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MARINE ECOTOXICOLOGICAL TESTS WITH SEAWEEDS

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ABSTRACT

Marine macroalgae (seaweeds) are best suited for field tests because of their size and requirement for large volumes of seawater. Their composition is dominated by acidic hydrocolloids and they are very lipophobic. Their role in ecotoxicological testing is therefore mostly limited to growth and accumulation studies in areas exposed to heavy metal pollution.

Seaweeds can function as useful indicators of biologically available toxic metal ions in seawater. The monitoring can be based on local populations of algae or on test specimens transferred from unpolluted sites. Uptake rates are used and have to be related to uptake at control sites. Growth rates and other physiological parameters are normally of little use in field studies since the gradients in toxicants are usually followed by changes in salinity, nutrient levels, light, and other environmental factors. To some extent these factors may also influence the uptake rate of metals by the algae.

KEYWORDS

Marine ecotoxicology, Hazard assessment, Bioassays, Methods, Macroalgae, Seaweeds, Review.

INTRODUCTION

Seaweed is a common designation for a large and variable group of macroscopic, mainly benthic marine algae. Taxonomically these primitive plants are grouped into three phyla, namely green algae (Chlorophytes), red algae (Rhodophytes), and brown algae (Phaeophytes). They often form dense underwater forests along the coast in tropical as well as temperate and cold waters, and are then responsible for a considerable part of the primary production in shallow regions. The seaweeds normally range from a few millimeter in lenght to several meters. The large kelps grow beyond 30 m and are among the largest plants in the world.

Chemically the seaweeds are dominated by carbohydrates, the so-called hydrocolloids, which frequently are acidic polysaccharides such as alginic acid, various fucoidans, agar, and carrageenan. These render the seaweeds very hydrophilic and give them ion-exchange properties. This, together with their large size and relatively slow growth, determine their role in ecotoxicological testing. They bind metal ions and may concentrate these against strong gradients. Generally, they do not accumulate lipid toxins, and in principle are not well suited for tests which are based on growth measurements, mainly because they require large-size tanks and huge quantities of seawater for their cultivation. A semimicro technique which circumvents some of these problems has, however, been developed.

DESCRIPTION OF THE TESTS

Although many seaweeds, both green, and red or brown are easily kept alive and grow well in aquaria under laboratory conditions, the maintainance of seaweed cultures must be regarded as fairly difficult and quite expensive. Long-term operation of seawater laboratories always leads to much corrosion and expensive maintainance.

Seaweed cultures are therefore seldom used for laboratory screening of toxic chemicals. Their major use in toxicology is as indicator organisms for heavy metal pollution of inshore waters. For this purpose wild populations may be studied in situ, or selected plants may be transplanted and their development followed. No standardization of the tests have been attempted yet.

LABORATORY TESTS FOR EFFECT OF CHEMICALS ON GROWTH OF SEAWEEDS

At the present time the author sees only one procedure which appears promising for the routine testing of chemicals on the basis of seaweed growth, and that is the system worked out by Strömgren (1977a, 1979a). The method works well with algae which have apical growth and a clearly defined shape. Many of the intertidal Fucaceae and a number of red algae form suitable test organisms. Young stages of the Laminariaceae may also be used.

Vegetative apices some 20 mm long are cut from the test plants and mounted on plexiglass frames as described by Strömgren (1977a). Each apice is clamped onto the plate with a second thin plate, and secured with a string (Fig. 1). The apice protrudes 3 to 4 mm into a circa 10 mm hole in the middle of the frame. Opposite to the sample an adjustable polyethylene diffraction edge is mounted. The algal samples with the plexiglass frames are placed in trays or aquaria with running seawater and illuminated continuously or in a day : night regime. The light conditions must be adjusted to fit the species studied. For Fucus species from the intertidal zone Strömgren (1977b) found 12.5 Wm^{-2} from white fluorescent light tubes suitable.

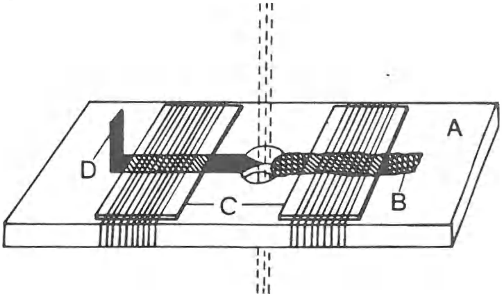

Fig. 1. Plexiglas frame (A) with apice (B) mounted under thin plates (C). The diffraction edge (D) is adjustable.

The growth is measured by a laser diffraction technique originally developed to study mussels (Strömgren, 1975). The algal apices are mounted on plexiglass frames with the growing tip close to an adjustable diffraction edge. Laser light sent through the narrow opening will be diffracted, and the diffraction pattern is recorded on a film strip placed under the opening. Growth of the alga will narrow the slit and change the pattern accordingly. The technique has been improved recently by Strömgren and Carry (1984). In the new version, the signals are stored on tape and processed in a minicomputer which presents growth rates by the hour. Increments in growth down to circa 1 µm are measured with satisfactory accuracy. Each measurement takes less than 30 s, and a couple of hundred apices may be handled daily.

For toxicity testing control groups are run in pure seawater and compared to the test groups in trays which are flushed continuously with seawater containing known concentrations of the chemical to be tested. Then, the growth is followed for 1 to 3 days. From the data obtained, the concentration of the chemical giving a 50 % reduction in growth is estimated or the highest concentration which does not give a reduction computed.

TESTS BASED ON UPTAKE (ACCUMULATION) OF CHEMICALS IN SEAWEEDS

Many seaweeds accumulate toxic metals but it is unknown when the accumulated metals become toxic to the algae. Therefore tests based on metal uptake by seaweeds are not really toxicological tests. In ecotoxicology their value lies in the fact that the accumulation in the algae reveals the presence of the chemical in the environment. It can indicate the severity of the contamination, and since accumulation starts at sublethal levels of the metal and leads to efficient concentration of the element in question, studies of metal content in seaweeds may be very useful, especially in monitoring programs.

The seaweeds to be used should be abundant, identifiable and easy to harvest, quite tolerant to environmental adverse influence, react in proportion to the load, and be available most of the year. Some of the intertidal brown seaweeds, such as Ascophyllum nodosum and several of the Fucus species are ideal for North European and North American waters.

A fairly large number of specimens is needed for the tests (20 to 30) to reduce the effect of individual variation in the material (Baardseth and Haug, 1953). Epiphytes and other contamination must be removed prior to drying. Drying cabinets with forced air at 40 to 50 °C are suitable and should be used to produce bone-dry, brittle material which can be broken into small pieces by hand. At this point quarting out a suitable subsample may be practical before it is ground to a fine powder in a porcelain ball mill or a similar nonmetal device. The dry matter content of the product is determined, and subsamples are weighed out for metal analysis, normally by atomic absorption, X-ray spectroscopy or activation analysis.

Whole plants, or in the case of *Ascophyllum nodosum* and a few others, tissue divided into age classes, may be used. By separate analysis of material of known age and comparison of their metal content, information on the trends in metal contamination may be obtained from a single sample without having to set up a time-series of samples.

APPLICATION OF TESTS. VIRTUES AND WEAKNESSES

The literature contains a rather limited number of records of studies on the toxic effect of heavy metals on seaweeds (Bryan, 1969 ; Russel and Morris, 1970 ; North *et al.*, 1972 ; Goodman *et al.*, 1976 ; Strömgren, 1977ab, 1980 ; Reed and Moffat, 1983). These studies show that zinc, copper and some other metal ions become toxic to seaweeds at fairly high concentrations (0.1 - 5 $mg.l^{-1}$), but they also demonstrate the many problems involved in the use of seaweeds in ecotoxicological tests, *e.g.* the need for large samples, the requirement for large tanks in laboratory experiments, and the extended test-time required to measure effects on growth or changes in biomass in the conventional way.

LABORATORY TESTS BASED ON GROWTH AND VIABILITY

The introduction of the laser method to determine lenght increments of growing tips of seaweeds has allowed the development of a practical toxicity test with these plants. By this semimicro method Strömgren (1979ab, 1980) studied the tolerance levels of a number of intertidal seaweeds to copper and zinc ions *A. nodosum* did not react to 30 $\mu g.l^{-1}$ of copper or to 100 $\mu g.l^{-1}$ of zinc, while all species investigated showed reduced growth at 1.4 $mg.l^{-1}$ zinc. The reduction in growth was particulary significant during the first 2 to 3 days of exposure. This method can easily be standardized to become a rapid test for toxic effects of industrial waste water on marine macroalgae. A large number of samples can be handled in a short time, and the experimental conditions are easily controlled. The equipment and operational expenses are reasonable. Continuous addition of toxicant to the medium will provide a constant concentration of available metal (or toxicant), and will also secure that most of the metal is in the free ionic form.

A problem is that the relationship between the increase in lenght of the apical part and the growth (increase in biomass) of the whole plant may be quite complex and is not known in most cases. It may also be questioned to what extent the removal of the apice from the rest of the plant will influence the growth of the apice.

Furthermore it must be admitted that the seaweeds seem to be rather insensitive to many chemicals and will probably survive pollution better than many other organisms in the marine environment. The hydrophilic character of the seaweeds will in generally protect them against lipoid toxicants, and no accumulation of such compounds as petroleum hydrocarbons, PCB, DDT, PAH, and lipid-soluble pesticides can therefore be expected.

The discovery by Taylor and West (1980) that the pigment, Evan's blue, is taken up by dead cells and excluded from living cells, has been used by Reed and Moffat (1983) to develop a viability test for macroalgae. In this way the prolonged incubation periods needed in conventional growth tests was avoided. Reed and Moffat (1983) applied this method in studies on the toxicity of and tolerance to copper in the green seaweed _Enteromorpha compressa_.

Other physiological parameters may also be quite sensitive indicators of toxicity and may be used in toxicity studies. The rate of net photosynthesis is easily determined using an oxygen electrode (Reed _et al._, 1980), and this looks promising for the development of a rapid toxicity test.

Rhizoid formation in _E. compressa_ and related green algae may also form the basis for a quick bioassay (Reed and Moffat, 1983).

TESTS BASED ON UPTAKE OF CHEMICALS IN SEAWEEDS

During the last decade seaweeds have been repeatedly used as indicators of heavy metal pollution in estuaries and coastal waters. Research teams from Great Britain (Preston _et al._, 1972 ; Bryan and Hummerstone, 1973 ; Ireland, 1973 ; Fuge and James, 1974 ; Morris and Bale, 1975) were among the first together with Norwegian researchers (Haug _et al._, 1974) to study the metal concentration in seaweeds in relation to environmental pollution problems. Phillips (1977) has reviewed the progress up to 1977. Seelinger and Edwards (1977) determined correlation coefficients and concentration factors of copper and lead in some benthic algae, and the Norwegian teams continued their studies on the use of seaweeds as biological indicators of heavy metal contamination in Norwegian fjords (Melhuus et al., 1978 ; Myklestad et al., 1978 ; Eide et al., 1980). Similar studies based on a brown seaweed Cystoceira crinito, to monitoring heavy metal pollution in the Black sea were also initiated by Burdin et al. (1980). Recently, Woolston et al. (1982) carried out a sampling study on the brown seaweed Ascophyllum nodosum as a monitor for trace metals in the St Croix Estuary and Passamaquoddy Bay which form the boundary between Canada and the USA on the Atlantic coast.

It has been possible to establish baseline levels for the content of several heavy metals in A. nodosum and a few Fucus species in Norwegian waters (Haug et al., 1974 ; Melhuus et al., 1978). The baseline data for A. nodosum are summarized in Table I. Analyses of local samples of seaweed can, in comparison with these figures, demonstrate high metal contents which are to be attributed either to natural sources, i.e. the presence of special mineral resources along the coast, or to man-made sources i.e. mining or metallurgical industries in the area. The location of the metal input can often be revealed through studies on the metal content in the seaweeds in combination with studies on the hydrography of the area. This is clearly demonstrated in the distribution of the metal concentration in seaweeds in Sörfjorden in Western Norway (Haug et al., 1974), as shown in Fig. 2 and Table II.

Table I. Heavy metal content of Ascophyllum nodosum from uncontaminated waters in Norway, based on Haug et al.(1974)

Metal	Content ($mg.kg^{-1}$ dry wt)
Zn	60 – 100
Cu	5 – 10
Pb	< 3
Cd	< 0.7
Hg	0.05 – 0.08

Table II. Heavy metal content of Ascophyllum nodosum (mg.kg^{-1} dry wt) from five districts in the Hardangerfjord (Haug *et al.*, 1974)

Metal	Area I	 II	 III	 IV	 V
Zn	3 220	2 140	2 140	1 420	315
Cu	111	29	15	6	5
Pb	81	22	5	< 3	< 3
Cd	14	7	6	4	1
Hg	12	2	1	0.3	0.08

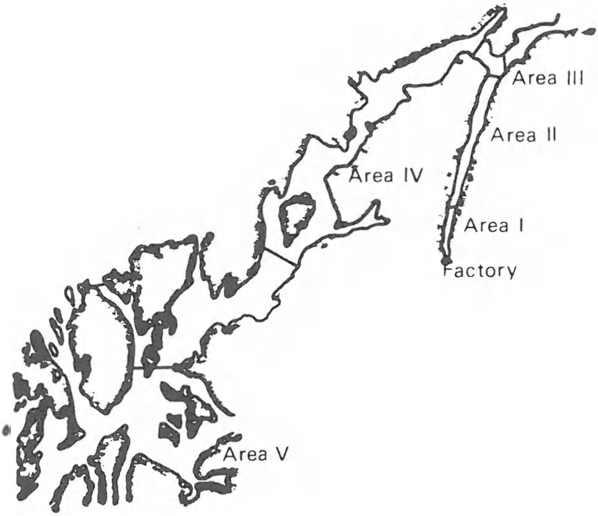

Fig. 2. Distribution of metal concentration in seaweeds in the Hardangerfjord (Haug *et al.*, 1974).

It is important that the algal material in the various samples of a series is of the same age since accumulation of metals is very much a one-way process, *i.e.* old tissue has higher contents than younger material. This means, however, that when the algal material can be separated into age classes, as is the case with *A. nodosum*, more information may be obtained. Analysis of a single sample, divided into subsamples of 0, 1, 2, 3, 4 years and older age classes, can identify any heavy metal pollutant and indicate the seriousness of the contamination as well as show whether the situation is improving or deteriorating. A mathematical model for the uptake of heavy metals in benthic algae has been worked out (Seip, 1979), and the heavy metal pollution in several Norwegian fjords is routinely monitored through analysis of the metal content in local seaweeds. The monitoring is carried out by the mining and metallurgical industries in the areas as part of the official regulations controlling their activity.

Myklestad and collaborators (Myklestad *et al.*, 1978 ; Eide *et al.*, 1980) have used a relatively easy transfer technique in studies on heavy metal uptake and release in seaweeds. Whole plants and the piece of rock they were attached to, were transferred from polluted to pure waters and *vice versa*. The authors concluded that the uptake of zinc and cadmium was slow in winter and rapid in summer, while lead accumulated at a nearly constant rate throughout the seasons. Some seasonal variation also occurred in the release processes of these metals.

Studies on the kinetics and mechanisms of metal uptake and release have been initiated in several laboratories. The insight obtained has improved the value of the test in ecotoxicological monitoring. A two-compartment model seems to fit the uptake of zinc in *A. nodosum* very well. (Skipnes *et al.*, 1975). A major part of the zinc is probably bound to polyphenols in the alga, while alginic acid seems to be the major binding site for strontium and maybe lead in the brown seaweeds (Skipnes *et al.*, 1975 ; Eide *et al.*, 1980). Filion-Myklebost and Norton (1981) have shown that *A. nodosum* may shed its epidermis under certain conditions, and since zinc and some other heavy metals tend to accumulate in the phenol-rich epidermis cells, this shedding may have some influence on the test results and may also be of some ecological significance for the alga.

Whereas the correlation between the metal content in the seaweeds and the source of the contamination has been established in most cases, the relationship to the metal content in the surrounding seawater appears to be more dubious. The former correlation has beyond doubt been mediated by the seawater, but the variations in the concentration of metals in the water are frequently too large to allow any correlation to be detected in the sampling programs normally applied. Eide (1977) introduced integrated sampling and used chelating resins to obtain good average values. This technique is recommended in attempts to correlate metal content in the seaweeds with that of the surrounding seawater.

CONCLUSIONS

In the present context seaweeds seem to be best suited for in situ monitoring of heavy metal pollution in coastal waters, although the speed and efficiency of the semimicro laser-based method of Strömgren (1975) makes toxicity testing with seaweeds in the laboratory both practicable and rapid. The common seaweed A. nodosum is particularly useful when age classes of tissue are analyzed separately. The author is not aware of any other method which can give information on both the identity and concentration as well as on the trend in heavy metal pollution in seawater on the basis of one single sample.

The enrichment of the metals in the alga, which may be in the order of 10^3 to 10^5 times above the level in the seawater, also considerably simplifies the determination of the metals. The seaweeds give some sort of average picture of the metal load at a particular site, and also react only to biologically available metal. This is the type of information needed in ecotoxicology.

It must be admitted that the response of seaweeds has no predictive value for the toxic effects on other organisms exposed to the same pollution, but tests with seaweeds certainly appear more informative and relevant than simple chemical monitoring.

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