

STRIVE

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Macroalgal Biomonitoring - Applying Phenolic Compounds as Biomarkers for Metal Uptake Characteristics in Irish Coastal Environments

STRIVE

Environmental Protection
Agency Programme

2007-2013

Environmental Protection Agency

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EPA STRIVE Programme 2007–2013

Macroalgal Biomonitoring – Applying Phenolic Compounds as Biomarkers for Metal Uptake Characteristics in Irish Coastal Environments

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STRIVE Report

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The EPA STRIVE Programme addresses the need for research in Ireland to inform policymakers and other stakeholders on a range of questions in relation to environmental protection. These reports are intended as contributions to the necessary debate on the protection of the environment.

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Executive Summary

Metals occur naturally in sea water at low concentrations and some act as essential micronutrients for marine biota, but they may become toxic when absorbed or ingested by plants and animals at high concentrations. Despite some recent research on metal contents in seaweeds from Ireland, few data exist for Irish coastal and transitional waters. The 2003 *National Environmental Monitoring Programme for Transitional, Coastal and Marine Waters. A Discussion Document* by the Environmental Protection Agency of Ireland proposes the inclusion of seaweeds in an extended assessment of future monitoring programmes for metal concentrations in Ireland and recommends the monitoring of hazardous substances and contaminants, including metals, by using biomonitoring organisms such as shellfish and seaweeds. Currently no standardised monitoring protocol exists in Ireland, although some limited biomonitoring of metals using shellfish has been conducted. In this 3-year project, seasonal, spatial and inter-site variations in phenolic and metal (copper, zinc, cadmium, chromium) contents and in phenolic composition in the ecologically and economically most important intertidal seaweeds in Ireland, *Ascophyllum nodosum* and *Fucus vesiculosus*, were observed,

demonstrating their suitability as biomonitors of metal contamination. The experimental approach taken in this project established a quantitative link between physiological responses in intertidal brown seaweeds and phenolic production, composition, exudation and the potential of phenolics to bind metals under natural environmental conditions. The effect of copper enrichment and its interaction with salinity, and the effect of iron and zinc contamination, were closely investigated. However, neither the intracellular phenolic content of the seaweed nor phenolic exudation could be used as biomarkers of metal contamination, whereas the cell-wall phenolic content of brown seaweeds seems promising but needs further investigation. This project has produced data on the contaminant status of selected Irish coastal and transitional waters, including sites of active seaweed harvesting by the Irish seaweed industry. Finally, some recommendations are proposed for the sampling methodology when using seaweeds as biomonitors of metal contamination, and some general comments are made on the utilisation of a series of biomonitors (seaweed, animal filter-feeders such as mussels), as well as sediment, to characterise the metal contamination of a site in more detail.

1 Introduction

1.1 Background

Metals occur naturally in sea water at low concentrations and some metals, such as copper, zinc and iron, act as essential micronutrients for marine biota, contrarily to cadmium and chromium (Lobban and Harrison, 1994). However, elevated concentrations that may occur in coastal water and sediments can pose a threat to marine life as metals may become toxic when absorbed or ingested by plants and animals. Few data exist on metal contents in Irish coastal and transitional waters (Marine Institute, 1999), but substantial metal contamination occurs in coastal waters near mines (e.g. Avoca, Arklow River: Morrison, 2004) and industrial activity (e.g. Shannon Estuary, Cork Harbour: Morrison, 2004; Morrison et al., 2008). European legislation, such as the Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR) of 1992 and the Water Framework Directive (2000/60/EC), has focussed on improving the quality of all waterbodies, including coastal and transitional waters, with the aim to achieve 'good quality' of European waters by 2015. This requires environmental assessment, monitoring and an improved, integrated management of waterbodies. The *National Environmental Monitoring Programme for Transitional, Coastal and Marine Waters. A Discussion Document* (EPA, 2003) proposes the inclusion of seaweeds in an extended assessment of metal concentrations in future monitoring programmes in Ireland and recommends the monitoring of hazardous substances and contaminants, including metals, by using biomonitoring organisms, such as shellfish and seaweeds. Currently, no standardised monitoring protocol exists in Ireland, although some limited biomonitoring of metals using shellfish has been conducted.

Metal analyses of biomonitors, instead of direct analyses of water samples, have significant advantages where economic considerations exclude the use of complex and expensive methods for water analysis, and/or when seasonal or spatial fluctuations in concentrations need to be integrated. Seaweeds are

particularly useful where there is an interest in the bioavailable (usually the soluble) fraction of metals because they can also accumulate in the food chain; however, this fraction is not measured when analysing filter- and deposit-feeding animals which only accumulate metals bound to particulates (Luoma et al., 1982). Moreover, seaweeds concentrate and integrate short-term temporal fluctuations in metal concentrations. Seaweeds are also able to release metal ions as well as to uptake them, especially if the metal concentrations in the water fluctuate; this is true for certain metals such as cadmium or zinc, but only to a limited extent for others such as lead (Lobban and Harrison, 1994; Baumann et al., 2009). Metal accumulation by seaweeds, e.g. *Fucus vesiculosus* and *Ascophyllum nodosum*, is also of ecological importance in the marine coastal system as these seaweeds are key primary producers and habitat providers.

Brown algae bind metals with high affinities. The cell composition, as well as the composition and structure of the cell wall, are important factors in determining the ability of a seaweed species to absorb metals. Their metal binding ability, and especially cation binding, is linked to polysaccharide (alginates and fucoidan) contents in the cell wall (Davis et al., 2003) and to polyphenol, also named phlorotannin, contents (Ragan et al., 1979; Hider et al., 2001) localised either in specialised vacuoles called physodes, free in the cytoplasm (Ragan et al., 1979; Ragan and Glombitza, 1986), or in the cell wall (Schoenwaelder, 2002; Koivikko et al., 2005; Salgado et al., 2009). Intracellular phenolic compounds can make up to 20% of algal (dry) biomass in temperate brown seaweeds (Ragan and Glombitza, 1986; Connan et al., 2004) and cell wall phenolic compounds less than 2% of algal (dry) biomass in *F. vesiculosus* (Koivikko et al., 2005). In situ, macroalgae are exposed to a range of environmental conditions, and phenolic contents have been previously reported to fluctuate according to environmental factors. Advances in the understanding of the environmental control of phenolic production are

therefore essential in optimising the application and reliability of algal biomonitoring. Some previous laboratory studies have shown that phenolic production in brown algae can be induced by changes in irradiance, temperature and salinity (Ragan and Jensen, 1978; Ragan and Glombitza, 1986). Being able to quantify reliably the environmental effects on the production of phenolics that bind divalent metals, and, therefore, contribute to seasonal and local variation in algal metal concentrations, would significantly improve the application of seaweeds as biomonitors.

This project for the first time quantified annual/seasonal variation in phenolic levels, composition and fluctuations of phenolic substances in two ecologically and economically important intertidal seaweeds in Ireland, *A. nodosum* and *F. vesiculosus*. The experimental approach taken in this project established a quantitative link between physiological responses in intertidal brown seaweeds and phenolic production, composition, exudation and the potential of phenolics to bind metals under natural environmental conditions.

Although some evidence suggests that seaweed metal binding is reversible (Rai et al., 1981; Baumann et al., 2009), the role of exudated metal-binding phenolics, which could be a major contributor to this apparent 'release of metals', has so far remained unexplored. Phenolic exudation occurs under natural conditions (Ragan and Jensen, 1979; Ragan and Craigie, 1980; Carlson and Mayer, 1983; Swanson and Druehl, 2002; Abdullah and Fredriksen, 2004; Shibata et al., 2006), and in coastal sea water exudated phenolics may be present in large quantities (2.5 mg/l; Sieburth, 1969) and thus represent a major source of organic matter. Their metal-binding properties can detoxify divalent metals in sea water and thus *enhance* phytoplankton productivity (Ragan et al., 1980), while, on the other hand, they may limit trace metal availability (Ragan et al., 1980) and reduce light penetration, and thus *reduce* productivity. These contradictory effects of phenolic exudation are also a major unknown in coastal phytoplankton ecology, and being able to quantify environmental impacts on exudation of seaweed phenolics will improve our understanding of

the effects of phytochelators and sporadically increased organic matter in coastal waters.

1.2 Objectives

This project addressed the problem of natural variation in metal accumulation by algal biomonitors due to environmental control of phenolic production, composition and exudation. The research approach included the monitoring of natural variation in situ, and experimental manipulation of phenolic content and metal uptake by exposing plants to different controlled environmental conditions, relevant to natural Irish coastal environments. The objectives of this project therefore are listed below.

- **Characterise natural phenolic and metal contents (copper, zinc, cadmium, chromium) in the ecologically and economically important intertidal seaweed species *A. nodosum* and *F. vesiculosus* on the Irish west coast, focussing on seasonal, spatial and within-plant variation.**
- **Quantify environmental influences on phenolic production, exudation and composition** under a range of ecologically relevant conditions and combinations thereof, focussing on salinity, temperature and desiccation time. Besides culture experiments in the laboratory, transplant experiments between sites with different environmental conditions were performed to study in situ the effect of environment on phenolic content and composition.
- **Assess metal uptake characteristics of actively growing tips of *A. nodosum* and *F. vesiculosus* (Phaeophyceae, Fucales) under a range of environmental conditions, such as salinity, in culture experiments in the laboratory.**
- **Quantify the contribution of different phenolic fractions to the metal-binding capacity of brown seaweeds.** Phenolics are observed in intracellular vacuoles (physodes), with some of these physodes linked to the cell wall, and some phenolics in the cell wall.
- **Develop a protocol to standardise brown algal biomonitoring** under environmental conditions prevailing in potentially contaminated sites

(harbours, estuaries), taking into account spatial and seasonal fluctuations in algal phenolic and metal contents.

Preliminary data are reported on the contaminant status of selected Irish coastal and transitional waters, including sites of active seaweed harvesting by the Irish seaweed industry, biodiversity hotspots and Special Areas of Conservation (SACs) or Special Protection Areas (SPAs). Based on improved scientific

understanding of the environmental control of metal binding by phenolics, recommendations have been made on the development of a standardised protocol for brown algal biomonitoring under realistic Irish coastal conditions, and on improved management of coastal zones with regard to metal contamination that potentially threatens Irish aquaculture, seaweed harvesting, biodiversity and public health.

2 Materials and Methods

2.1 Seaweed Species

Ascophyllum nodosum (Linnaeus) Le Jolis and *Fucus vesiculosus* Linnaeus are perennial temperate brown seaweeds belonging to the Fucales. They grow at mid-tide level on sheltered and moderately exposed shores on the west and east coasts of the North Atlantic Ocean. They are commonly found around Ireland in sheltered bays and estuaries. Both species exhibit apical growth with the maximum growth rates observed in spring and early summer (Stengel and Dring, 1997).

2.2 Biochemical Assays

The quantifications of phenolic and metal content in the seaweed tips were common to the field survey and culture experiments in the laboratory. The estimation of phenolic exudation was made only to water samples from culture experiments.

2.2.1 Phenolic content, composition and exudation

Seaweed tips were rinsed with Milli-Q water and stored in a freezer until processing. Depending on the experiments, either one or two pools of phenolic compounds were extracted: phenolics localised in the cytoplasm (intracellular phenolic, either free or contained within physodes) and those linked to the cell wall. A three-step extraction was then applied to each tip as described in Cerantola et al. (2006) and Koivikko et al. (2005). Phenolics were then spectrophotometrically assayed using the method described in Sanoner et al. (1999) and expressed in percentage of seaweed dry weight (DW). The seaweed fresh weight to dry weight ratio was determined by freeze-drying seaweed tips.

The intracellular phenolics from samples collected during the within-plant study in spring 2006 were further purified using solvents with different polarities allowing a distribution of phenolics in organic or aqueous solvents. ^1H Nuclear magnetic resonance

(^1H -NMR; NMR laboratory, University of Western Brittany, France) spectra of purified intracellular phenolics were performed to study the structural variations of phenolic compounds between the two sites and seaweed parts (Cerantola et al., 2006).

Phenolic exudation was estimated by measurement of the absorbance of the water at 274 nm (Wiencke et al., 2007).

2.2.2 Metal content

Metals were assayed either on a flame atomic absorption spectrometer (flame AAS; SpectrAA-600 Varian, USA; copper in the within-plant study) or on an inductively coupled plasma with optical emission spectrometer (ICP-OES, Varian 710, USA; other studies) in collaboration with the Estuarine Research Group, Waterford Institute of Technology, Co. Waterford. Copper, zinc, cadmium and chromium contents were measured in samples collected from the field and in culture experiments (and iron content for one culture experiment). The quality of the methodology was tested using a certified reference material (CRM NIES No. 9, National Institute for Environmental Studies, Japan) from the brown seaweed *Sargassum fulvellum*. The recovery of each metal was then calculated and for each metal assayed the recovery was higher than 95% except for iron (72%).

2.3 Physiological Measurements

The measurements were applied to seaweed tips during culture experiments to assess the effect of culture and different environmental parameters and/or metal contamination on seaweed tips. Growth and photosynthetic parameters (i.e. chlorophyll fluorescence measurement, maximum quantum yield which estimates the efficiency of the photosystem II) were measured at the start of the experiments and at each sampling occasion.

3 Seasonal and Spatial Variation in Metal Content, Intracellular Phenolic Content and Composition

In order to study in situ the metal contamination monitoring capacity of two brown seaweeds (*A. nodosum* and *F. vesiculosus*) and the link with their phenolic contents, eight sites were chosen on the Irish west coast presenting different environmental conditions. All these sites were chosen in accordance with previous studies that were already conducted in the laboratory on seaweeds and metal contamination (Morrison, 2004; Stengel et al., 2004, 2005; Morrison et al., 2008).

3.1 Site Descriptions and Sampling Methodology

Eight sites were chosen around Ireland (Fig. 3.1): one site in Connemara (Ballyconneely), three sites within Galway Bay (Oranmore, Kinvara and Finavara), three sites along the Shannon Estuary (Ringmoylan, Foynes, Beal Point–Carrig Island), and one site in Cork Harbour (Cobh). For a comparison, two sites with only

A. nodosum were chosen in the Bay of Brest, Brittany, France (Moulin Blanc and Le Dellec).

Tips of five plants of *A. nodosum* and *F. vesiculosus* were collected at each site at four sampling times corresponding to two seasons (summer and winter) over 2 years from summer 2006 until winter 2008 to monitor the seasonal variation in metal and phenolic content. Seaweed tips were processed as described in Section 2.2.

On each sampling date, water samples were collected at mid-tide in acid-rinsed plastic bottles and environmental water parameters (salinity, pH, temperature; YSI 556 MPS) were measured at the Irish sites. After filtration, water was analysed for nutrients using a Lachat nutrient analyser (Department of Earth and Ocean Sciences, National University of Ireland, Galway). Data for the French sites were provided by

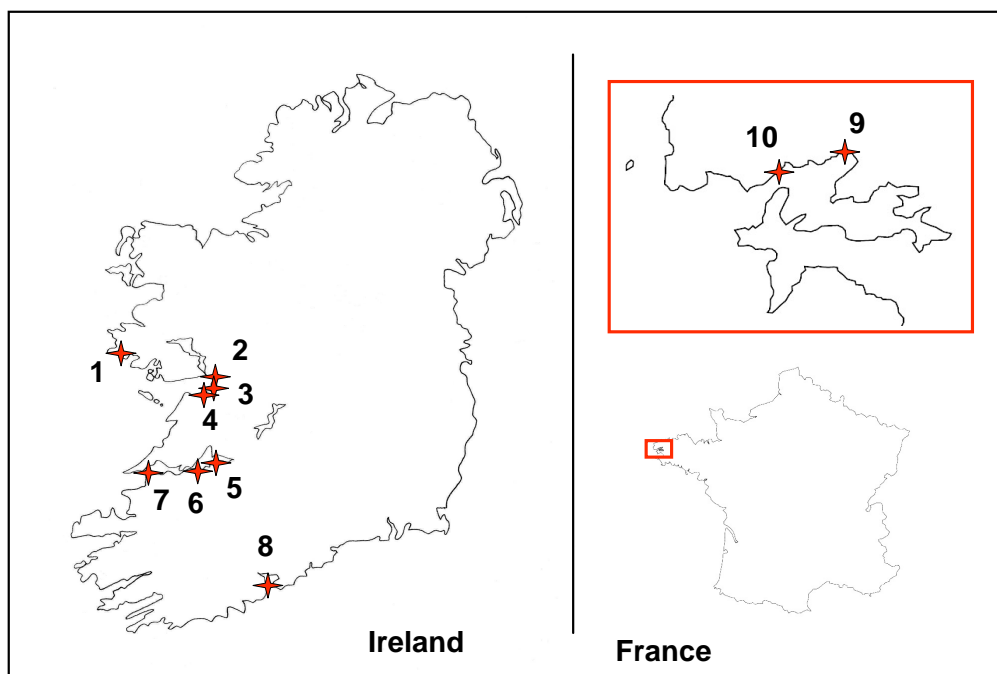


Figure 3.1. Location of the different sites studied in the inter-site survey. 1, Ballyconneely; 2, Oranmore; 3, Kinvara; 4, Finavara; 5, Ringmoylan; 6, Foynes; 7, Beal Point–Carrig Island; 8, Cobh; 9, Moulin Blanc; 10, Le Dellec.

Ifremer/Quadrige/REPOM hydrology for the Moulin Blanc and SOMLIT group (Service d'Observation en Milieu Littoral, INSU-CNRS, Portzic) for Le Dellec.

3.2 Environmental Parameters

The different sites presented very different environmental conditions and could be split into three groups according to their salinity and nutrient contents:

1. Sites with high salinity (>25 PSU) and a low nutrient content: Ballyconneely, Finavara, Beal Point, and Le Dellec. Carrig Island has also a high salinity, but its nutrient levels were a little higher.
2. Sites presenting with low salinity (<20 PSU) and a high nutrient content: Oranmore, Ringmoylan, Cobh, and Kinvara. At Kinvara, the groundwater river gave to this site its unique characteristic with a very high salinity variation over a tidal cycle.
3. Sites with lowest salinity, between 20 and 25 PSU, and intermediate nutrient levels: Foynes and Moulin Blanc.

Over the 2 years of sampling, nutrient concentrations were increasing in the winter concomitant with a decrease in pH levels due to a higher rainfall. Moreover, a decrease in nutrient concentrations was observed from the inner to the outer parts of Galway Bay, the Shannon Estuary and the Bay of Brest.

3.3 Intracellular Phenolic Content

Overall, high phenolic content was measured in both species with levels up to 17% of DW of seaweed tissues. Large variation was found between sampling sites, with higher intracellular phenolic contents in seaweeds from the outer part of the bays and estuary, especially for *A. nodosum*. These results indicated a clear relationship for this species between salinity and phenolic content, with an increase in phenolics at elevated salinity. Also, higher levels were observed in winter compared with summer. However, only phenolics of *A. nodosum* (and not *F. vesiculosus*) showed this seasonal variation.

3.4 Metal Content

Copper, zinc, cadmium and chromium were assayed in all samples collected on the shore. The four metals

were found in all seaweed samples with variations depending on seaweed species, site and season.

Copper contents were between 0.6 and 8.9 µg/g DW, with large variation between sampling sites and season. For both seaweed species, copper levels were higher in winter than in summer. In general, *F. vesiculosus* contained higher copper levels than *A. nodosum* when collected at the same site. Higher copper levels were found in the Shannon Estuary and Cork Harbour than in the other Irish sites. In the Bay of Brest, *A. nodosum* from Moulin Blanc contained higher copper level than plants from Le Dellec.

Zinc contents were much higher than copper contents and were between 5.0 and 66.2 µg/g DW. Again, seasonal and site-specific variations were observed with much higher zinc contents in winter in both species and in the inner part of Galway Bay (Oranmore and Kinvara for *A. nodosum* and just Oranmore for *F. vesiculosus*) and in Cork Harbour. *Ascophyllum nodosum* collected at Moulin Blanc had a very high content of zinc compared with the Irish sites and Le Dellec. Contrary to copper, except for a few sampling dates, there was no difference in zinc content between the two seaweed species.

In contrast to the two previous metals, cadmium contents were lower and showed greater differences between the two species: cadmium levels (between 0.1 and 1.2 µg/g DW) in *A. nodosum* were two and five times lower than levels in *F. vesiculosus*. There was also a great variation between sampling sites, with *A. nodosum* from Kinvara containing the highest cadmium level; for *F. vesiculosus*, plants collected at the three sites within Galway Bay (Oranmore, Kinvara and Finavara) and also Beal Point showed higher cadmium levels than the other sites. As for copper and zinc, cadmium content varied seasonally, with higher levels in winter and lower in summer.

Chromium contents were between 0.1 and 2.6 µg/g DW and showed considerable variation between the two seaweed species, especially in winter 2008. Seasonal variation was also found, with higher levels in winter than in summer, with some exceptions such as *F. vesiculosus* collected at Oranmore.

4 Seasonal, Spatial and Within-Plant Variation in Metal Content, Intracellular Phenolic Content and Composition

To study in more detail the variation of metal and phenolic content and phenolic composition, for *A. nodosum*, different plant parts were analysed from two sites that presented very different environmental conditions.

4.1 Site Descriptions and Sampling Methodology

The two sites were located close to and in Galway Bay: Ballyconneely in Connemara and Kinvara in the inner part of the bay.

Entire plants of *A. nodosum* were collected at seven seasons on these two shores over 18 months from spring 2006. On each sampling occasion, plants were brought to the laboratory where they were measured, aged (i.e. by counting of the air bladders) and divided into three or four parts: base, median, apex and/or receptacles (when present).

Due to different environmental conditions, *A. nodosum* thalli presented very different shape, colour and toughness at both sites:

1. At Ballyconneely, *A. nodosum* was tough and healthy, olive green in winter and bright yellow in summer, with narrow round axes.
2. At Kinvara, *A. nodosum* was fragile, black in winter to dark brown in summer, with very flat and wide axes.

Receptacles were found only during the reproductive season which also differed between sites and occurred in late winter–spring at Ballyconneely and late summer–autumn at Kinvara.

4.2 Intracellular Phenolic Content and Composition

High intracellular phenolic contents were measured in the different parts of *A. nodosum* thalli, with levels up to 13.0% DW of seaweed tissues. No within-plant

variation was observed at each sampling date. However, the intracellular phenolic content was considerably higher in plants collected at Ballyconneely than in plants collected at Kinvara. In plants from both sites, phenolic contents exhibited a seasonal variation, with higher levels in autumn and lower levels in spring or summer, according to the site.

The distribution of phenolics between extraction solvents differed according to the sampling site. ¹H-NMR spectra of purified intracellular phenolics showed differences in the phenolic signals of the same thallus part between the two sites and between different parts of plants collected at the same site, corresponding to a difference in their chemical structure.

4.3 Metal Content

Copper contents in all samples were analysed using a flame AAS, whereas zinc, cadmium and chromium contents were assayed using an ICP-OES only for samples collected in summer 2007.

Copper contents were higher in plants collected in Kinvara than in Ballyconneely; however, the overall copper contents were low (0.18–2.64 µg/g DW). Within-plant variation of copper content was also observed. Copper contents also exhibited seasonal variation, with an increase in winter and spring, and a decrease in summer and autumn in plants at both sites. Receptacles contained the same copper levels as vegetative parts even though they were present only for few months.

In summer 2007, receptacles were only found in *A. nodosum* collected at Kinvara. Higher zinc, cadmium and chromium contents were measured in different thallus parts of *A. nodosum* from Kinvara, compared with Ballyconneely plants. Except for copper, metal levels varied considerably between plant parts in seaweeds from both sites.

5 Effect of Environmental Parameters on Phenolic Content, Composition and Exudation

To investigate the effect of the environment on phenolic content, composition and exudation, two transplant experiments were performed with *A. nodosum* at Ballyconneely and Kinvara, in addition to some culture experiments under controlled environmental conditions in the laboratory. These culture experiments allowed us to study the effect of salinity, temperature, and the combined effect of salinity, temperature and desiccation time on *A. nodosum* and *F. vesiculosus* tips.

5.1 Transplant Experiment

5.1.1 Design

Two 1-year transplant experiments starting at different seasons were carried out to investigate the effect of the transplant season on the behaviour of transplanted seaweeds.

The two sites chosen for these experiments were Ballyconneely and Kinvara, both sites located either close to or in Galway Bay and representing very different environmental conditions. Fifteen plants of *A. nodosum* were transplanted from one site to the other, where they were attached to naturally growing plants. At the same time, 15 additional plants were transplanted within the same site (control plants). Tips were sampled at the start of the experiments and again after 0.5 month, 1 month, 1.5 months, 3.5 months, 6 months and 1 year. Collected tips were brought back to the laboratory where they were rinsed with distilled water and frozen until processing for phenolic content.

5.1.2 Plants

At the start (and the end) of the first transplant experiment, *A. nodosum* plants exhibited very different colours at both sites, a bright yellow at Ballyconneely and a dark brown at Kinvara. At the end of the experiment, seaweeds from Kinvara transplanted to Ballyconneely were the same colour as the surrounding seaweeds, suggesting an effect of the environment (nutrients or light) on their pigment

content. On the other hand, seaweeds from Ballyconneely transplanted to Kinvara became red after 6 months and were dead or had disappeared after 1 year. Changes in pigmentation were confirmed by pigment analyses (personal observation).

5.1.3 Intracellular phenolic content and composition

Within-cell phenolic contents (up to 16.6% DW) varied depending on collection time at both sites (control) without showing almost any considerable difference between the two sites. No difference was observed between the control transplant plants (not transplanted) and the control plants (transplanted in their native site) at both sites. Plants originally from Kinvara and transplanted to Ballyconneely had phenolic contents similar to control plants and control transplants. On the other hand, plants originally from Ballyconneely and transplanted to Kinvara exhibited considerably lower phenolic contents after 1.5 months than control plants and control transplants.

No major change due to transplantation was observed in the phenolic composition of plants: for example, the proportion of phenolics extracted by 100% methanol varied during the experiment according to the time of sampling, but no effect of transplantation was observed.

5.2 Culture Experiments

Culture experiments were performed in the laboratory to quantify the potential environmental effects on phenolic exudation: salinity, temperature, and the combined effect of salinity, temperature and desiccation time were tested on *A. nodosum* and *F. vesiculosus*. The latter experiment also investigated the effect of combined parameters on phenolic content and photosynthesis.

Ascophyllum nodosum and *Fucus vesiculosus* tips were collected at Finavara (53°09'25" N; 09°06'58" W), Co. Clare.

5.2.1 Salinity experiment

Four salinities were tested (35, 25, 15 and 5 PSU) at 15°C over 42 days. Water samples were collected for phenolic exudation estimation. Fresh weights of the seaweed tips were measured at the start and the end of the experiment.

Throughout the experiment, the specific growth rate of *F. vesiculosus* was higher than that of *A. nodosum*, except at 5 PSU. The specific growth rate of both species varied according to the salinity, and decreased with the salinity. After 3 and 6 weeks at 5 PSU, *F. vesiculosus* tips became green and friable.

Phenolic exudation of *F. vesiculosus* was higher than that of *A. nodosum*. For *A. nodosum*, no considerable difference in exudation was observed between salinity treatments, whereas salinity had an effect on phenolic exudation of *F. vesiculosus*. However, after Day 28, no difference between salinity treatments was found.

5.2.2 Temperature experiment

Four temperatures were tested (10, 15, 20 and 25°C) under normal salinity over 42 days. Water samples for phenolic exudation estimation were collected. The fresh weight of seaweed tips was measured at the start and the end of the experiment.

Higher specific growth rates were observed for *F. vesiculosus* at 10 and 20°C than for *A. nodosum*. Temperature considerably affected specific growth rates of both seaweed species. At the end of the experiment, *F. vesiculosus* tips growing at 25°C were, again, friable and green.

As for the salinity experiment, phenolic exudation was higher for *F. vesiculosus* tips than for *A. nodosum*. Temperature affected considerably phenolic exudation of both seaweed species, with an increase in exudation at higher temperatures.

5.2.3 Combined temperature, salinity and desiccation experiment

This experiment was conducted to investigate the combined effect of temperature and desiccation time on tip weight and photosynthesis, and the combined effect of salinity, temperature and desiccation time was tested on phenolic content of *A. nodosum* and *F. vesiculosus* tips. Two salinities (normal and half), four temperatures (10, 15, 20 and 25°C) and three or two (depending on the parameter) desiccation times (0 and 6 and 18 h) were tested over 28 days.

No difference in the maximum quantum yield was observed between the different treatments at the start of the experiment. After the experiment, maximum quantum yield was similar after 6 h at 10°C to values at the start, and considerably decreased for the other treatments, with the lowest values for 20°C whatever the desiccation time. After rehydration for 1 h, this maximum quantum yield recovered for all treatments, but it recovered fully only for the 10°C, 18-h treatment.

The effective quantum yields also showed an effect of the treatment (except after 6 h at 10°C), with a decrease after the experiments after 18 h at 20°C and 10°C, and after 6 h at 20°C. Recovery occurred after all treatments except after 18 h at 20°C.

Within-cell phenolic contents of 0.36–8.34% DW of seaweed were measured, with a decrease over time irrespective of the condition. At 10°C, a considerable effect of experimental conditions was only measured at half salinity and 18 h of desiccation per day. On the other hand, at 20°C, both salinity treatments using 18 h-desiccated tips considerably affected intracellular phenolic contents.

Within the intracellular phenolic content, the distribution between the two fractions varied regarding the salinity: the proportion of phenolics extracted with 50% methanol increased with the decrease in salinity.

6 Impact of the Environment and Exposure to Metal on Metal Content, Phenolic Production, Exudation and Composition

Culture experiments were carried out in the laboratory to investigate the effect of copper enrichment on copper and other metal (zinc, cadmium and chromium) contents, on phenolic content, composition and exudation of *A. nodosum* tips, and the interaction of copper enrichment and salinity on *A. nodosum* and *F. vesiculosus* tips. To test the response of the *A. nodosum* tips to exposure to different metals, zinc and iron were also used in culture experiments.

6.1 Sampling

Ascophyllum nodosum and *Fucus vesiculosus* tips were collected at Finavara (53°09'25" N; 09°06'58" W), Co. Clare. Only results for *A. nodosum* are presented here; however, results for *F. vesiculosus* followed the same pattern.

6.2 Experimental Design

Tips were cultured at 15°C over 2 weeks. Copper, zinc and iron were added to the water when appropriate to obtain four metal enrichments: 0 (normal sea-water level; control), 0.1, 1 and 5 mg/l (N = 3).

At Day 0, 100 ml of water and four tips were disposed into 125-ml flasks, which were placed on an Mk V Orbital Shaker (LH Engineering, UK) at maximum speed over the 2 weeks of experiment. Light (100 $\mu\text{mol photons/m}^2/\text{s}$; 12 h light/12 h dark cycle) was provided by Fluotone cool light lamps (TLD15W/33, Philips, UK).

Plants were removed from flasks for analysis on Days 0, 1, 2, 7 and 15 after the start of the experiments. Each time, water was sampled to estimate phenolic exudation. After the photosynthetic measurements, tips were rinsed with Milli-Q water and stored in the freezer: one tip was used for the phenolic extraction and three tips were freeze-dried and used for the metal assessment. The culture water was changed on Day 7

for the last set of samples to limit the depletion of nutrients in the water to the seaweed tips.

6.3 Copper Enrichment at Different Salinities

6.3.1 Growth and photosynthesis

Growth rates of *A. nodosum* tips decreased considerably with the increase in copper concentration and the decrease in salinity. However, growth rates were higher at 25 PSU than at 35 PSU.

The increase in copper concentration caused discoloration of *A. nodosum* tips which exhibited some red patches after 1 week of experiment, with 0.1 and 1 mg/l copper and turned completely orange with 5 mg/l copper at all salinities.

A considerable effect of copper concentration and salinity was also observed on the maximum quantum yield (representing the efficiency of the photosynthesis): maximum quantum yield decreased together with the increase in copper concentration and the decrease in salinity. At 5 PSU or 5 mg/l copper, the maximum quantum yield reached 0.

This decrease in the maximum quantum yield was linked to the decrease in the minimal and the maximal fluorescence. These coincided with the decrease in the relative electron transport rate, the effective quantum yield, the photochemical quenching and the increase in the non-photochemical quenching; all these changes were considerably affected by the copper concentration (especially 5 mg/l) and the salinity (especially 5 PSU).

6.3.2 Total phenolic content, composition and exudation

In these experiments, intracellular and cell-wall phenolics were extracted and assayed. No seasonal variation was observed in total phenolic content at the

start of the experiments, with levels varying between 9.1 and 14.2% DW of seaweed.

Copper concentration and salinity both affected the total phenolic content, with a decrease in phenolic content at increased copper concentration and decreased salinity.

During the experiment, the phenolic composition also changed; cell-wall phenolics increased and intracellular phenolics decreased, due to an increase in copper concentration and decrease in salinity. The three fractions of phenolics were affected by the increase in copper concentration and/or the decrease in salinity.

At the start of the experiments, the water absorbance was affected by the copper concentration and the salinity. Initially exudation was observed at all conditions, even at normal salinity without copper. Phenolic levels exuded into the water increased during the experiment, with the exception of Day 15 at certain conditions due to the change of water at Day 7 (to limit the depletion of nutrients). Phenolic exudation was considerably affected by the copper concentration and salinity, with an increase in exudation at high copper levels and low salinity; the combined effect of both parameters caused higher phenolic exudation to occur earlier.

6.3.3 Metal content

A considerable accumulation of copper was observed as soon as after 1 day, and the accumulation rate was related to the copper concentration in the surrounding water. After copper was added to the water, the maximum copper content was reached after 2 days; after the addition of new water on Day 7, the copper content increased again. The salinity also considerably affected the copper content, with a decrease with a decrease in salinity.

A release of zinc was observed after 1 day in all treatments (also without copper) with the exception of 15 PSU and 1 mg/l copper. At 5 PSU, this content decreased considerably on Day 15 without copper and with 0.1 mg/l copper and on Day 7 when 1 mg/l copper was added. At 5 mg/l copper, the zinc content decreased considerably after 1 day irrespective of the salinity.

Cadmium contents of *A. nodosum* were very low, often close to the limit of detection (LOD), and some values were lower than the LOD. The effect of copper and salinity on cadmium content was similar as for zinc: cadmium contents considerably decreased together with an increase in copper enrichment and/or a decrease in salinity.

Contrary to the two previous metals (cadmium and zinc), copper enrichment had no effect on chromium content, whereas the salinity considerably affected these contents (especially on Day 1 followed by a recovery of these contents).

6.4 Metal Experiments

The effect of copper, zinc or iron enrichment was investigated on growth of *A. nodosum* tips, photosynthesis, phenolic exudation and metal content.

6.4.1 Growth and photosynthesis

The specific growth rate of *A. nodosum* tips was considerably affected by the type of metal enrichment and concentration. The increase in copper and zinc concentrations caused a considerable decrease in growth, with a greater effect caused by copper. On the other hand, a small increase in iron concentration enhanced the growth of the seaweed, whereas higher iron concentrations caused a decrease in the specific growth.

Maximum quantum yield in control plants (without the addition of metals) was stable over the 15 days of experiments. However, the maximum quantum yield was differentially affected by the type of metal and its concentration. When added at 5 mg/l, the strongest and fastest effect was observed for copper, followed by iron; zinc showed an effect only at the end of the experiment.

The increase in copper and iron enrichment had also an effect on the other photosynthetic parameters measured (effective quantum yield, relative electron transport rate, photochemical quenching and non-photochemical quenching). On the other hand, zinc enrichment caused no effect on the other photosynthetic parameters measured, even after 15 days.

6.4.2 Phenolic exudation

As in the previous experiment (salinity–copper enrichment experiment), phenolic exudation was observed in all treatments, even without metal enrichment, and exudation increased over time. At low metal concentrations up to 1 mg/l, seaweed tips exposed to copper and zinc enrichment exhibited higher phenolic exudation than those exposed to iron. At 5 mg/l, zinc enrichment caused the smallest effect on phenolic exudation.

6.4.3 Metal content

The copper content of the seaweed tips increased with copper concentration in the water, and an increase in copper content was measured even after 1 day. After this, the copper content remained stable until the change of the surrounding sea water (adding new available copper). On the other hand, copper enrichment caused a considerable decrease in

cadmium and zinc content. No effect of copper was observed on chromium content.

As for copper, the zinc content of the seaweed tips followed the zinc concentration of the water even after 1 day with, however, an increase in zinc content under high zinc concentrations over the experiment. In contrast to the copper experiment, no effect of zinc enrichment was observed on copper, cadmium or chromium contents.

As for the copper and zinc experiments, the iron content of the seaweed tips depended on the iron concentration in the water, but with a much greater difference between the two highest iron concentrations compared with the experiments with copper and zinc. Also, the maximum iron content never exhibited saturation over the 2 weeks of the experiment. The copper and chromium contents of the seaweeds were not affected by iron enrichment, whereas the cadmium and zinc contents decreased considerably.

7 Summary of Results

In the field monitoring study, high phenolic contents up to 17% DW of seaweed tissue were measured in *A. nodosum* and *F. vesiculosus* but clear differences were observed between sampling sites. Considerable positive effects of salinity on phenolic contents were observed for *A. nodosum* but not for *F. vesiculosus*. Seasonal variations of phenolic contents of the two brown seaweeds were observed, with the highest levels occurring in winter and the lowest at the end of spring. Generally, *F. vesiculosus* contained higher copper and cadmium levels than *A. nodosum*, whereas the zinc and chromium contents of both species were similar.

Metal contents exhibited considerable variation between the different sites, suggesting the different contamination levels at these sites. However, these sites also represented different environmental conditions, which were likely to affect the metal bioavailability in the water, as well as the ecophysiological stage of the seaweed. At Ballyconneely, considered an almost pristine site with only few residential homes, *A. nodosum* and *F. vesiculosus* (with the exception of copper for *F. vesiculosus*) contained the lowest levels of copper, zinc, cadmium and chromium. The metal content of *A. nodosum* from Ballyconneely represented the baseline metal content of *A. nodosum*. In the inner Galway Bay, copper and chromium contents were still low, but an increase in zinc and cadmium was observed for both seaweed species. In the Shannon Estuary, elevated levels of copper content were measured, whereas the zinc and cadmium levels were lower than in Galway Bay. No difference in the cadmium and chromium contents was observed within the estuary; increases in the cadmium contents of *F. vesiculosus* from the inner to the outer part of the estuary were observed during both winter collections. Cork Harbour is considered a contaminated site, and copper and zinc contents there were the highest of all sites, whereas cadmium and chromium were either the lowest (cadmium) or similar to those at the other sites (chromium). Copper and zinc levels in *A. nodosum*

collected from the two sites in the Bay of Brest, France, showed large variability, whereas cadmium and chromium levels were similar.

Even though differences were observed in phenolic content between Ballyconneely and Kinvara and between sampling seasons, no within-plant variation in phenolic content of *A. nodosum* was shown at either site. However, to our knowledge, this study is the first showing evidence for within-plant and inter-site variation in phenolic composition or chemical structure based on $^1\text{H-NMR}$ spectra and this requires further investigation. Overall, higher copper levels were measured in plants from Kinvara and copper content exhibited seasonal variation, with an increase in winter and spring and a decrease in autumn and winter in all thallus parts. Copper, zinc, cadmium and chromium contents exhibited within-plant variation with higher levels near the base of the plant.

Transplantation from Ballyconneely to Kinvara affected *A. nodosum* plants considerably: their pigment content increased as indicated by a change in colour, and, after a decrease in phenolic content, plants died. On the other hand, transplants from Kinvara to Ballyconneely survived until the end of the experiment and their pigmentation changed to adopt the colour of the Ballyconneely plants (yellow in summer), without any changes in their phenolic content.

In the present study, culture experiments were conducted to quantify such environmental effects on metal and phenolic levels. Under high nutrient concentrations, salinity affected growth of *A. nodosum* and *F. vesiculosus* tips. Also, phenolic exudation from *F. vesiculosus* increased at lower salinities. At 5 PSU, *F. vesiculosus* tips started to die after 3 weeks. In the second set of experiments, growth rates were strongly influenced by temperature in these two seaweed species. As for salinity, *F. vesiculosus* exhibited a lower adaptive ability to high temperature and plants turned green and disintegrated at 25°C after 6 weeks. A positive effect of temperature on phenolic exudation

was also observed for both species in the present study.

The final series of experiments suggested a considerable effect of temperature, and the temperature \times desiccation time interaction, on the photosynthesis efficiency (maximum and effective quantum yields), with a decrease together with the increase in temperature and desiccation time. However, after 6 h and even 18 h of desiccation at 10°C, seaweed tips were still able to photosynthesise, and a complete recovery at this temperature was observed after 1 h of rehydration.

In culture experiments, a decrease in salinity considerably affected growth, photosynthesis, total phenolic contents, phenolic composition, phenolic exudation, and uptake and accumulation of copper by *A. nodosum* tips. It is likely that the phenolic contents and the effect on copper uptake were reduced due to lower metabolic activity at decreased salinity. An enhanced or synergistic response to copper was observed in this study under conditions of osmotic stress, a response supported by the considerable interaction between salinity and copper toxicity. Clearly, a decrease in salinity affected the physiology of the seaweed and its biochemical composition, influencing availability of both intra- and extracellular metal-binding sites. Increased copper concentration considerably affected growth, photosynthesis, pigment contents, total phenolic contents, and phenolic composition; it also influenced the accumulation of copper (reflecting the external copper concentration), and internal zinc and cadmium contents, whereas chromium accumulation by *A. nodosum* tips seemed to be unaffected by the increased copper concentration.

The copper content of *A. nodosum* tips corresponded to the copper concentration of the surrounding water; the maximum copper content was achieved after 1 day of experiment and increased only when the water was changed (adding new available copper), suggesting that some copper-binding sites were still available even after the maximum of copper content was reached within the first 2 days of the experiment.

When comparing copper effects on *A. nodosum* tips with those of zinc and iron, copper was the most toxic metal. At the highest concentration, growth was greatly reduced, and also a faster and stronger inhibition of photosynthesis and a faster and higher exudation of phenolics occurred, as well as a decrease in cadmium and zinc seaweed contents. Zinc was less toxic and, at the highest concentration, had a weaker effect on growth, and photosynthesis was only affected on the last day of experiment. High concentrations of zinc also resulted in lower exudation of phenolics; a slower accumulation of zinc over the experiments was also observed, which had no effect on the content of copper, cadmium and chromium. However, the zinc content of the seaweed reflected the zinc concentration in the surrounding water. Finally, the addition of a small amount of iron to the water initially enhanced the growth of *A. nodosum* tips, whereas addition of higher concentrations of iron eventually reduced and even inhibited growth. At the highest concentration, iron decreased the photosynthesis after 2 days, increased the phenolic exudation and the iron content of the tips, and decreased the cadmium and zinc contents whereas no change in the accumulation of chromium or copper was observed.

8 Conclusions and Recommendations

8.1 Conclusions

In this 3-year project, seasonal, spatial and inter-site variations were observed in phenolic and metal (copper, zinc, cadmium, chromium) contents and in phenolic composition of two intertidal brown seaweeds, *A. nodosum* and *F. vesiculosus*, indicating their suitability as biomonitors of metal contamination. The effects of environmental parameters on the physiology of the seaweeds, on phenolic contents and composition and on phenolic exudation were assessed. Finally, the effect of copper enrichment and interactions with salinity and iron and zinc contamination were investigated.

Ideal biomonitors for metal contamination in sea water should be sedentary, easy to identify, abundant, long lived, available for sampling throughout the year, large enough to provide sufficient material for analysis, resistant to handling stress caused by laboratory studies of metal kinetics and/or field transplantation, and tolerant of exposure to environmental variations in physico-chemical parameters such as salinity. Moreover, they should be net accumulators of the metal in question, with a simple correlation between metal concentration in tissue and average ambient bioavailable metal concentration over a recent time period (Rainbow, 1995). Brown seaweeds could comply with almost all of these requirements, except that their physiology is influenced by changes in environmental parameters. However, the physiology of all other biomonitors (including animals) is also affected by the environmental parameters.

The justification for using seaweeds as biomonitors or bioindicators of metal contamination has three main bases (Luoma et al., 1982):

1. Metal concentrations in solution are often near the limits of analytical detection and may vary from time to time, and seaweeds concentrate metals from solution and integrate short-term temporal fluctuations in concentration.
2. Methods to distinguish the biologically available

fraction of the total concentration of a dissolved metal have not been developed for natural systems; seaweeds will accumulate only those metals that are biologically available.

3. Seaweeds do not ingest particulate-bound metals (as animals do); they will accumulate metals only from solution. Animals, such as mussels, uptake metals not only from solution and food but also from the ingestion of inorganic particulate material (Rainbow and Phillips, 1993; Struck et al., 1997).

Moreover, seaweeds are able to release metal ions as well as uptake them, especially if the metal concentrations in the water fluctuate; this is true for certain metals such as cadmium or zinc, but only to a limited extent for others such as lead (Lobban and Harrison, 1994).

Because they are photosynthetic organisms, the physiology of seaweeds, and thus the metal accumulation, is influenced by environmental variations. Metal levels in seaweeds may thus vary with season, temperature and salinity (Burdon-Jones et al., 1982), age of the frond and position in the intertidal zone (Bryan and Hummerstone, 1973; Barreiro et al., 1993), interactions with other metals (Foster, 1976) or growth rate (Rice and Lapointe, 1981). Any factor that tends to alter the growth rates of seaweeds will thus interfere with the accuracy of inter-site comparison and the results of a transplantation experiment. Environmental parameters, such as salinity or pH or nutrient enrichment, may also exert physico-chemical influences on metals in the water, rendering them less available for uptake (Seeliger and Edwards, 1977).

In brown seaweeds, the cell composition, as well as the composition and structure of the cell wall, is an important factor in determining the ability of a seaweed species to absorb metals. Their metal-binding ability, and especially cation binding, is mainly linked to the content of two types of compounds: polysaccharides (alginate and fucoidan) in the cell wall (Davis et al., 2003) and polyphenols located either in specialised

vacuoles, the physodes, in the cytoplasm (Ragan et al., 1979; Ragan and Glombitza, 1986) or in the cell wall (Schoenwaelder, 2002; Koivikko et al., 2005; Salgado et al., 2009). This study tested the ability of using polyphenols of two intertidal brown seaweeds, *A. nodosum* and *F. vesiculosus*, to act as biomarkers of metal contamination. Recently, another biomarker of metal contamination, the phytochelatins (peptides involved in the detoxification of metals in cells from higher plants), was identified in brown Fucales from contaminated sites (Pawlik-Skowronska et al., 2007).

In this study, the intracellular phenolic content was shown not to be a good biomarker for metal contamination probably due to the exudation of these phenolics coinciding with an increase in metal contamination. The binding capacity of purified intracellular phenolics was demonstrated: they contained copper, zinc, cadmium and chromium; in the presence of 1 and 5 mg/l copper, the cadmium and zinc contents of phenolics decreased, and no change in the chromium content was observed, similar to the experience with the seaweeds. By contrast, the increased phenolic exudation together with the metal content could be a good biomonitor of metal contamination; however, this exudation is also increased by other stresses including a decrease in salinity. Moreover, even if high exudation rates were measured (e.g. over 1 mg/kg fresh weight seaweed per day; Ragan et al., 1980), the dilution of exuded phenolics in the surrounding sea water makes phenolic exudates difficult to assay. Finally, the cell-wall phenolic contents also increased due to copper enrichment, but not to salinity. A detailed assessment of cell-wall phenolics in seaweeds from clean and contaminated sites will ascertain their involvement in metal detoxification of brown seaweeds.

8.2 Recommendations

Ascophyllum nodosum and *Fucus vesiculosus* are good candidates for acting as biomonitors of copper, cadmium, chromium and zinc contaminations because they are readily available on Irish shores, and are able to concentrate these metals to reflect ambient concentrations; however, they are not good biomonitors for iron because of the lack of relationship between the iron–water concentration and iron–seaweed contents.

When applying seaweeds as bioindicators, the sampling design needs to consider the use of seaweed tissue of the same age (youngest tips for a status on the contamination stage of the site, tissues of different age from the same plants to follow the history of contamination of the site) to avoid artificial higher metal contamination (Melhuus et al., 1978). Seaweed samples should also be collected at two seasons (e.g. winter and summer, or autumn and spring) to remove the effect of dilution or concentration of metals due to the seasonal growth pattern of seaweeds.

For a comparison between sites, the sampling should be conducted at the same seasons and the same plant parts should be collected. Environmental parameters which influence both metal availability and seaweed physiology, such as salinity, nutrient enrichment and pH, should also be recorded on the sites. Moreover, some metals could originate from the rocks and the ground, such as nickel or chromium (Morrison et al., 2008); thus, the degree of accumulation depends not only on human activities but also on geology of the sites. To avoid that problem, the geology of the sampling sites should be taken into consideration. As seaweeds only accumulate bioavailable fractions of heavy metals, to obtain a full understanding of metal bioavailabilities and partitioning, their use should be complemented by the collection of animal filter-feeders and sediments. According to previous studies, contamination from adherent particles on seaweed surface (bacteria, microalgae, fine sediment) can account for a considerable part of some metal levels, such as chromium and iron, in seaweeds but does not normally affect copper, cadmium, or zinc. The measurement of metals in the first centimetre of the sediment can additionally allow a characterisation of contamination and help to quantify the bioavailable fraction of these metals such as chromium and iron (Gledhill et al., 1998; Giusti, 2001; Daka et al., 2003; Morrison et al., 2008).

This project has contributed to baseline data on the contamination stage of coastal sites around Ireland. Even though their physiology and thus their metal accumulation could be influenced by radical environmental changes, this research has demonstrated the suitability of *A. nodosum* and *F. vesiculosus* as biomonitors of metal contamination.

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Acronyms

¹H-NMR	¹ H Nuclear magnetic resonance
AAS	Atomic absorption spectrometer
CRM	Certified reference material
DW	Dry weight
ICP-OES	Inductively coupled plasma with optical emission spectrometer
LOD	Limit of detection
OSPAR	Convention for the Protection of the Marine Environment of the North-East Atlantic
PSU	Practical salinity unit
SAC	Special Area of Conservation
SOMLIT	Service d'Observation en Milieu LI ^T toral
SPA	Special Protection Area

An Gníomhaireacht um Chaomhnú Comhshaoil

Is í an Gníomhaireacht um Chaomhnú Comhshaoil (EPA) comhlachta reachtúil a chosnaíonn an comhshaoil do mhuintir na tíre go léir. Rialaímid agus déanaimid maoirsiú ar ghníomhaíochtaí a d'fhéadfadh truailliú a chruthú murach sin. Cinntímid go bhfuil eolas cruinn ann ar threochtaí comhshaoil ionas go nglactar aon chéim is gá. Is iad na príomh-nithe a bhfuilimid gníomhach leo ná comhshaoil na hÉireann a chosaint agus cinntiú go bhfuil forbairt inbhuanaithe.

Is comhlacht poiblí neamhspleách í an Gníomhaireacht um Chaomhnú Comhshaoil (EPA) a bunaíodh i mí Iúil 1993 faoin Acht fán nGníomhaireacht um Chaomhnú Comhshaoil 1992. Ó thaobh an Rialtais, is í an Roinn Comhshaoil agus Rialtais Áitiúil a dhéanann urraíocht uirthi.

ÁR bhFREAGRACHTAÍ

CEADÚNÚ

Bíonn ceadúnais á n-eisiúint againn i gcomhair na nithe seo a leanas chun a chinntiú nach mbíonn astuithe uathu ag cur sláinte an phobail ná an comhshaoil i mbaol:

- áiseanna dramhaíola (m.sh., líonadh talún, loisceoirí, stáisiúin aistrithe dramhaíola);
- gníomhaíochtaí tionsclaíocha ar scála mór (m.sh., déantúsaíocht cógaisíochta, déantúsaíocht stroighne, stáisiúin chumhachta);
- diantalmhaíocht;
- úsáid faoi shrian agus scaoileadh smachtaithe Orgánach Géinathraithe (GMO);
- mór-áiseanna stórais peitreal.
- Scardadh dramhúisce

FEIDHMIÚ COMHSHAOIL NÁISIÚNTA

- Stiúradh os cionn 2,000 iniúchadh agus cigireacht de áiseanna a fuair ceadúnas ón nGníomhaireacht gach bliain.
- Maoirsiú freagrachtaí cosanta comhshaoil údarás áitiúla thar sé earnáil - aer, fuaim, dramhaíl, dramhúisce agus caighdeán uisce.
- Obair le húdaráis áitiúla agus leis na Gardaí chun stop a chur le gníomhaíocht mhídhleathach dramhaíola trí chomhordú a dhéanamh ar líonra forfheidhmithe náisiúnta, díriú isteach ar chiontóirí, stiúradh fiosrúcháin agus maoirsiú leigheas na bhfadhbanna.
- An dlí a chur orthu siúd a bhriseann dlí comhshaoil agus a dhéanann dochar don chomhshaoil mar thoradh ar a gníomhaíochtaí.

MONATÓIREACHT, ANAILÍS AGUS TUAIRISCIÚ AR AN GCOMHSHAOIL

- Monatóireacht ar chaighdeán aer agus caighdeán aibhneacha, locha, uisce taoide agus uisce talaimh; leibhéil agus sruth aibhneacha a thomhas.
- Tuairisciú neamhspleách chun cabhrú le rialtais náisiúnta agus áitiúla cinntiú a dhéanamh.

RIALÚ ASTUITHE GÁIS CEAPTHA TEASA NA HÉIREANN

- Caimníochtú astuithe gáis ceaptha teasa na hÉireann i gcomhthéacs ár dtiomantas Kyoto.
- Cur i bhfeidhm na Treorach um Thrádáil Astuithe, a bhfuil baint aige le hos cionn 100 cuideachta atá ina mór-ghineadóirí dé-ocsaíd charbóin in Éirinn.

TAIGHDE AGUS FORBAIRT COMHSHAOIL

- Taighde ar shaincheisteanna comhshaoil a chomhordú (cosúil le caighdeán aer agus uisce, athrú aeráide, bithéagsúlacht, teicneolaíochtaí comhshaoil).

MEASÚNÚ STRAITÉISEACH COMHSHAOIL

- Ag déanamh measúnú ar thionchar phleananna agus chláracha ar chomhshaoil na hÉireann (cosúil le plannanna bainistíochta dramhaíola agus forbartha).

PLEANÁIL, OIDEACHAS AGUS TREOIR CHOMHSHAOIL

- Treoir a thabhairt don phobal agus do thionscal ar cheisteanna comhshaoil éagsúla (m.sh., iarratais ar cheadúnais, seachaint dramhaíola agus rialacháin chomhshaoil).
- Eolas níos fearr ar an gcomhshaoil a scaipeadh (trí cláracha teilifíse comhshaoil agus pacáistí acmhainne do bhunscoileanna agus do mheánscoileanna).

BAINISTÍOCHT DRAMHAÍOLA FHORGHNÍOMHACH

- Cur chun cinn seachaint agus laghdú dramhaíola trí chomhordú An Chláir Náisiúnta um Chosc Dramhaíola, lena n-áirítear cur i bhfeidhm na dTionscnamh Freagrachta Táirgeoirí.
- Cur i bhfeidhm Rialachán ar nós na treoracha maidir le Trealamh Leictreach agus Leictreonach Caite agus le Srianadh Substaintí Guaiseacha agus substaintí a dhéanann ídiú ar an gcrios ózóin.
- Plean Náisiúnta Bainistíochta um Dramhaíl Ghuaiseach a fhorbairt chun dramhaíl ghuaiseach a sheachaint agus a bhainistiú.

STRUCHTÚR NA GNÍOMHAIREACHTA

Bunaíodh an Gníomhaireacht i 1993 chun comhshaoil na hÉireann a chosaint. Tá an eagraíocht á bhainistiú ag Bord lánaímseartha, ar a bhfuil Príomhstíúrthóir agus ceithre Stíúrthóir.

Tá obair na Gníomhaireachta ar siúl trí ceithre Oifig:

- An Oifig Aeráide, Ceadúnaithe agus Úsáide Acmhainní
- An Oifig um Fhorfheidhmiúchán Comhshaoil
- An Oifig um Measúnacht Comhshaoil
- An Oifig Cumarsáide agus Seirbhísí Corparáide

Tá Coiste Comhairleach ag an nGníomhaireacht le cabhrú léi. Tá dáréag ball air agus tagann siad le chéile cúpla uair in aghaidh na bliana le plé a dhéanamh ar cheisteanna ar ábhar imní iad agus le comhairle a thabhairt don Bhord.

Science, Technology, Research and Innovation for the Environment (STRIVE) 2007-2013

The Science, Technology, Research and Innovation for the Environment (STRIVE) programme covers the period 2007 to 2013.

The programme comprises three key measures: Sustainable Development, Cleaner Production and Environmental Technologies, and A Healthy Environment; together with two supporting measures: EPA Environmental Research Centre (ERC) and Capacity & Capability Building. The seven principal thematic areas for the programme are Climate Change; Waste, Resource Management and Chemicals; Water Quality and the Aquatic Environment; Air Quality, Atmospheric Deposition and Noise; Impacts on Biodiversity; Soils and Land-use; and Socio-economic Considerations. In addition, other emerging issues will be addressed as the need arises.

The funding for the programme (approximately €100 million) comes from the Environmental Research Sub-Programme of the National Development Plan (NDP), the Inter-Departmental Committee for the Strategy for Science, Technology and Innovation (IDC-SSTI); and EPA core funding and co-funding by economic sectors.

The EPA has a statutory role to co-ordinate environmental research in Ireland and is organising and administering the STRIVE programme on behalf of the Department of the Environment, Heritage and Local Government.