INVESTIGATIONS ON THE INFLUENCE OF ORGANIC SUBSTANCES PRODUCED BY SEAWEEDS ON THE TOXICITY OF COPPER

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Introduction

Toxic effects of heavy metals to marine organisms are considerably influenced by the presence of organic substances in the sea water. It is generally recognized that this is due to the influence of organic compounds on chemical speciation of the metals, in particular by the formation of metal organic complexes (Siegal, 1971; Sunda and Guillard, 1976; Gillespie and Vaccaro, 1978; Schmidt and Forster, 1978; Kremling, 1983; Kramer and Duinker, 1984; Bernhard and George, 1986). The biological effectiveness of trace metal binding by artificial chelators is well known and of practical importance for the culture of aquatic organisms (Steemann-Nielsen and Wium-Andersen, 1970; Fängström, 1972; Morris and Russel, 1973; Sunda and Guillard, 1976).

The possible ecological relevance of metal organic complexation by natural organic compounds in the open sea has been discussed by various authors (e.g. Johnston, 1964; Barber and Ryther, 1969; Siegal, 1971; Davey et al., 1973; Bernhard and George, 1986).

In off-shore regions, production of dissolved organic substances produced by plankton, especially phytoplankton, seems to play the most important role (e.g. Kremling et al., 1983). In shallow coastal waters, however, benthic organisms contribute significantly to the dissolved organic carbon budget (Langston and Bryan, 1984).

Benthic primary producers, especially seaweeds, release a considerable proportion of the photosynthetic products into the surrounding seawater (exudates). Normally, 1-5 % of photosynthetically fixed carbon is released as exudate (DOC) by benthic marine plants. Under extreme environmental conditions, such as osmotic stress, desiccation or extreme temperatures, release of up to 40% of net primary production has been observed (Khailov and Burklakova, 1969; Sieburth, 1969; Brylinski, 1971; Moebus, 1974; Guterstam et al., 1977; Schramm et al., 1984).

Own measurements showed that in the Baltic Sea the DOC concentration ranges from 0.5 - 2 mg C l^{-1} in the open sea. In shallow coastal waters, in the vicinity of seaweed or seagrass beds, DOC contents up to 6 mg C l^{-1} have been determined.

On the other hand, heavy metal pollution due to industrial and municipal waste water discharge, primarily effects coastal areas.

This paper presents investigations on the effects of organic compounds produced by seaweeds on the toxicity of copper.
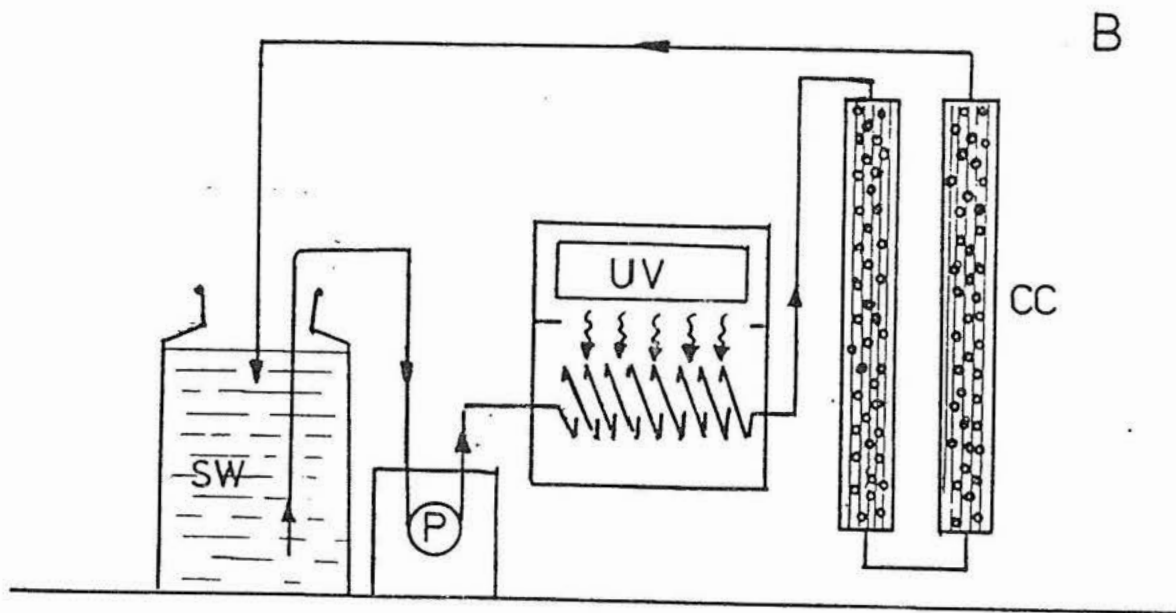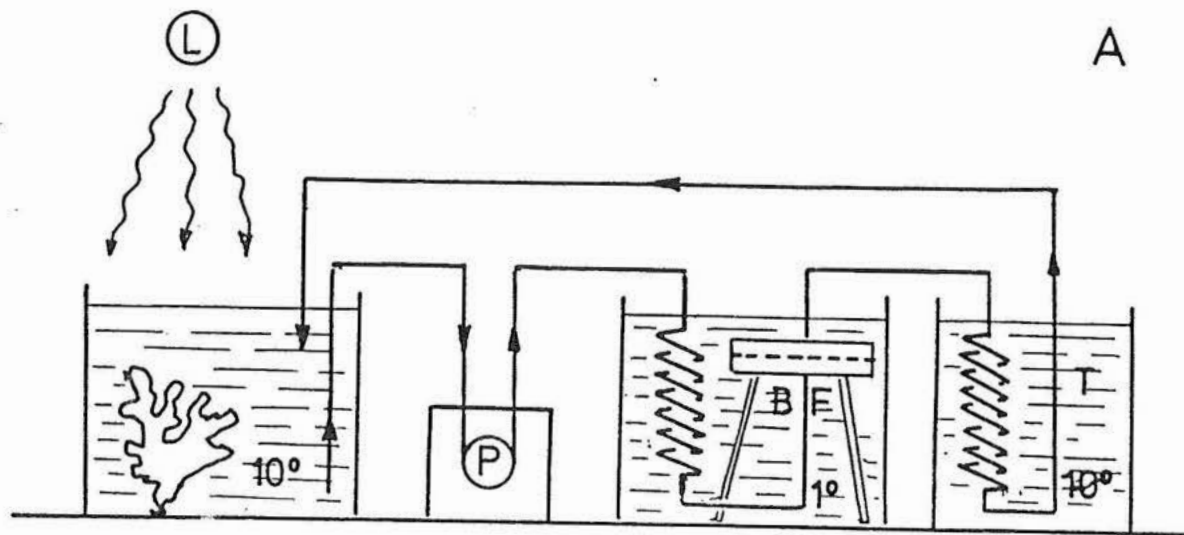
Fig. 1. Device for the enrichment of seaweed exudates in sea water **(a)**; peristaltic pump (P); aquarium with seaweeds (A); light source (L); bacteria filter cooled to 1°C (BF); thermostat 10°C (T). Device for preparation of DOC "free" sea water media **(b)**; peristaltic pump (P); high-energy UV lamp for destruction of organic compounds (UV); activated charcoal filters (CC).

Methods

Among the various biological tests which have been used to study the toxicity of heavy metals, in particular copper, to marine organisms (e.g. Steemann-Nielsen and Wium-Andersen, 1970; Erickson *et al.*, 1970; Raff, 1975; Vaccaro, 1978; Brown *et al.*, 1988), the method introduced by Schwenke (1960) proved to be especially suitable for our experiments. Damaged cells in the highly sensitive tissue of young phylloids of the red seaweeds *Delesseria sanguinea* or *Phycodrys rubens* show a characteristic discoloration due to the release of plastid pigments into the cell vacuole. The proportion of destroyed cells visible under the microscope can easily been estimated and placed in one of for example 5 categories (damaged cells 0, 5, 20, 50 and 100%). The advantages of this method are the following. A large number of samples can be tested within short periods of time, which permits large test series with a high statistic significance. Large numbers of the small phylloids can be held in small amounts of water (10-20 phylloids in 100 ml medium) owver several weeks. The high copper specific sensitivity enables testing of ecologically relevant Cu concentrations (1-2 μg l^{-1}). The phylloids, which develop *in situ* only during the winter months, can easily be cultured over several months, so that test material is available over a longer period of the year. It should be pointed out, however, that the sensitivity of the copper test, as in all bioassays, depends to great extent on the environmental conditions (temperature, salinity etc.) as well as on the physiological state of the test material. This means that only relative values in comparison to control samples will be obtained.

For the toxicity tests, Baltic seawater (S = 15-18 $^o/_{oo}$) was collected off-shore in the open Kiel Bight, where the Cu background usually was below 1 μg l^{-1}. The content of dissolved organic carbon (DOC) after filtration through 0.2-μm membrane filters ranged from 0.5-1 mg l^{-1}. This background DOC was reduced to nearly undetectable levels by filtering through activated charcoal columns and subsequent UV oxidation of the seawater, employing a circulating system (Armstrong *et al.*, 1966; Gillespie and Vaccaro, 1978) as shown in Fig. 1a. After UV oxidation the seawater medium was "aged" for several days, because immediate use in some cases was detrimental to the algae (Davey *et al.*, 1973).

The toxic effects of copper on the young *Delesseria* phylloids was tested in the presence of organic substances from the seaweeds *Fucus vesiculosus*, *Laminaria digitata*, *Ulvaria obscura*, or from a mixture of the dominant forms of a typical Baltic red algal community (*Phyllophora truncata*, *Phycodrys rubens*, *Delesseria sanguinea*), respectively. The algal dissolved organic substances were obtained either as seawater soluble extracts from finely chopped fresh algal material, or by enriching algal exudates. For this purpose the seaweeds were incubated in recirculating "DOC free" seawater at 15°C and at a light intensity of 0.2 kW m^{-2}. Bacterial activity during enrichment was kept as low as possible by passing the circulating seawater through a cooled bacterial filter (0.2°C, 2 μm pore size; Fig. 1a).

In other experiments, natural DOC compositions of sea water collected in the vicinity of seaweed communities or off-shore in the open Kiel Bay were tested. All seawater media were prepared or collected, filtered and then kept frozen (-18°C) in one litre glass bottles till use for the experiments.

During the 3 to 6 weeks lasting toxicity experiments, the test algae were incubated at 5°C and a quanta flux density of 100 μE m^{-2} s^{-1} (fluorescent lamps, 12:12 hours light:dark) in 200 ml of the respective media, which were renewed every second or third day.

Copper was added as a concentrated CuCl$_2$ standard solution each time after renewing the seawater media. Copper concentrations were measured on APDC extracts by atomic absorption spectrometry (AAS: Perkin Elmer). DOC was determined employing a CENCO DOC analyzer (Schreurs, 1978).

In order to estimate the Cu binding capacity of organic substances produced by seaweeds, a semi-quantitative bioassay method was made adopting the method used by Davey et al. (1973): the sigmoid titration curve of EDTA versus Cu showed that the point of inflection corresponded to the equivalence point (EP), where the moles of copper added, equal the moles of EDTA present in solution. By analogy, Davey and co-workers used the point of inflection of similar shaped curves of growth response of the algae *Thalassiosira pseudonana* to copper concentrations in the presence of EDTA or natural DOC as a measure of copper binding capacity of DOC. This method was employed to estimate the copper binding capacity algal exudates in comparison to EDTA.

Results

The applicability and sensitivity of the toxicity test employed, is demonstrated in Fig. 2. In "DOC free" seawater (residual DOC in this case less than 0.1 mg C l^{-1}, background Cu 0.4 μg l^{-1}) toxic effects of copper as low as 1 μg l^{-1} of sea water were detectable. The sensitivity of the test, however, was influenced by environmental factors, such as temperature (Fig. 3) and salinity (Fig. 4), or by the physiological state of the test material.
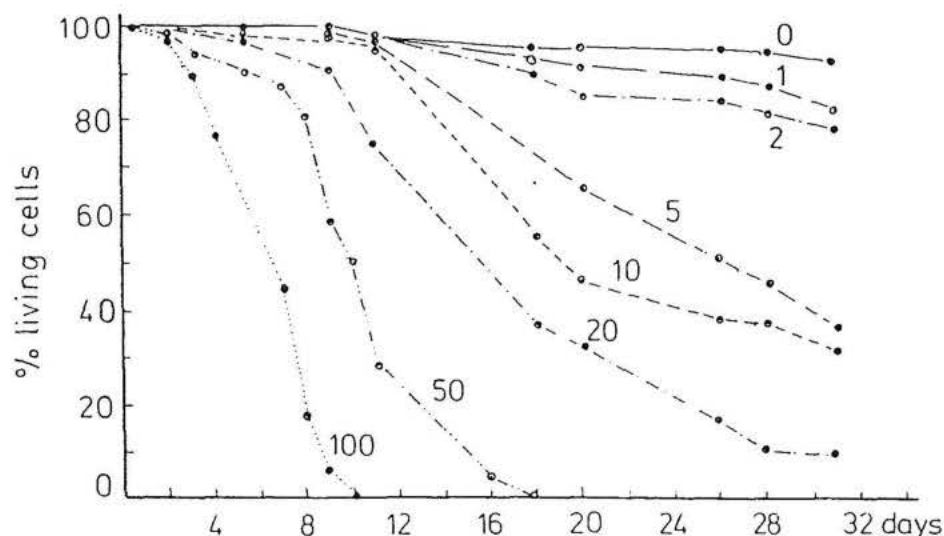

Fig. 2. Visible detrimental effects (discolorisation of cels) of varying Cu concentrations (0-100 μg Cu l^{-1}) on young phylloids of *Delesseria sanguinea*. Medium: DOC-reduced sea water (rest DOC < 0.1 mg l^{-1}); salinity S= 15.5 $^\circ/_{\circ\circ}$; light-dark period 12:12 hrs; illumination 0.03 kW m^{-2}; water exchange every 2nd day.
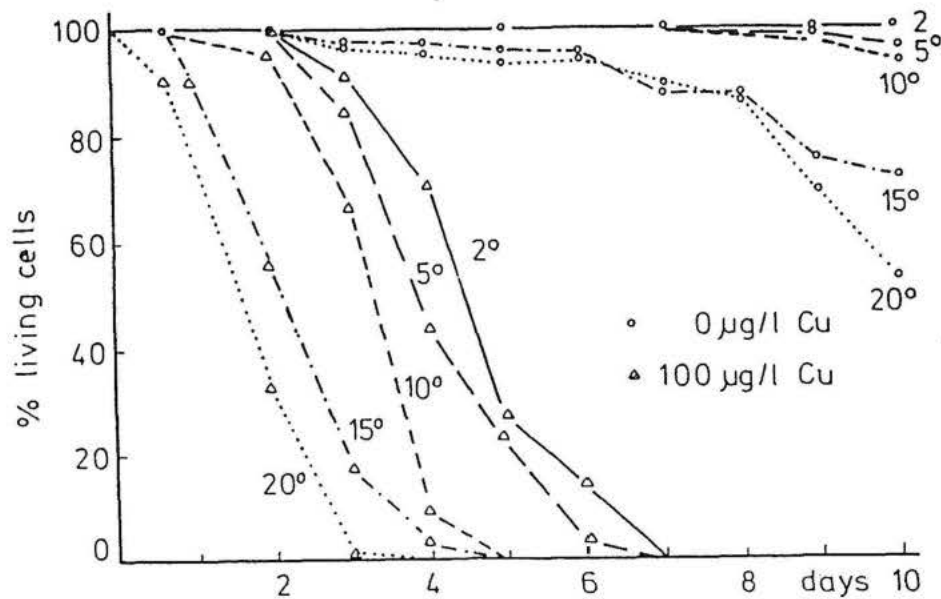
Fig. 3. The influence of temperature on visible detrimental effects of copper (100 µg Cu l^{-1}) on young phylloids of *Delesseria sanguinea*. Medium: DOC-reduced sea water (rest DOC < 0.1 mg l^{-1}). For further details see Fig. 2.
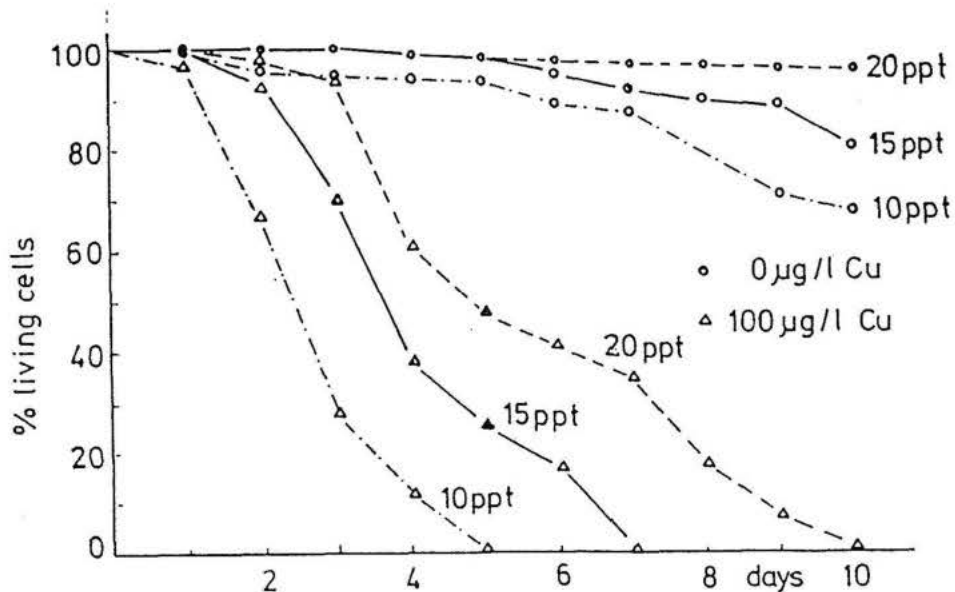

Fig. 4. The influence of salinity on visible detrimental effects of copper (100 µg Cu l^{-1}) on young phylloids of *Delesseria sanguinea*. Medium: DOC-reduced sea water (rest DOC < 0.1 mg l^{-1}). For further details see Fig. 2.

Addition of seawater soluble extracts from *Fucus*, *Laminaria*, or *Ulvaria* considerably reduced the toxicity of copper in the test range of 5 to 100 µg Cu l[-1], while extracts from red algae were less effective (Figs. 5 and 6).
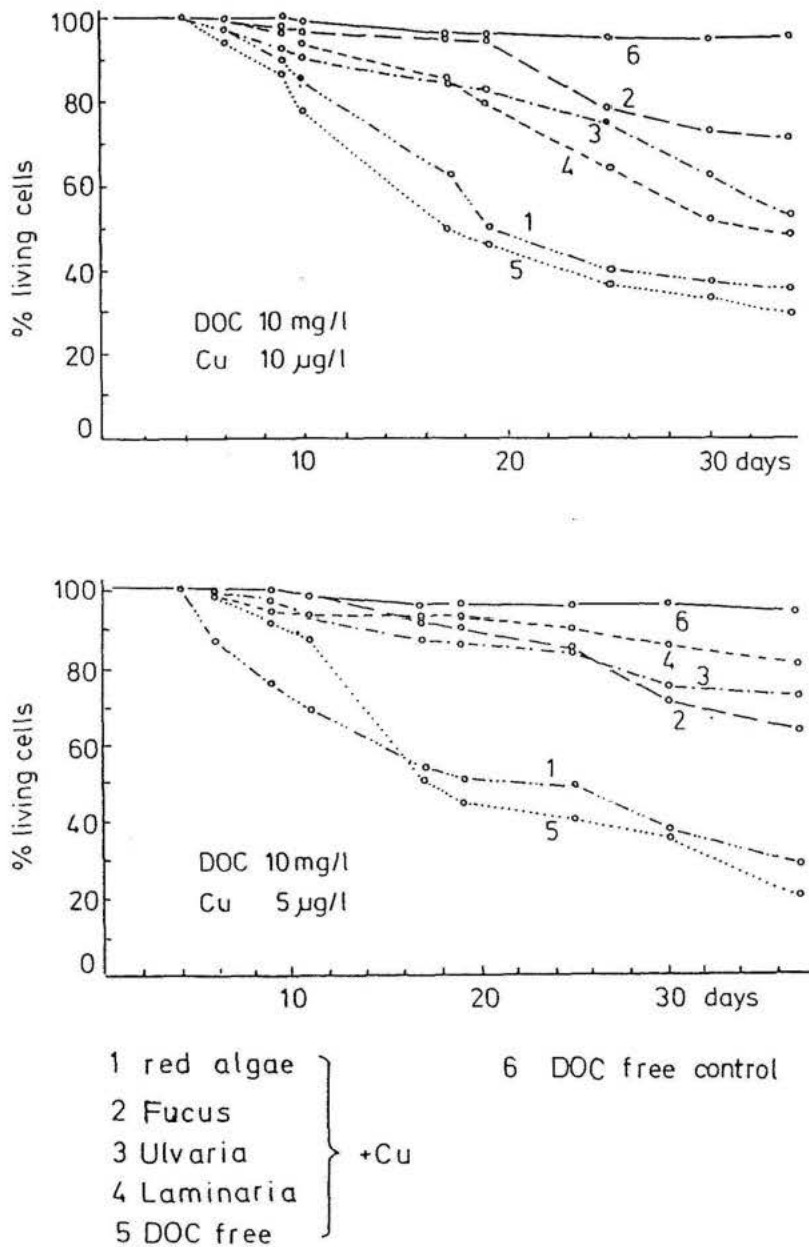

Fig. 5/6. The influence of seawater extracts (DOC 7-8 mg l^{-1}) from red algae (*Delesseria sanguinea*, *Phycodrys rubens*), *Fucus vesiculosis*, *Laminaria saccharina* or *Ulvaria obscura*, on visible detrimental effects of copper (5 or 10 µg Cu l[-1]) on young phylloids of *Delesseria sanguinea*. For further details see Fig. 2.
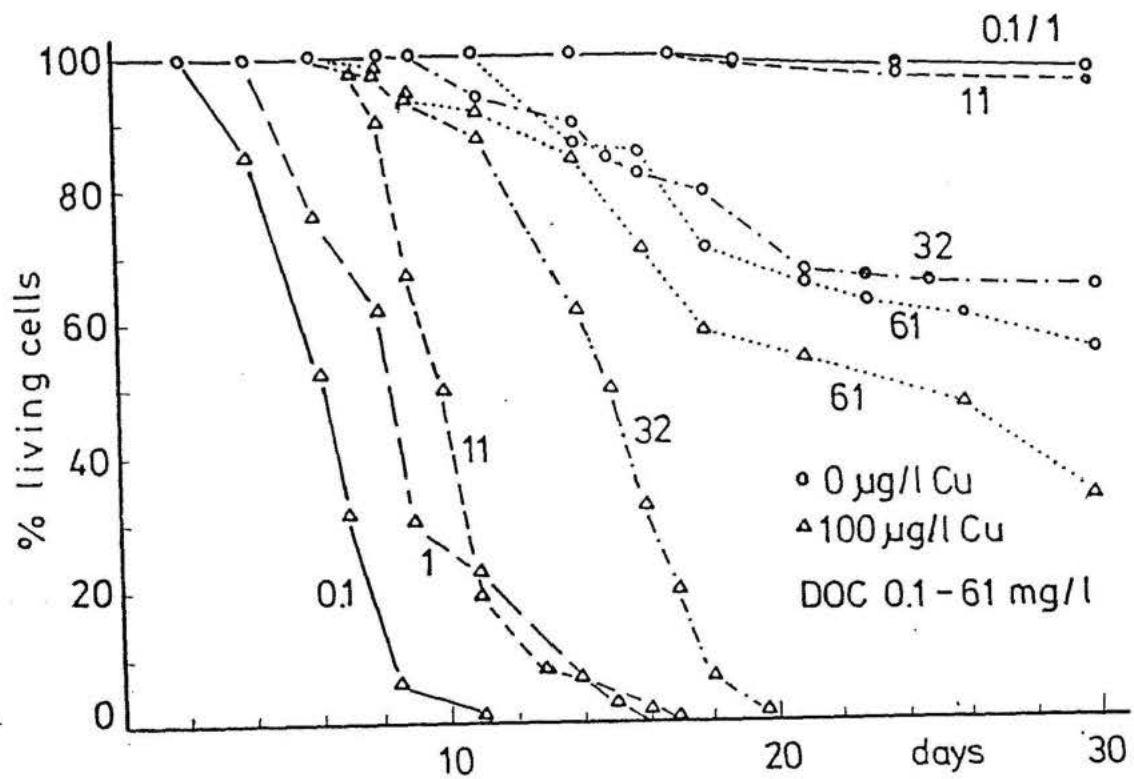
Fig. 7. The influence of varying concentrations of seawater extracts (DOC 0.1, 1, 11, 32, 61 mg l⁻¹) from *Fucus vesiculosis* on visible detrimental effects of copper (100 µg Cu l⁻¹) on young phylloids of *Delesseria sanguinea*. For further details see Fig. 2.

The influence of varying concentrations of algal extracts (average DOC values <0.1, 1, 11, 32, 61 mg l^{-1}) on copper toxicity is shown in Fig. 7. Very high extract concentrations (DOC >30 mg l^{-1}), however, were detrimental to the algae, even without addition of copper.

Like the extracts, algal exudates enriched in seawater to about 6-7 mg l^{-1} DOC reduced the toxicity of copper in a similar way. Organic substances produced by red algae again showed the least protective effects (Fig. 8).

The effectiveness of natural DOC concentrations was tested with seawater collected in the vicinity of algal communities or in open sea, respectively (Fig. 9). In this experiment the DOC concentrations were 1.2 mg C l^{-1} for the open sea water, 1.4 mg C l^{-1} for the red algae community water, 2.4 mg C l^{-1} for *Laminaria* and 3.2 mg C l^{-1} for *Fucus* bed water, respectively. The DOC content of the "DOC free" control medium did not exceed 0.1 mg C l^{-1}. Even at Cu concentrations (50 µg l^{-1}) far above the average values known for coastal waters, natural DOC contents considerably decreased the Cu toxicity.
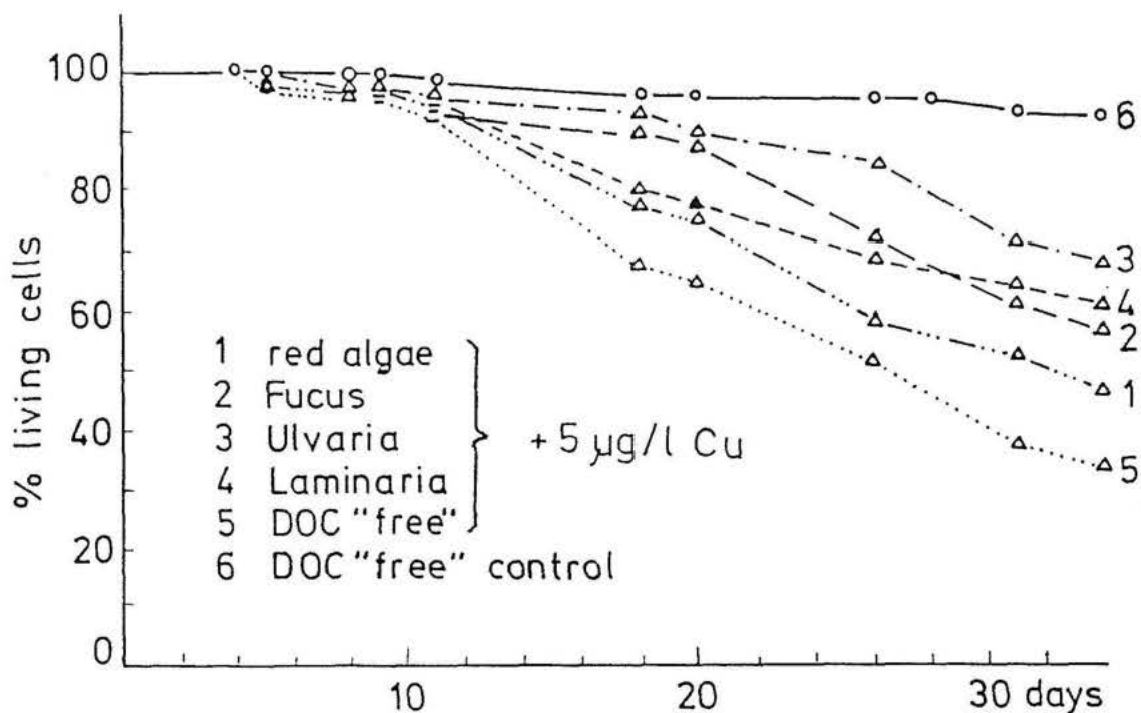

Fig. 8. The influence of exudates from red algae (*Delesseria sanguinea, Phycodrys rubens*), *Fucus vesiculosis, Laminaria saccharina* or *Ulvaria obscura* on visible detrimental effects of copper (5 µg Cu l^{-1}) on young phylloids of *Delesseria sanguinea*. The exudates from the different seaweeds were enriched in DOC-reduced sea water up to 9 mg l^{-1} DOC. For further details see Fig. 2.
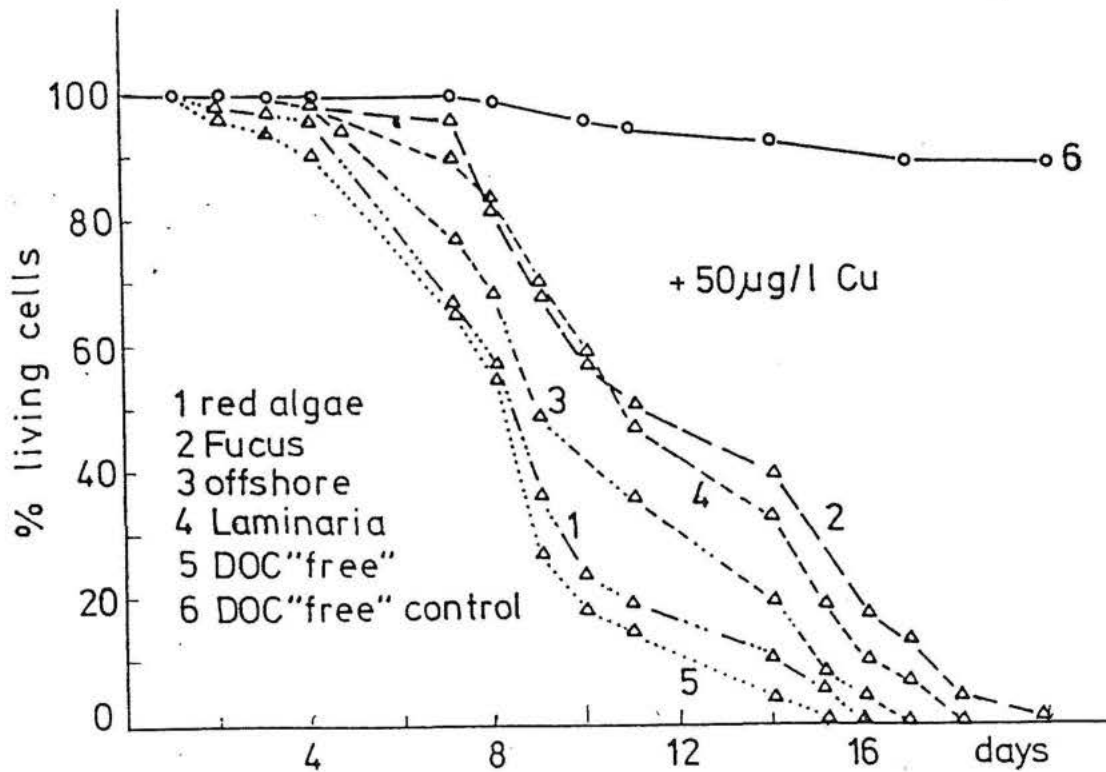
Fig. 9. The influence of natural sea water of different origin on visible detrimental effects of copper (50 µg l⁻¹) on young phylloids of *Delesseria sanguinea*. DOC concentrations (mg l⁻¹): off-shore water (1.2); red algae community (1.4); *Laminaria* bed (2.4); *Fucus* community (3.2). For further details see Fig. 2.

The results clearly show that organic substances released from seaweeds have the potential to modify the Cu toxicity or bioavailability.

Preliminary experiments employing ultrafiltration and gel filtration methods on algal exudates indicated that in our experiments a major proportion of the copper is is reacting with the lower molecular fractions (<500 MW) of the exudates.

The bioassay method introduced by Davey *et al.* (1973) was employed in our experiments to estimate the relative binding capacity of a *Fucus* exudate for copper, in comparison to the strong complexant EDTA. In three replicate experiments, the expected complexation of 0.50 and 0.25 µM Cu by the molar equivalent of EDTA occurred over a time range from 1 to 4 days, probably depending on the physiological state of the test material. Figs. 10 and 11 show the results of one of the experiments, where the expected complexation was already observed after one day. In the further time course of the experiments, the binding capacity or protective effects both of the exudate and EDTA decreased.

In other words, the presence of the organic compounds and the assumed chemical or physico-chemical reaction with copper seems not to lower, but rather to retard the toxic effects of copper. One explanation may be the influence on uptake rates, depending on the molecule size of the Cu species. This hypothesis is supported by the results of experiments on the uptake of copper by *Fucus vesiculosus* depending on the type and the concentration of organic complexants (Fig. 12).
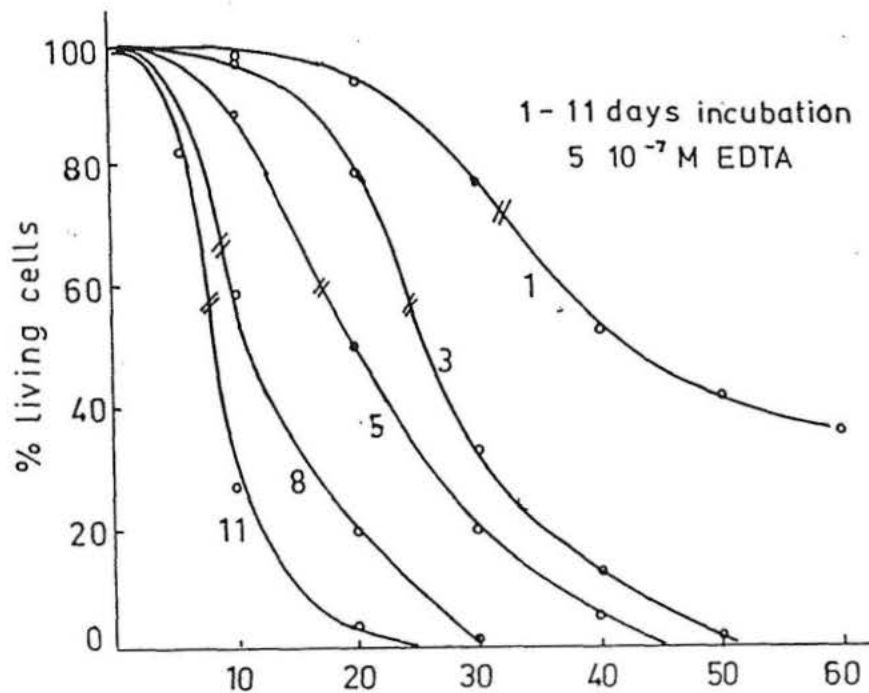
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Fig. 10. Visible detrimental effects of varying Cu concentrations (0-60 μg l⁻¹) in the presence of 5 x 10⁻⁷ M EDTA on young phylloids of *Delesseria sanguinea* after 1, 3, 5, 8 and 11 days of incubation. EP= point of inflection (equivalence point). For further details see Fig. 2.
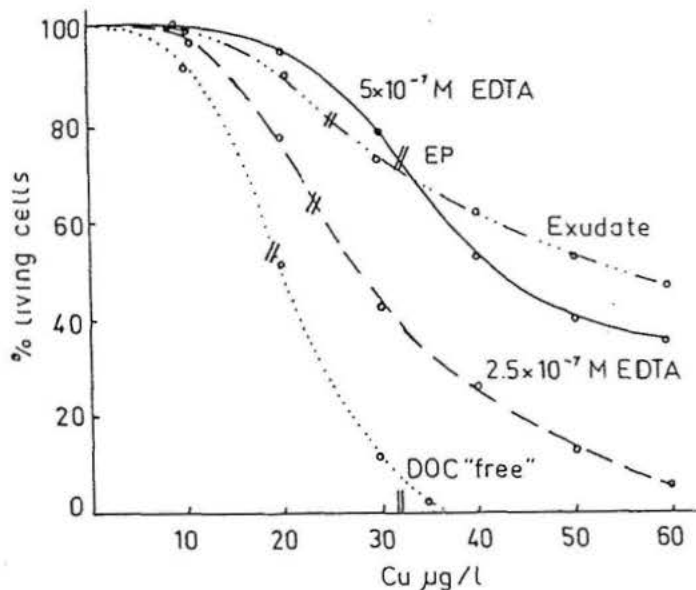
Fig. 11. The influence of dissolved organic substances (*Fucus vesiculosis* exudate, 3 mg l⁻¹ DOC; EDTA 2.5 and 5 x 10⁻⁷ M; DOC-reduced sea water, rest DOC <0.1 mg l⁻¹) on visible detrimental effects of varying Cu concentrations (0-60 μg Cu l⁻¹) on young phylloids of *Delesseria sanguinea* after 1 day incubation. For further details see Fig. 2.
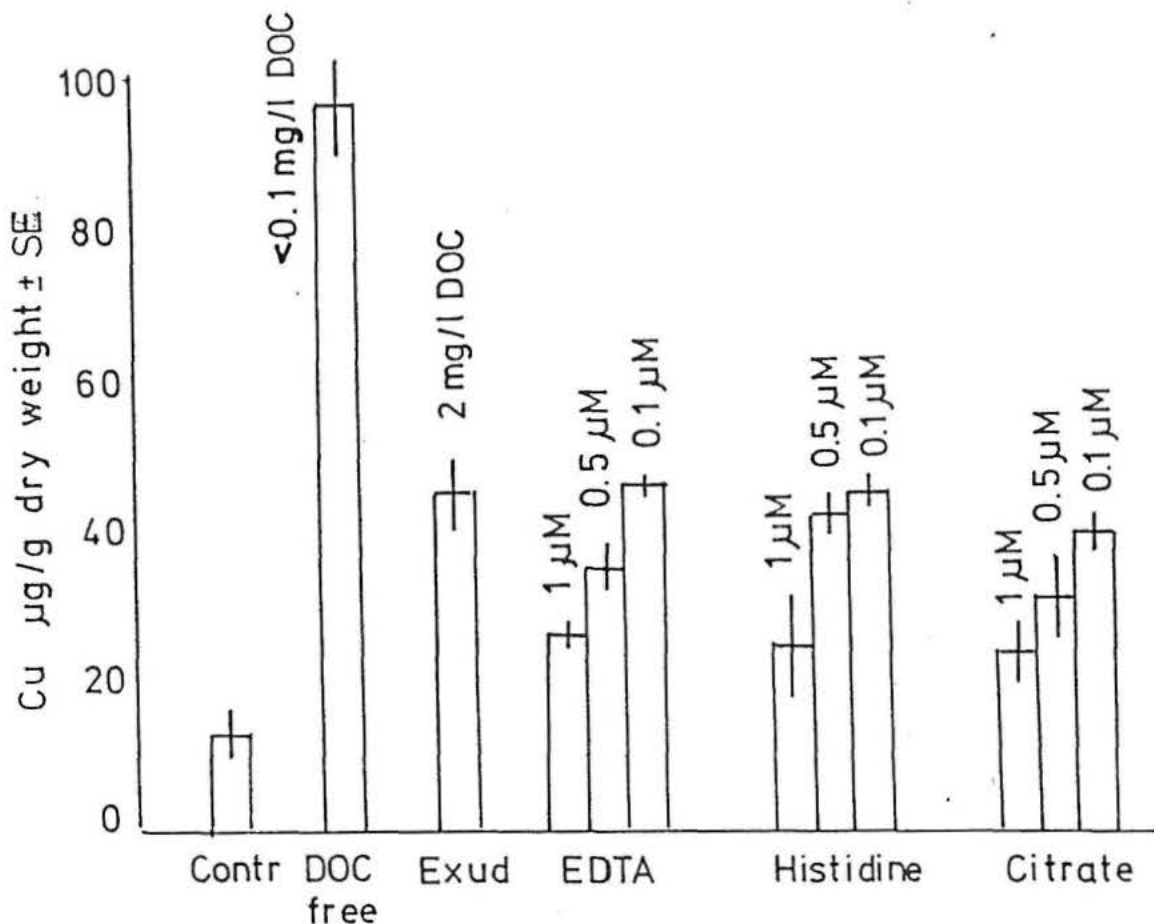
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Fig. 12. The influence of different types and concentrations of organic complexants (*Fucus* exudate, EDTA, histidine, citrate) on the uptake of copper by *Fucus vesiculosis* (μg g^{-1}dwt $\pm$ SD) during a 3-week incubation period. DOC-reduced sea water medium (rest DOC <0.1 mg l^{-1}) spiked with 5×10^{-7} M Cu, and addition of *Fucus* exudate, 9 mg l^{-1} DOC; EDTA, histidine, citrate, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, respectively.

Discussion

Investigations on the effects of copper on marine organisms, very often did not take into account the role of natural organic compounds contained in seawater. This may explain, why detrimental or lethal copper concentrations vary considerably, and are usually far beyond average copper concentrations in seawater.

Our results as well as those of some other investigations (e.g. Steemann-Nielsen and Wium-Andersen, 1970; Erickson *et al.*, 1970) show that copper concentrations even as low as usually occur in oceanic or off-shore waters, i.e. in the range of 0.5-1 μg l^{-1}, may have detrimental effects on certain particularly sensitive marine organisms, if dissolved organic substances in the sea water were reduced, or at low concentrations. The results also show that dissolved organic material in concentrations as usually occur in the open Baltic, i.e. 1-2 mg l^{-1} DOC, can significantly reduce detrimental effects of copper, even at extreme concentration levels as may occur in some highly polluted areas, as for example along the SW-coast of Sweden (Öresund), where local extremes as high as 50 μg Cu l^{-1} (Hägerhall, 1973) and 125 μg Cu l^{-1} (Wachenfeldt, 1975) have been measured.

In shallow coastal areas, probably the marine benthic plants, in particular seaweeds and seagrasses, are the main source of dissolved organic material. In the close neighbourhood of macrophytobenthic communities of the Baltic, we have measured DOC concentrations up to 6 mg l^{-1}. The low molecular fraction such as amino acids or sugars is usually significantly higher (amino acids 20-30 μg l^{-1}), compared to off-shore water, and the distribution pattern of amino acids and sugars in the seawater suggests the origin from marine macrophytes (Liebezeit, 1980; Dawson, pers. comm.).

Of the organic compounds released by benthic macroalgae, known so far, several chemical groups, e.g. sulfonated polysaccharides, uronates, polyphenols, humic acids, peptides and proteins, fatty acids and amino acids theoretically have the potential to form metal organic complexes (Ragan et al., 1979; Sueur et al., 1982; Langston and Bryan, 1984; Ragan and Glombitza, 1986). Although for thermodynamical reasons, inorganic ligands may be of greater importance for the speciation of heavy metals in sea water (Lerman and Child, 1973), the formation of metal-organic complexes in situ, under natural conditions, is significant. Numerous speciation studies employing e.g. extraction methods, ultrafiltration, dialysis, AAS, or inverse voltammetry, show that in seawater over 90% of the total copper can be present in organic forms (Slowey et al., 1967; Williams, 1969; Morris, 1974; Duinker and Kramer, 1976; Hasle and Abdullah, 1981; Kremling, 1981, 1983).

Only little can be said about the ecological significance of organic copper complexation. Still, little is known about the chemical nature and biological effectiveness of different organic copper comlexes, and even less about the physiological and biochemical mechanisms (Leppard, 1983). Copper is not only toxic in its ionic form, but may vary considerably with the chemical type of the organic copper complexes, resulting in bimodal response curves (e.g. Goksoyr, 1955, in Fängström, 1972). Florence et al. (1983) for example found, for a wide variety of natural and artificial organic complexants, no consistent correlation between the toxicity of copper as determined in bioassays, and free or labile copper fractions measured with physico-chemical methods. In some cases, organic ligands, in particular lipid-soluble compounds, even increased the toxicity of copper (Stauber and Florence, 1987; Florence et al., 1992).

From our results, in particular from the uptake and accumulation experiments, it appears that besides possible masking effects through organic chelation, particularly the bioavailability may be altered, for example by retarded uptake of the bigger metal-organic molecules.

As the heavy metal content of seaweeds has frequently been proposed and used a measure of metal pollution in coastal waters (Bryan, 1983; Kangas and Autio, 1986; Rönnberg et al., 1990), under the aspect of these results, possibly the meaning and reliability of seaweeds as bioindicators should be reconsidered.

Even though organic substances produced and released by seaweeds may not have the potential to permanently detoxify copper, they may be important in the marine ecosystems, because of the temporary buffering capacity. Particularly in coastal and estuarine systems with rapid changes of concentrations and pronounced gradients of pollutants, the dissolved organic materials probably contribute significantly to the well recognized general buffer function of the transition zone between land and oceans.

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