

The Kelp *Laminaria hyperborea* as a Bioindicator

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Abstract

During the period of 2015-2017, concentrations of the toxic or essential elements Cd, Hg, Pb, Al, Cr, Fe, Ni, Cu, Zn and As were analysed in the species kelp *Laminaria hyperborea* in an upwelling and nutrient-rich area at Mausund in Frøya municipality in Trøndelag, Norway. Samples were extracted by HNO₃ and analysed using inductively coupled plasma mass spectrometry (ICP-MS). In this study, the mean levels in *L. hyperborea* were significantly different between all years for the elements Hg, Pb, Cr, Fe, Ni and Zn, and the study indicated a temporal increase in the concentrations of Hg, Pb, Fe and Zn during the three-year period. The study showed high levels of Cd and Hg, according to levels set by the Regulation of fertilisers, etc. of organic origin [1]. According to European feed legislation regarding the maximum permitted levels in feed ingredients and complete feed stuff, there were elevated levels for Cd in 2016, for Hg in 2017 and for As throughout the three-year period. The European food legislation also sets maximum permitted levels for Cd, Pb and Hg in food supplements. The Hg levels in 2017 also exceeded these levels for supplements. The lamina of the species *L. hyperborea* can be used as a bioindicator for short-term exposure, and the results from this study can be considered to be reference levels.

Keywords

Bioindicator; Pollution; Bioaccumulation; Macroalgae; Toxic elements; Monitoring

Introduction

The Government of Norway and The Foundation for Industrial and Technical Research at the Norwegian University of Technology (SINTEF), believe that the seafood industry in Trøndelag in Norway will be six-fold by 2050. SINTEF is one of Europe's largest independent research organisations. The background for this claim is the value creation based on a productive sea in 2050 [2]. Environmental monitoring of seafood will remain important in the coming years with increasing production. Traditionally, the levels of essential or toxic elements in seafood are monitored by taking samples from the meat in fish and crab. A supplement for environmental monitoring of marine resources is to analyse the same elements using samples taken from macroalgae. By analysing toxic or essential elements in macroalgae and comparing the concentrations between years, macroalgae can be used as a bioindicator. A bioindicator is an organism that shows the results of environmental impact. The use of a bioindicator can provide information on a wide range of items, including both organic and inorganic pollutants. Pollutants can be toxic elements, such as trace metals, or essential elements that individuals need, but which give negative consequences if excessive concentrations of them occur. The elements selected for analysis in this study are cadmium (Cd), mercury (Hg), lead (Pb), aluminium (Al), chrome (Cr), iron (Fe), nickel (Ni), copper (Cu), zinc (Zn) and arsenic (As). They are inorganic trace elements, which are either essential or toxic. The selection of these elements was based on elements specified in the Norwegian regulation "Regulation of fertilisers, etc. of organic origin".

The purpose of this regulation (Regulation of fertilisers, etc. of organic origin) is to ensure satisfactory quality of products covered by the regulations, to prevent pollution, health and hygiene disadvantages in the manufacture, storage and use of fertilisers, etc. of organic origin and facilitate the use of these products as a resource. The regulations will also contribute to environmentally management of the soil and to take into account the importance of biodiversity [1]. The elements specified in this regulation are shown in Table 1.

The regulation states maximum limits for the elements Cd, Pb, Hg, Ni, Zn, Cu and Cr. Materials containing these elements are placed within quality classes 0, I, II, and III, where 0 is the best and III is the worst (Table 1).

Maximum permitted levels for the elements As, Cd, Pb and Hg in feed ingredients and complete feedstuff are set by the European feed legislation as follows (for 12% moisture content): As (total) is 40 mg kg⁻¹ in feed materials from seaweed and products thereof, 2

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Quality classes:	0	I	II	III
	mg/kg dry weight			
Cadmium (Cd)	0.4	0.8	2	5
Lead (Pb)	40	60	80	200
Mercury (Hg)	0.2	0.6	3	5
Nickel (Ni)	20	30	50	80
Zinc (Zn)	150	400	800	1500
Copper (Cu)	50	150	650	1000
Chrome (Cr)	50	60	100	150

Table 1: Maximum levels (mg kg⁻¹ dry weight) given for Cd, Pb, Hg, Ni, Zn, Cu and Cr in quality classes 0, I, II and III

mg kg⁻¹ in feed and 10 mg kg⁻¹ for fish feed; inorganic As is 2 mg kg⁻¹ in feed ingredients; Cd is 1 mg kg⁻¹ in feed and 1 mg kg⁻¹ in fish feed; Pb is 10 mg kg⁻¹ in feed and 5 mg kg⁻¹ in fish feed; and Hg is 0.2 mg kg⁻¹ in feed and 0.1 mg kg⁻¹ in fish feed. The European food legislation regulates food stuffs and food supplements, and the maximum levels for Cd, Pb and Hg in macroalgae are set at 3.0, 3.0 and 0.1 mg kg⁻¹ (wet weight), respectively [3].

Lately, there has been increasing interest in the cultivation and harvest of wild stocks of macroalgae in Norway. High levels of inorganic arsenic and cadmium have been reported in brown algae, and this content may limit the use of brown algae as food and feed ingredients, but there are no regulations for As in macroalgae used as a food supplement [3]. *L. hyperborea* is an important species used in the alginate industry [4]. Alginate is mainly used for pharmaceuticals, food and industrial products. Macroalgae grow along the coast of Norway, and the kelp forests include other kelp species, epiphytes and animals at different trophic levels, which use the kelp forest as their nursing and feeding habitat [4]. E.g. sea urchin feeds on *L. hyperborea*. When edible crab, birds and otter eats sea urchin, toxic elements from *L. hyperborea* will accumulate in the food chain.

Algae are divided into brown, red and green algae. The macroalgae *L. hyperborea* is a brown algae that grows below low tide and can grow up to 20 years old. The macroalgae consist of stipe, lamina and hapter (holdfast). The old lamina falls off in winter. Growth occurs in the lower part of the lamina, called the meristem, and the growth zone pushes the lamina upwards. The large algae filter water and nutrients throughout their lamina, stipe and hapter [5]. There are regional differences in the large algal population structure (size and age), which is due to regional differences in the ecological consequences of macroalgae harvesting by trawling. In mid-Norway, there are optimal temperature and light conditions, and *L. hyperborea* has most rapid stipe growth in this area [4]. When temperature and light are low in the winter season, but nutrient levels are high, *L. hyperborea* grow. The carbohydrates that are used for growth in winter are built up over the long days in summer [6].

Seaweeds accumulate dissolved trace metals from seawater via several binding sites to metallic elements in the polysaccharides in the cell walls [7]. In Trinidad Island, Brazil, background levels of the elements As, Cd, Cu, Pb, Zn and Hg were measured in brown and red algae, and one finding showed significant differences in Cu, Pb and Zn levels in seaweed from north and south of Trinidad Island, partially because of local hydrodynamics. Higher levels of As and Cd in brown algae, compared to red algae, confirm that brown algae accumulate higher concentrations of toxic trace elements [8]. Scheiner et al. [9] reported concentrations of the elements Al, Ti, Cr, Mn, Fe, Ni, Cu, Zn, As, Rb, Sr, Mo, Ba, Pb and I in four seaweed species (*L. digitata*, *L. hyperborea*, *S. latissima* and *A. esculenta*) from the Isle of Seil, Scotland. The concentrations were ranged in the order Sr>Fe>As>Al>Zn>Ti. Correlation analysis from *L. hyperborea* showed a strong correlation between the ash content and As level ($r=0.7$, $p<0.5$). There were no significant variations in the metals Na, K, Ca and Mg in *L. hyperborea* between seasons. Based on their results, concentrations of Al, Cr, Mn, Fe and Ni in *L. hyperborea* were high in September 2011.

Concentrations of the elements As, Cd, Cr, Pb, Mn, Hg and Se in the large alga *Alaria nana*, collected in Adak Island, Adak Harbor and

Clam Cove in Alaska, showed that the variations (14%-43%) were explained by the total length, part of the plant and location, except for lead. The different parts of the plant that contributed to variation were length for As and Se, location for Hg and part of the plant for As, Cd, Cr and Mn. To use large alga as a bioindicator, these data indicate that selection of the studied part of large alga must be consistent. For long-term exposure for As, Cd, Cr and Mn, it is recommended to use the hapter, and for short-term exposure, the lamina can be used to analyse all metals [10]. Chen et al. [11] recommend continual surveillance of metals in seaweed because of the toxicity of the elements Cd, Hg and Pb. They arranged the elements from red seaweed (*Porphyria*) and brown seaweed (*Laminaria* and *Undaria*) collected in Zhejiang, China, in the following order of concentration: Al>Mn>As>Cu>Cr>Ni>Cd>Se>Pb>Hg. Levels of the elements Cu, Ni, Mn and Cd were higher in red seaweed than in brown seaweed, and there was a moderate positive correlation between Ni and Cr ($r=0.59$, $p<0.05$).

In view of the fact that the lamina in *L. hyperborea* is new every year and reflects the uptake of elements throughout the whole organism, the results from analysis of a selection of segments items, where samples are taken from the meristem, will contribute to environmental monitoring.

The aim of this study is to investigate whether the macroalgae *L. hyperborea* can be used as a bioindicator for environmental monitoring of marine resources. The chosen analyses were used to examine whether there is (i) a difference in the concentration of the elements Cd, Hg, Pb, Al, Cr, Fe, Ni, Cu, Zn or As in the species kelp *L. hyperborea* in Mausund between the years 2015-2017 and (ii) a correlation between the elements Cd, Hg, Pb, Al, Cr, Fe, Ni, Cu, Zn or As in *L. hyperborea* in Mausund in the years 2015-2017.

Materials and Methods

During three consecutive years (2015-2017), teachers participating in a postgraduate course at the Norwegian University of Science (NTNU) conducted an annual research project (Kelp Forest Environmental Monitoring) designed to address the previously described aims. The samplings were conducted in the first half of September each year. The sampling region was in the inshore Mausund area, covering approximately 1 km² (Figure1). The kelp species *L. hyperborea* was collected by the students under supervision at one location (63°54'38" N, 8°36'43" E).

L. hyperborea pieces were brought to Mausund Field station prior to sampling from the meristem at lamina. Plant size and age for all species were not determined in this study. In total, 5-8 samples from the meristem, approximately one gram (g) for each sample, was taken from each individual. The samples were immediately frozen in 25 mL polystyrene cups. The samples were analysed using inductively coupled plasma mass spectrometry (ICP-MS) at the NTNU.

Nine *L. hyperborea* individuals, for a total of forty-eight samples, were collected at Mausund during 2012–2015. The concentrations of the ten elements Cd, Hg, Pb, Al, Cr, Fe, Ni, Cu, Zn and As are given in µg/g dry weight.



Figure 1: Mausund (63°N, 008°E), Frøya municipality in Sør-Trøndelag, Norway

Statistical analysis

Statistical tests were performed with IBM SPSS Statistics, Version 25. The set premises in SPSS assumed a normal distribution with skewness $-/+3$, kurtosis $-/+3$ and Pearson r $-/+1$. The data were normally distributed, except for Hg (skewness 3.105). Differences in element concentrations were tested between years for all 10 elements using hypothesis testing performed with independent samples t -tests with $p < 0.05$. Correlation analyses were conducted. To assess the relationship between variables, Pearson's r values were used, and a regression line was used to assess the relationship in a scatter chart. Principal component analysis (PCA) was performed.

Results

L. hyperborea were collected at one location (63°54'38" N, 8°36'43" E) in Mausund. The mean concentration, minimum and maximum concentration, median and standard deviation of the analysed elements, Cd, Hg, Pb, Al, Cr, Fe, Ni, Cu, Zn and As during 2012-2015, are presented in Table 2. The concentration in each sample is given in the Supporting Information (SI 1).

The mean concentration of Cd is within quality class I. The other elements are within quality class 0. The maximum concentrations of Cd and Hg are close to quality class II (Table 1).

There were significant differences ($p < 0.05$) (Table 3) between 2015 and 2016 for the elements Cd, Hg, Pb, Al, Cr, Ni, Zn and As and significant differences ($p < 0.05$) between 2016 and 2017 for the elements Cd, Hg, Pb, Al and As. Between 2015 and 2017, there were significant differences ($p < 0.05$) for the elements Hg, Pb, Cr, Fe, Ni and Zn. This pattern indicates a temporal increase in the concentrations of Hg, Pb, Fe and Zn during the three-year period.

There were moderate positive correlations between the following elements (Table 4): Cd and Fe ($r = 0.244$, $p < 0.05$), Hg and Ni ($r = 0.293$, $p < 0.05$), Hg and Al ($r = 0.307$, $p < 0.05$), Pb and Fe ($r = 0.483$, $p < 0.01$), Ni and Zn ($r = 0.404$, $p < 0.01$), Ni and Cu ($r = 0.429$, $p < 0.01$), Al and Fe ($r = 0.545$, $p < 0.01$), Pb and Al ($r = 0.656$, $p < 0.01$), and Hg and Pb ($r = 0.605$, $p < 0.01$).

There was a strong positive correlation between Cu and As ($r = 0.770$, $p < 0.01$) (Figure 2).

The PCA showed that Cu and As were clustered, and Fe, Al, Hg and Pb were relatively close together (Figure 3). This clustering may reflect a similar bioaccumulation mechanism in the macroalgal cell wall.

Discussion

Environmental monitoring is regularly conducted to investigate if seafood is exposed to contaminants, including toxic elements, and accumulates these contaminants to levels exceeding regulatory guidelines. Usually, the samples are taken from seafood, e.g., fish filets

Elements	N	Min.	Max.	Mean	S.D.	Median
Cd	48	0.096	1.885	0.822	0.487	0.722
Hg	48	0.001	2.800	0.302	0.597	0.080
Pb	48	0.017	0.102	0.041	0.018	0.037
Al	48	0.180	7.980	1.484	1.378	1.100
Cr	48	0.000	0.300	0.095	0.046	0.100
Fe	48	5.100	17.45	10.17	2.921	9.200
Ni	48	0.050	0.320	0.130	0.056	0.110
Cu	48	0.250	1.780	0.739	0.285	0.760
Zn	48	11.50	103.6	43.69	21.04	40.31
As	48	27.23	95.00	61.13	16.12	62.00

Table 2: The mean, median, minimum and maximum concentrations (N=48), and standard deviation from the years 2015-2017 in $\mu\text{g/g}$ dry weight for the elements Cd, Hg, Pb, Al, Cr, Fe, Ni, Cu, Zn and As in *L. hyperborea*

	Mean 2015	Mean 2016	Mean 2017
Cd	0.538	1.044	0.787
Hg	0.028	0.081	0.630
Pb	0.036	0.024	0.056
Al	1.467	0.911	1.969
Cr	0.112	0.102	0.080
Fe	9.303	9.640	11.07
Ni	0.174	0.131	0.111
Cu	0.687	0.803	0.708
Zn	24.19	46.64	49.36
As	57.59	70.93	54.45

Table 3: Mean concentrations from each year in 2015, 2016 and 2017 $\mu\text{g g}^{-1}$ dry weight for the elements Cd, Hg, Pb, Al, Cr, Fe, Ni, Cu, Zn and As in *L. hyperborea*

Elements	Correlation between the elements ($p < 0.05$)
Cd	Fe
Hg	Pb, Al, Cr, Ni
Pb	Hg, Al, Fe
Al	Hg, Pb, Cr, Fe
Cr	Hg, Al, Zn
Fe	Cd, Pb, Al, Zn
Ni	Hg, Cu, As
Cu	Ni, As
Zn	Cr, Fe, Ni,
As	Ni

Table 4: Correlation between the elements Cd, Hg, Pb, Al, Cr, Fe, Ni, Cu, Zn and As in *L. hyperborea* in Mausund in the years 2015-2017

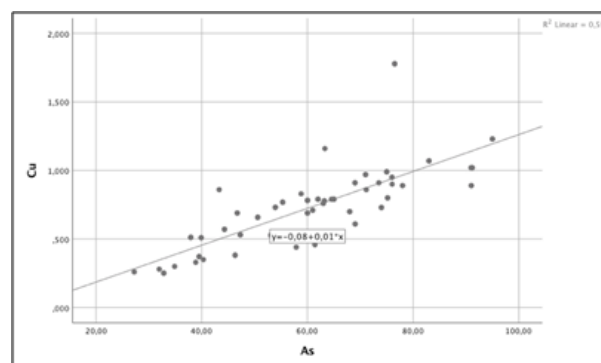


Figure 2: High positive correlation between As and Cu ($r = 0.770$, $p < 0.01$) in *L. hyperborea* during the years 2015-2017

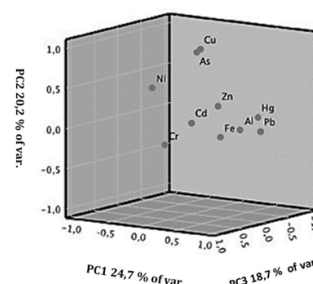


Figure 3: The elements Cd, Hg, Pb, Al, Cr, Fe, Ni, Cu, Zn and As in *L. hyperborea* in a PCA rotated plot

or meat from edible crab. A study from mid-Norway showed elevated levels of Cd in the brown meat from edible crab, (*Cancer pagurus*), and a temporal increase in the concentrations of the elements Se, Cs, Mn, Zn and As during a four-year period [12]. Macroalgae can be investigated in the same way to monitor the environment. As a bioindicator, macroalgae can be used to compare year-to-year variations but also to confirm the good quality of macroalgae used in pharmaceuticals, food and industrial products. There is concern about toxic elements exceeding regulatory guidelines in seafood and seaweeds, due to potential health risks. Chen et al. [11] suggest that because of the confirmed toxicity of some metals, such as Cd, Pb and Hg, surveillance of metals in seaweeds should be performed continually.

In the present study, the toxic or essential elements Cd, Hg, Pb, Al, Cr, Fe, Ni, Cu, Zn and As were analysed in the species kelp *L. hyperborea*. Using quality classes for the elements Cd, Pb, Hg, Ni, Zn, Cu and Cr (Table 1) specified in the Norwegian regulation 'Regulation of fertilisers, etc. of organic origin' [1], the results showed a significant difference between 2015 and 2016 and between 2016 and 2017 for the element Cd. The concentration increased in 2016 and decreased in 2017. The mean concentration of Cd was within quality class I, and the maximum concentration of Cd was close to quality class II.

There were significant differences between 2015 and 2016, 2016 and 2017, and 2015 and 2017 for the element Hg. The concentration increased in 2016 and again in 2017. The mean concentration of Hg was within quality class I, and the maximum concentration of Hg was close to quality class II.

For the element Pb, there was a significant difference between 2015 and 2016, 2016 and 2017, and 2015 and 2017. The concentration decreased in 2016 and increased in 2017. The mean concentration of Pb was within quality class 0.

There was a significant difference between 2015 and 2016 and between 2016 and 2017, but not between 2015 and 2017, for the element Al. The concentration decreased in 2016 but increased in 2017. For the elements Cr and Ni, there were significant differences between 2015 and 2016, 2016 and 2017, and 2015 and 2017. The concentrations increased in 2016 and decreased in 2017. The mean concentrations of Cr and Ni were within quality class 0.

There was a significant difference between 2015 and 2016 and between 2015 and 2017 for the element Zn. The concentration increased in 2016 and again in 2017. The mean concentration of Zn was within quality class 0.

For the element As, there were significant difference in the concentrations between 2015 and 2016 and between 2015 and 2017. The concentration increased in 2016 but decreased in 2017.

There was a significant difference between 2015 and 2017 for the element Fe. The concentration increased in 2016 and again in 2017.

According to European feed legislation regarding the maximum permitted levels in feed ingredients and complete feed stuffs, there were elevated levels for Cd in 2016, for Hg in 2017 and for As throughout the three-year period.

The levels for Hg in 2017 also exceeded maximum permitted levels for food supplements given by the European food legislation.

Conclusion

Comparing the concentrations of toxic or essential elements in the kelp species *L. hyperborea*, collected in an upwelling and nutrient-rich area in Mausund, showed that there were between-year (2015-2017) variations in the concentrations of some toxic or essential elements. The mean ranked concentrations in *L. hyperborea* were significantly different between all years for the elements Hg, Pb, Cr, Fe, Ni and Zn, and there was a temporal increase in the concentrations of Hg, Pb, Fe and Zn during the three-year period. The study showed elevated level for Cd and Hg, according to the 'Regulation of fertilisers, etc. of organic origin'. According to European feed legislation regulating the maximum permitted levels in feed ingredients and complete feed stuff, there were elevated levels for Cd in 2016, Hg in 2017 and for

As throughout the three-year period. The European food legislation also states maximum permitted levels for Cd, Pb and Hg in food supplements. The levels of Hg in 2017 exceeded these levels. There was a temporal increase in the concentrations of Hb, Pb, Fe and Zn in 2015-2017, and there were correlations between Cu and As but also Hg, Al, Pb and Fe.

A follow-up study should include samples from the species *L. hyperborea* at times other than early autumn. Different levels of elements may occur in different periods, especially in the spring when the light is strong and there is great photosynthesis activity. The same analyses should also be conducted in seawater and sediments.

The lamina of the species *L. hyperborea* can be used as a bioindicator for short-term exposure, and the results from this study can be considered reference levels. Marine biological resources are becoming increasingly important to meet the food demands of the increasing human population, but there have to be revised regulations to determine safe levels of toxic, e.g., As, and essential elements in the environmental monitoring of seafood.

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