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Chlorophyll fluorescence analysis and cadmium-copper bioaccumulation in *Ulva rigida* (C. Agardh)

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ABSTRACT

The objective of the present study was to determine the usefulness of the alga *Ulva rigida* (C. Agardh) in monitoring dissolved cadmium and copper in seawater, as well as the physiological stress caused by both metals. Discs from the alga (d = 19 mm) were cultured in different Cd and Cu growth media during a 12-day period. The concentrations established were 0.1, 0.3, 0.9, 2.7 mg metal l^{-1}, as well as the corresponding controls. Chlorophyll fluorescence readings were performed on days 1, 2, 5, 9 and 12. Discs were frozen for later AAS analysis (Perkin-Elmer). Treatments with higher Cd concentration (0.3, 0.9, 2.7 mg l^{-1}) presented clear signs of disturbing photosystem II, since optimum quantum yields (Fv/Fm) were lower than the control responses. Similarly, Cu treatments diminished the alga's photosynthetic efficiency. Cu and Cd internal levels in *U. rigida* increased successively during the experiments, which also were proportional to the treatments. These levels were transformed into contamination factors (CF), calculated by dividing each concentration by its respective control and adjusting with Michaelis-Menten kinetics.

Key words: *Ulva rigida*, bioaccumulation, cadmium, copper, contamination factor, PAM chlorophyll fluorescence, photosynthesis, photosystem II, Michaelis-Menten.

RESUMEN

Análisis de fluorescencia de clorofila y bioacumulación de Cd y Cu en* Ulva rigida *(C. Agardh)

Mediante este estudio se pretende determinar la utilidad del alga Ulva rigida *(C. Agardh) para monitorizar Cd y Cu disueltos en agua de mar, así como el estrés fisiológico causado por ambos metales. Para ello, discos de dicha alga (d = 19 mm) se colocaron en diferentes soluciones de Cd y Cu, durante un periodo de 12 días, siendo las concentraciones seleccionadas: 0,1; 0,3; 0,9; 2,7 mg metal l^{-1} de solución, así como los respectivos controles. Las lecturas de fluorescencia de clorofila se efectuaron los días 1, 2, 5, 9 y se analizó el contenido metálico de los discos mediante espectrofotometría de absorción atómica con llama (AAS). Los tratamientos de Cu y los de mayor concentración de Cd (0,3; 0,9; 2,7 mg l^{-1}) presentaron claras señales de perturbación del fotosistema II, con reducciones claras del rendimiento cuántico óptimo (Fv/Fm). De igual forma, los tratamientos de Cu redujeron la eficiencia fotosintética del alga. Los niveles corporales de Cd y Cu en* U. rigida *registraron incrementos sucesivos durante el periodo experimental y fueron proporcionales a los tratamientos. Las cinéticas de carga fueron modelizadas mediante ajuste a Michaelis-Menten.*

Palabras clave: Ulva rigida, *bioacumulación, cadmio, cobre, factor de contaminación, fluorescencia de clorofila PAM, fotosíntesis, fotosistema II, Michaelis-Menten.*

INTRODUCTION

The use of certain organisms as indicators of trace-metal pollution has become a useful tool in monitoring levels of such elements in coastal zones, where the major pollution sources are river mouths, industrial and municipal discharges and ship traffic (Bryan *et al.*, 1985). Therefore, physiological responses from many organisms are used as pollution level parameters in coastal ecosystems. *Ulva* algae have been useful heavy-metal biomonitors in different parts of the world (Haritonidis, Häger and Schwantes, 1983; Bryan *et al.*, 1985; Ho, 1990; Scoullos and Caberi, 1991; Weis and Weis, 1992; Webster and Gadd, 1992, 1996a,b).

The present study aims to demonstrate the usefulness of *Ulva rigida* (C. Agardh) as a bioaccumulator of cadmium and copper, as well as to determine, under experimental conditions, the alga's metabolic stress, using chlorophyll fluorescence and accumulation kinetics.

MATERIALS AND METHODS

Ulva rigida discs with a diameter of 19.0 mm were set in different concentrations of Cd and Cu, over a 12-day period. Selected concentrations were: 0.1, 0.3, 0.9 and 2.7 mg element l^{-1}, as well as the corresponding controls. Chlorophyll fluorescence measurements were performed on days 1, 2, 5, 9 and 12, using a fluorometer (PAM-400, Waltz). Samples were kept in total darkness for 30 min at room temperature prior to the measurements of fluorescence parameters. For measurements of Chl fluorescence, measuring light of 0.1 μmol m^{-2} s^{-1} and white saturating flushes lasting 0.8 s at 2 400 μmol m^{-2} s^{-1} were applied. Subsequently, discs were frozen until analysis. Samples were dried at 55 °C for 24 hours, and immediately pulverised, then dried again at 105 °C for 24 h, and dry weight was determined. Samples were mineralised into 20-ml glass containers with 10 ml of HNO_3 Suprapur (Merck). We left the samples at room temperature for 12 h, but a glass ball was placed on the top to minimise evaporation of the sample, which could disturb the organic matter digestion. The samples were arranged on a hot plate (110 °C) during 48 h; subsequently the balls were withdrawn and evaporation occurred. We then resuspended the material with 20 ml of 1N HCl and metals were determined using AAS.

RESULTS

Cadmium and copper treatments with higher concentrations presented clear evidence of disturbing photosystem II, since fluorescence responses (Fv/Fm) were much lower than controls (figures 1 and 2). However, during the first 24 h, Cu reflected a higher toxic impact on photosynthesis, due to the rapid decreases in Fv/Fm, which produced measurements lower than 0.40 relative

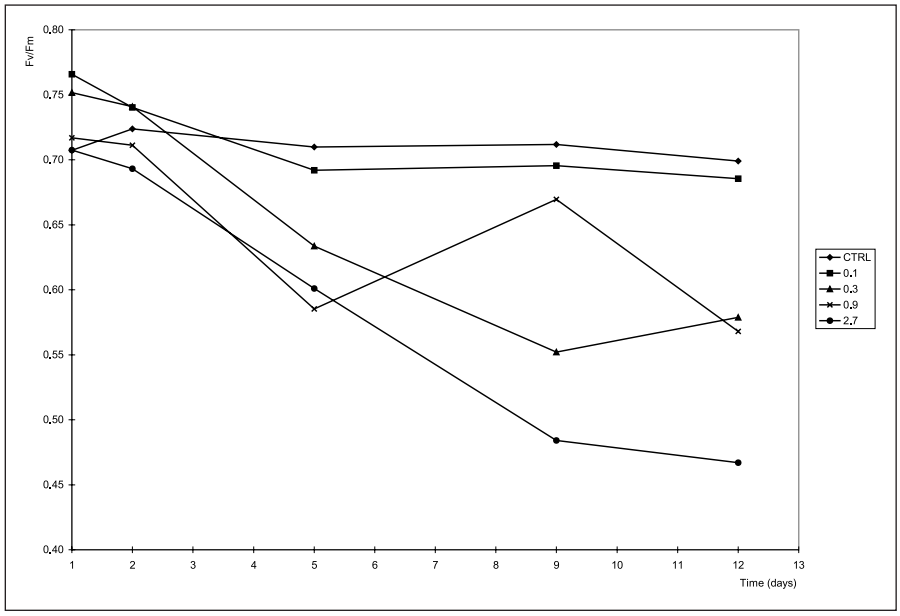

Figure 1. Fv/Fm variations in *U. rigida* under Cd treatments (mg l^{-1}) and control

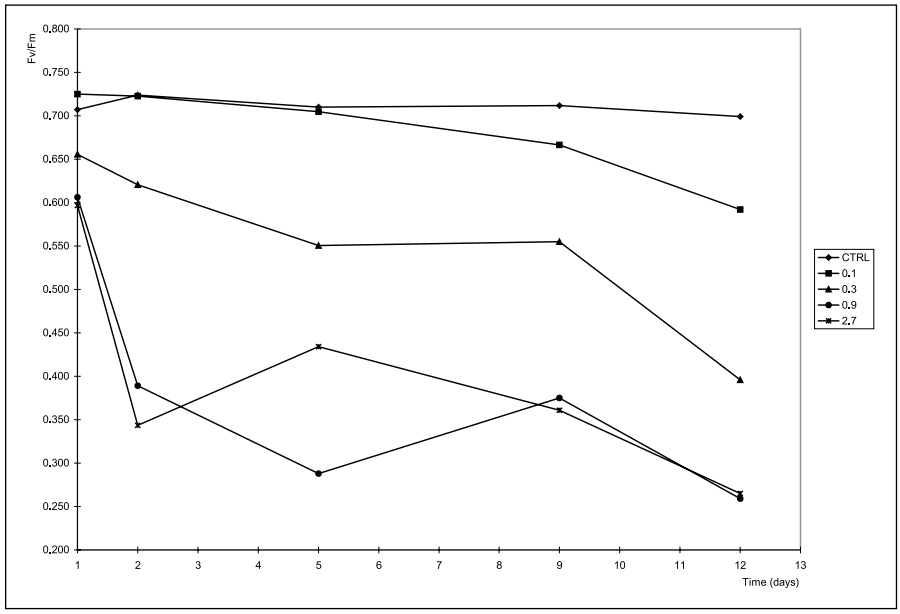

Figure 2. Fv/Fm variations in *U. rigida* under Cu treatments (mg l^{-1}) and control

units. On the other hand, fluorescence responses in the most concentrated Cd solution (2.7 mg l^{-1}) showed decreases lower than 0.500 relative units, which occurred from day 9.

Cadmium bioaccumulation levels transformed into contamination factors (CF), registered successive increases during the experiment. Figure 3 reflects the accumulation kinetics adjusted according to Michaelis-Menten, where the highest accumulation rates within the discs occurred in the most concentrated solutions, such as treatment at 2.7 mg l^{-1} in which *U. rigida* registered amounts of Cd equivalent to 120 times the control levels. Copper presented low accumulation levels, since the discs in the same concentration accumulated 70 times more Cu than controls (figure 4). Lower rates were observed at less concentrated solutions.

DISCUSSION

Decreases in chlorophyll fluorescence indicate the sensitivity of *U. rigida's* photosynthetic metabolism for Cd and Cu in seawater. In the case of Cd, the most important were 0.3, 0.9 and 2.7 mg l^{-1}, which caused low Fv/Fm responses (figure 1).

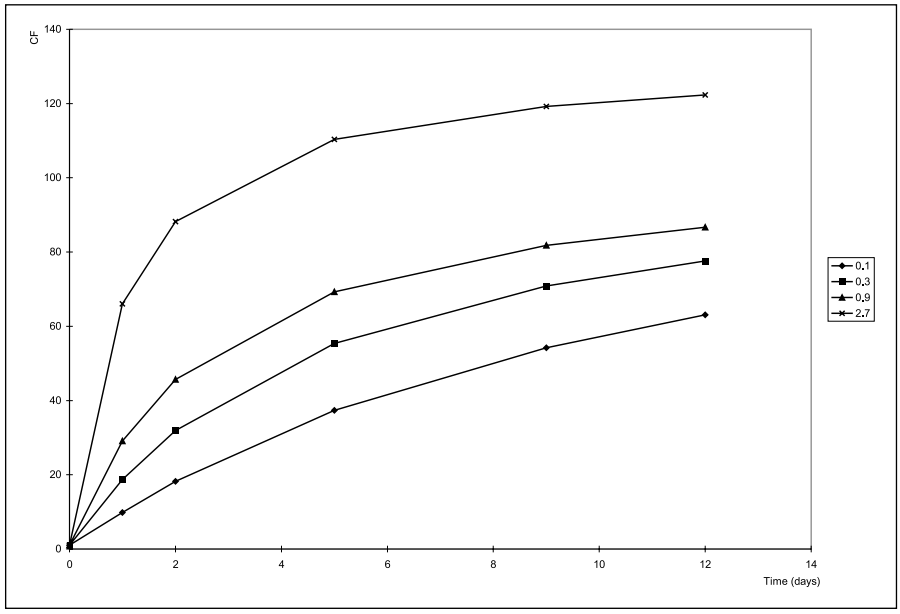

Figure 3. Cd bioaccumulation in *U. rigida*, adjusted according to Michaelis-Menten. Concentrations in mg l^{-1}

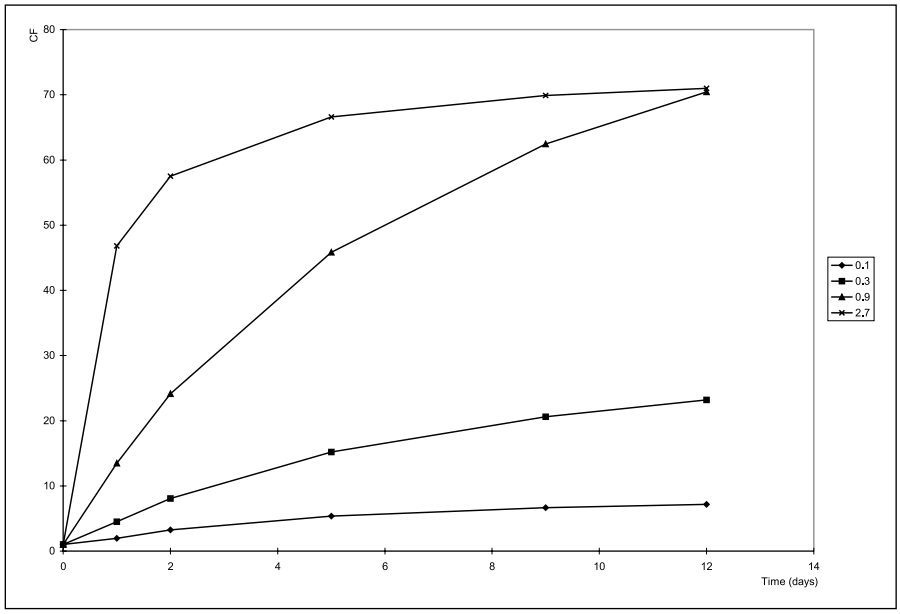

Figure 4. Cu bioaccumulation in *U. rigida*, adjusted according to Michaelis-Menten. Concentrarions in mg l^{-1}

Different trends were observed in treatment 0.1 and control, where levels were above 0.700 relative units. We assumed that the decreases in chlorophyll fluorescence are related to Cd presence, which caused disturbances in algae's photosystem II and also in the electrochemical balance of cells (Trevors, Stratton and Gadd, 1986; Webster and Gadd, 1992, 1996a).

Figure 2 shows responses as expected: higher concentrations of Cu yielded lower values of chlorophyll fluorescence. At the end of the experimental period, only control discs presented values above 0.70, whereas the others showed evidence of photochemistry disturbance. Unlike Cd, the toxic effect of Cu was immediate, since after the first 24 h, discs reflected Fv/Fm values lower than 0.70 in solutions 0.3, 0.9 and 2.7 mg l^{-1}. Wilson and Freeberg (1979) detected toxic concentration ratios (24-48 h) for four microalgae using fluorescence techniques. Such values varied from 0.07 to 5.00 mg Cu l^{-1}; however, *U. rigida* appears to be more resistant to that metal (lowest toxic concentration: 0.3 mg l^{-1}).

Figure 3 shows Cd accumulation in *U. rigida* discs, adjusted according to Michaelis-Menten. This alga showed high affinity for Cd, despite its high toxicity in live organisms (Förstner and Witmann, 1979; Haritonidis, Häger and Schwantes, 1983; Chandra and Grag, 1992; Webster and Gadd, 1992, 1996a,b; Krupa, Öquist and Huner, 1993). Levels of 120 units of CF were observed for treatment at 2.7 mg l^{-1}. These results coincide partially with those of Scoullos and Cabieri (1991), who found that Cd accumulation in *Ulva lactuca* is higher in the most concentrated solutions.

U. lactuca experienced the uncoupler effect of Cd in respiration, as well as interference on the cell electrochemical balance (Webster and Gadd, 1992, 1996a,b).

Unlike Cd, treatments with Cu caused low accumulation rates (figure 4). An example is the concentration 2.7 mg l^{-1}, in which the discs registered 70 times more Cu than controls. Even though Cu is essential for certain organisms, high concentrations are extremely toxic (Förstner and Wittmann, 1979; Stauber and Florence, 1987; Webster and Gadd, 1996a). As in Cd, these results coincide with those of Scoullos and Caberi (1991), where higher accumulation levels occurred in the discs placed in the most concentrated Cu solutions.

Copper toxicity mechanisms vary according to species and environmental conditions (Lepp, 1981; Stauber and Florence, 1987). In elevated concentrations, this metal disturbs the cell membrane permeability of *U. lactuca*, leading to K+ loss. This creates a severe ionic unbalance, causing gradual cell death (Webster and Gadd, 1996a), as well as having direct toxic impact on photosynthetic metabolism.

When comparing both accumulation rates, we assumed that Cu had caused a major impact on *U. rigida's* metabolism, because the stress reflected by chlorophyll fluorescence was higher than that observed for Cd.

In the present study, we found that under experimental conditions, bioaccumulation rates of

Cd and Cu in *U. rigida* discs are proportional to metal concentration in seawater, proving the utility of this alga for monitoring such metals in coastal zones.

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