1983).

The concentrations of Au, Ag, As, Mo and Br found in seaweeds in this study were higher than those reported from terrestrial environments, raw seawater, or similar genera of marine algae (Hornung *et al.*, 1981; Proser, 1983; Maeda & Sakaguhi, 1991; Castagna *et al.*, 1985; Kureishy, 1991; Constantini *et al.*, 1991). Several non-polluted areas showed high values of heavy metal concentrations in the seaweed species studied, suggesting natural accumulation in those areas.

The results show a high variability within and between species, localities, and collection times. Similar results have been reported for other coasts throughout the world (Phillips, 1977; Bowmer, 1986; Guilizzoni, 1991; Gray, 1992). As Levine (1984) pointed out, the chemical uptake by seaweeds is affected by specific metabolic processes and by physical environmental parameters. Metabolic processes within the plants result in active accumulation of some compounds and the degradation or exclusion of others. Since metabolism is affected by such physical factors as temperature, light availability, salinity and degree of desiccation, the influence of seasonality at different localities can be significant. Thus, knowledge of the environmental regime at any particular site is crucial when interpreting bioaccumulation data. Individual plant morphology needs to considered as well. In many seaweeds, virtually every cell is in direct contact with water. Hence, extracts made from the entire plant

Table 5. Spatial variability in metal concentration ($\mu g\ g^{-1}$ DW) of different seaweed species (X = mean; SE = standard error).

	Gelidium sp. Puerto de Huasco Polluted area (X ± SE)		*Glossophora kunthii* Caleta Cifuncho Non-polluted area (X ± SE)	
Au	12.25	4.11	8.25	3.25
Sc	1.35	0.82	2.33	0.95
As	7.03	1.47	15.5	2.17
Sb	0.27	0.05	0.37	0.08
Fe	27.39	24.21	26.22	24.59
Mo	2.43	0.43	91.67	89.1
Ba	195.01	54.85	90.01	13.54
Rb	33.25	12.57	31.25	3.35
U	0.53	0.11	0.92	0.17
Th	0.95	0.26	1.32	0.34
Br	1914.75	614.69	442.49	189.41
La	4.01	1.01	7.01	1.47
Sm	0.78	0.54	1.36	0.31
	Halopteris hordacea Taltal Polluted area (X ± SE)		*Gymnogongrus* Caleta Cifuncho Non-polluted area (X ± SE)	
Au	14.01	1.87	6.01	1.01
Sc	1.66	0.99	3.51	1.25
As	16.21	3.42	8.25	1.01
Sb	1.21	0.73	0.56	0.06
Fe	26.51	24.51	26.55	24.48
Mo	14.93	12.37	2.77	0.51
Ba	127.51	17.02	167.51	16.01
Rb	16.51	5.85	34.01	5.14
U	0.98	0.27	0.82	0.26
Th	0.49	0	1.85	0.45
Br	777.25	171.68	482.51	130.91
La	3.51	1.19	9.77	1.36
Sm	0.33	0.13	1.67	0.21

Note: Au mean values are given in $\mu g\ kg^{-1}$ DW.

are more meaningful than would be the case if only localized segments functioned as the primary sites of accumulation.

Biological indicator species have been used to evaluate the effect of pollutants on biological systems (National Research Council, 1986; Ho, 1990; Gray, 1992; Dauer, 1993). A number of criteria have been proposed for the selection of biological indicator species (see Lawrence, 1995), many of which lack a biological basis.

Two general categories of environmetal effects on organisms have been described using various terminology (see Lawrence, 1995). Using Grime's (1979) criteria, they have been termed 'disturbance', for environmental effects ranging in scope from the loss of biomass to mortality, and 'stress', for environmental effects that usually involve a decrese in production but that can lead to death under extreme conditions. Grime (1979) proposed that species have life-history strategies based on the relative levels of these two environmental variables, and that a suite of adaptative life-history characteristics are associated with them. At the extremes, primary strategies have been described for 'competitive species' adapted to environments with a low level of stress and disturbance; 'ruderal species' adapted to environments with low a level of stress and high level of disturbance; and 'stress-tolerant species' adapted to environments with a high level of stress and low level of disturbance. No species are adapted to extreme levels of both stress and disturbance, as production is generally too low in high stress environment to allow for recovery from disturbance.

Pollutants are environmental compounds that decrease production and thus cause stress. The most important changes associated with pollutant exposure are those that adversely affect growth and organism survival and thus the ability to contribute to the population gene pool (Capuzzo, 1988). As stress is an integral part of life history theory, life-history strategies could be applied to evaluate species as biological indicators and to interpret results in studies of pollution impacts. If sensitivity to a toxicant is the primary criterion for selecting a species for biotesting, ruderal species would be the best choice as they allocate the least resources to decrease the effect of toxicants. In turn, stress-tolerant species would be the worst choice as they allocate the most resources to decrease the effect of toxicants (Grime, 1979; Lawrence, 1995). This biological criterion has not been considered previously when selecting seaweeds as bioindicators.

Since *Lessonia nigrescens* does not occur in polluted areas this macroalga should be used as a bioindicator to detect natural accumulation only. In contrast, *Enteromorpha* is present in both contaminated and non-contaminated areas and could be used as an indicator of both anthropogenic pollutants and metals derived from natural accumulation. Both species have a wide distribution range, and most of the characteristics reported by Reisch (1988) for selecting suitable species for biotesting. Based on their life-history characteristics, the intertidal species *Lessonia nigrescens* is a ruderal species, and *Enteromorpha* is a stress-tolerant species. Hence, in intertidal areas (with a high level of disturbance) *Lessonia nigrescens* can be used as a biological indicator of heavy metals. In polluted areas, affected by anthropogenic impacts, *Enteromorpha* can be used as a biological indicator of pollution. Consequently, we suggest the use of stress tolerant seaweed species as biological indicators of polluted environments and ruderal species for non-contaminated ones. This links ecology and physiology to ecotoxicology through the examination of life-history strategies.

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