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Kinetic measurements of metal accumulation in two marine macroalgae

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Abstract We measured the uptake kinetics of four metals (Cd, Cr, Se and Zn) in two marine macroalgae (the green alga *Ulva lactuca* and the red alga *Gracilaria blodgettii*). Metal uptake generally displayed a linear pattern with increasing exposure time. With the exception of Cr, which exhibited comparable uptake rate constants at different concentrations, uptake rate constants of Cd, Se and Zn decreased with increasing metal concentration, indicating that the seaweeds had a higher relative uptake at lower metal concentration. Uptake of Cd and Zn was higher in *U. lactuca* than in *G. blodgettii*, whereas uptake of Cr and Se was comparable between the two species. Only Cd and Zn uptake in *U. lactuca* was significantly inhibited by dark exposure. A decrease in salinity from 28 to 10‰ enhanced the uptake of Cd, Cr, Se and Zn in *U. lactuca* 1.9-, 3.0-, 3.6-, and 1.9-fold, respectively. In *G. blodgettii*, Cd uptake increased two-fold when salinity was decreased from 28 to 10‰, whereas uptake of Cr and Zn was not significantly affected by salinity change. The calculated depuration rate constants of metals in *U. lactuca* were 0.01 d^{-1} for Cd, 0.05 to 0.08 d^{-1} for Cr, 0.14 to 0.16 d^{-1} for Se, and 0.12 to 0.15 d^{-1} for Zn, and were relatively independent of the metal body burden in the algae. The predicted bioconcentration factor was 3×10^4 for Cd, 2×10^3 for Cr, 40 to 150 for Se, and 1 to 2×10^4 for Zn in *U. lactuca*. Our kinetic study suggested that *U. lactuca* would be a good biomonitor of Cr and Zn contamination in coastal waters.

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Introduction

Marine macroalgae (e.g. brown algae: *Fucus vesiculosus* and *Laminaria digitata*; green algae: *Ulva lactuca* and *Enteromorpha* sp.) have been used as biomonitors of metal contamination in many coastal waters (e.g. Fowler 1979; Phillips 1990, 1993; Rainbow and Phillips 1993). They are abundant in coastal and estuarine systems and can be relatively easily collected for metal analysis. An important assumption underlying biomonitoring programs is that metal concentrations in the seaweeds are directly proportional to the bioavailable metal concentrations in the water column. Many field studies have indicated that metal concentrations in these seaweeds respond rather faithfully to gradients of metal concentration in their environments (Bryan and Hummerstone 1973; Morris and Bale 1975; Foster 1976; Seeliger and Edwards 1977; Forsberg et al. 1988; Ho 1990; Say et al. 1990; Barreiro et al. 1993; Haritonidis and Malea 1995). Because metals are available to the seaweeds only from the dissolved phase, concentrations in these organisms may indicate the bioavailable levels of a metal in the solute phase. Biomonitors also integrate short-term temporal variation of metal concentration in the environments. Rainbow and Phillips (1993) proposed the employment of a suite of biomonitors from different ecological groups (e.g. macrophyte, suspension feeder and deposit feeder) to monitor contamination in a particular coastal and estuarine habitat (e.g. contaminants in the water, the seston and natural sediments).

Despite the importance of macroalgae as potential biomonitors, there are very few experimental studies of metal accumulation in these organisms, in contrast to other organisms such as mussels, which have been most extensively used as biomonitors of coastal contamination (Goldberg et al. 1983; O'Connor 1992). Most previous studies measured metal concentrations in field-collected macroalgae and assessed their suitability as biomonitors based on these field measurements (e.g. Talbot and Chegwidden 1982; Ho 1990). Few experi-

mental studies examined the kinetics of metal uptake in these species (Gutknecht 1965; Bryan 1969; Bunt 1970; Skipnes et al. 1975; Rice and Lapointe 1981; Boisson et al. 1997) and the factors (e.g. metal concentration, light, temperature, salinity) that can potentially affect metal accumulation remain less well quantified. Metal accumulation in seaweeds appears to be controlled by an initial absorption (e.g. with cell wall polysaccharide), followed by uptake to the membrane vacuoles containing polyphenols at high concentration (Phillips 1990), but the mechanisms of metal uptake are likely to vary for different metals and under different ecological conditions. The kinetics of metal uptake and factors affecting metal accumulation must be thoroughly investigated to assess the suitability of macroalgae as biomonitors for a variety of metal contaminants. Furthermore, the seaweeds are at the bottom of the food chain and are frequently exploited by man as they are harvested commercially for food, feed and fertilizers (Lobban and Harrison 1994). The potential transfer of contaminants (including metals) to higher trophic levels remains to be examined.

Both equilibrium and kinetic approaches have been used to study metal accumulation and bioavailability in aquatic organisms (Landrum et al. 1992; Luoma and Fisher 1997; Wang and Fisher 1997). The equilibrium approach assumes an equilibrium partitioning of metals between organism and ambient environment; thus the bioconcentration factor empirically obtained under laboratory conditions can be applied to predict metal concentration in organisms in the field. For many aquatic organisms including macroalgae, reaching equilibrium may require a significantly long period of time (e.g. months in the brown alga *Laminaria digitata*, Bryan 1969), and the study of bioaccumulation in these groups of organisms must rely on the kinetic approach. The kinetic model has been developed for various contaminants (reviewed in Landrum et al. 1992) and is now actively applied in studying contaminant accumulation in aquatic invertebrates (e.g. Boese et al. 1997; Wang and Fisher 1997; Reinfelder et al. 1998). The kinetic approach is not constrained by constant exposure or thermodynamic equilibrium and thus is more useful for the prediction of metal accumulation in natural environments where metal exposure is characterized by both temporal and spatial variations.

In the present study, we examined the kinetics of metal uptake (or accumulation) in two species of macroalgae, the green alga *Ulva lactuca* and the red alga *Gracilaria blodgettii*. *U. lactuca* has been previously employed as a biomonitor in the coastal waters of Hong Kong (Ho 1990). Four metals (Cd, Cr, Se and Zn) were selected for this study because of their great environmental impacts and availability of radioisotopes as tracers for these metals. Among the four metals, Cd and Zn are borderline hard/soft metals but tend to bind with proteins (Nieboer and Richardson 1980). Both Cr [Cr(III) and Cr(VI)] and Se [Se(IV), Se(VI), and organoselenium, Cutter and Bruland (1984)] have different redox species in aquatic systems. For Cr, we examined the uptake of Cr(VI) primarily because it is the dominant dissolved species in marine waters whereas Cr(III) is mainly associated with the particulate phase (Abu-Saba and Flegal 1995). For Se, we examined the uptake of Se(IV) (selenite) because of its much higher uptake rate than Se(VI) (selenate) in phytoplankton (Besser et al. 1993; Riedel et al. 1996).

Materials and methods

The green alga *Ulva lactuca* (Chlorophyte) and the red alga *Gracilaria blodgettii* (Rhodophyte) were collected from the Clear Water Bay, Kowloon, Hong Kong (22°20′N; 114°15′E). Upon arrival in the laboratory, the seaweeds were thoroughly cleaned and any epibiotics were carefully removed. The macroalgae were then maintained in the aquarium within the laboratory for 4 weeks before the experiments. During this acclimation period, no nutrient was added into the water, but the water was changed regularly. All experiments were conducted at 18 °C and 28‰ (except the salinity experiment described below).

General experimental approach

Radiotracers (^{109}Cd, $t_{1/2}$ = 462 d; ^{51}Cr, $t_{1/2}$ = 27.7 d; ^{75}Se, $t_{1/2}$ = 120 d; and ^{65}Zn, $t_{1/2}$ = 244 d) were employed to follow the kinetics of stable metal uptake by the seaweeds. The seawater used in the experiments was collected from the open waters 10 km east of Hong Kong, an area considered to be remote from any human activity. Seawater was then filtered through 0.2 μm Poretics membranes, and radioisotopes and their respective stable metals were added. Radioisotope additions were 6.2 to 9.2 kBq l^{-1} (corresponding to 0.75–1.1 n*M*) for ^{109}Cd (in 0.1 *N* HCl), 9.2 to 21.6 kBq l^{-1} (corresponding to 19–45 p*M*) for ^{51}Cr (in distilled water, chromate, $Na_2{}^{51}CrO_4$), 6.2 to 21.6 kBq l^{-1} (corresponding to 0.1–0.4 n*M*) for ^{75}Se (in distilled water, selenite, $Na_2{}^{75}SeO_3$), and 6.2 to 9.2 kBq l^{-1} (corresponding to 0.7–1.1 n*M*) for ^{65}Zn (in 0.1 HCl). The stable metals were from stocks of 1 mg ml^{-1}. Before the additions of the radioisotopes and stable metals, microliter amounts of 0.5 *N* Suprapur NaOH were added to maintain the final pH at 8.0. Radioisotopes and stable metals were then allowed to equilibrate in the dark for 12 to 16 h before the uptake experiments.

Ulva lactuca were cut into pieces (8 to 15 mg tissue dry weight), and their tissue surfaces were cleaned again using a cotton tip. No evidence indicated that uptake was significantly affected by cutting the algae into pieces (Ho 1993). They were then maintained in 0.2 μm filtered seawater for 1 d before the experiments. Because a previous study (Ho 1993) indicated that different tissues of seaweeds may have different rates of nutrient uptake, we only used the central thalli in the experiments; marginal tissues and the thallus near the holdfast were not used. Each tissue was then placed individually into 100 ml seawater containing both radioisotopes and stable metals. The uptake kinetics of the metals in the seaweeds were then followed for 1 to 2 d. At time intervals (2, 6, 11, 23, 35, 47 h), plants were removed from the radioactive waters, rinsed with 0.2 μm filtered seawater and then placed into vials containing 4 ml of 0.2 μm filtered seawater. The radioactivity was counted for 2 min (described in the following section). Individual seaweeds were then returned into new batches of seawater containing the same concentrations of metals. The renewal of radioactive waters at time intervals minimized the decline of metal concentration in the water due to seaweed uptake, thus maintaining a relatively constant metal concentration in the water throughout the exposure period. The amount of radioactivity in the particulate phase was also determined by filtering 10 ml of water onto 0.2 μm polycarbonate membranes. Results confirmed that very little radioactivity (<1%) was detected in the particulate

phase. Consequently, uptake by seaweeds was considered to be from the dissolved phase.

The influence of metal concentration on metal uptake was examined. Concentrations were 0.5, 2, 10, 50 μg l^{-1} for Cd, Cr and Se, and 1, 5, 20, 100 μg l^{-1} for Zn. The lowest concentrations employed in the experiments were about 2 to 25 times higher than the typical metal concentrations in the coastal waters (Bruland 1983; Flegal et al. 1991). Our preliminary results indicated that the light:dark cycle had a significant influence on metal accumulation in the seaweeds. All the kinetic measurements were therefore performed under light conditions (28.5 μE m^{-2} d^{-1}). Uptake rates were also determined by exposing seaweeds to light and dark, respectively, at a concentration of 2 μg l^{-1} for Cd, Cr and Se, and 5 μg l^{-1} for Zn. In addition, we also tested the effects of salinity on metal uptake in the seaweeds. Macroalgae were acclimated to different salinities (10, 15, 20, and 28‰) for 3 d before the uptake experiments. Lower salinity was prepared by diluting normal seawater (28‰) with Nanopure distilled water (18 mΩ-cm). Uptake rates were then determined at a concentration of 2 μg l^{-1} for Cd, Cr and Se, and 5 μg l^{-1} for Zn. At the end of the metal exposure, the seaweeds were rinsed with distilled water and dried at 70 °C for 1 d, and their dry weights were determined. Metal concentrations in the seaweeds were calculated on the basis of dry weight.

In another experiment, individual *Ulva lactuca* seaweeds were exposed to different metal concentrations for 2 d, and then placed into 250 ml filtered seawater to depurate the accumulated metals for 10 d. At time intervals, the radioactivity retained in each seaweed was determined and water was replaced with a new batch of seawater.

Radioactivity measurements

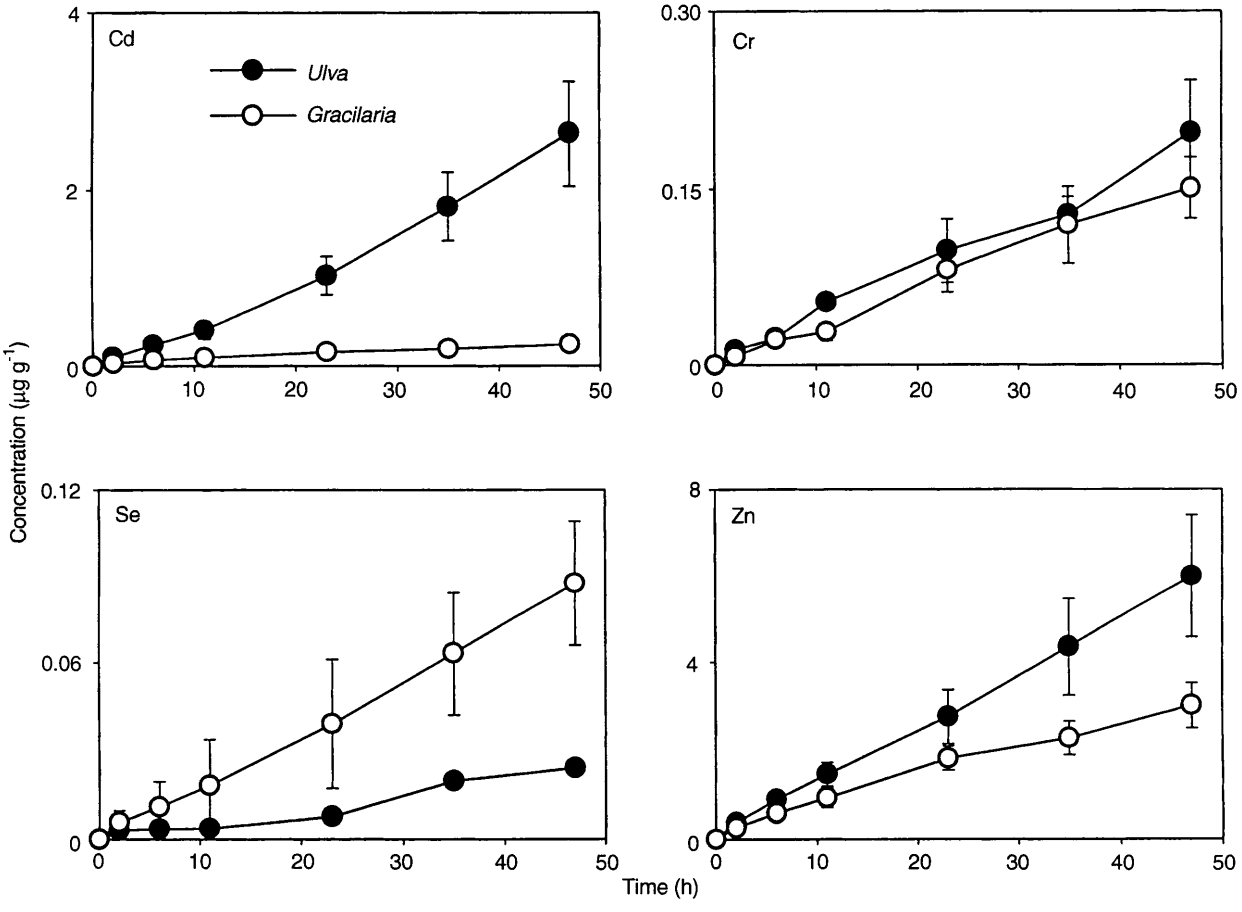
Radioactivity of ^{109}Cd, ^{51}Cr, ^{75}Se and ^{65}Zn in the water and in the seaweeds was determined by a Wallac Wizard 1480 NaI γ detector. Gamma emission was determined at 22 keV for ^{109}Cd, 320 keV for ^{51}Cr, 264 keV for ^{75}Se and 1115 keV for ^{65}Zn. Spillover of radioisotopes from a higher energy window to a lower energy window was calibrated and all counts were related to standards. Counting times were adjusted to result in propagated counting errors of <5 to 10%.

Results

Metal uptake at different metal concentrations

The uptake (accumulation) of trace elements in two seaweeds at the lowest concentration examined (4.46 n*M* for Cd, 9.61 n*M* for Cr, 6.33 n*M* for Se and 15.4 n*M* for Zn) is shown in Fig. 1. In general, a linear pattern of metal uptake over time was found during the 47 h exposure period. No steady state was evident at 47 h. At higher metal concentrations, however, there was an initial rapid adsorption of metals (especially for Cd, Se and Zn) within the first 2 h, followed by a linear pattern of metal uptake (which was presumably dominated by internalization) between 2 and 47 h (Fig. 2).

Fig. 1 *Ulva lactuca*, *Gracilaria blodgettii*. Metal accumulation in seaweeds over a 2 d exposure period; mean ± SD (n = 3). The concentration of metals in the dissolved phase was 4.46 n*M* for Cd, 9.61 n*M* for Cr, 6.33 n*M* for Se and 15.4 n*M* for Zn



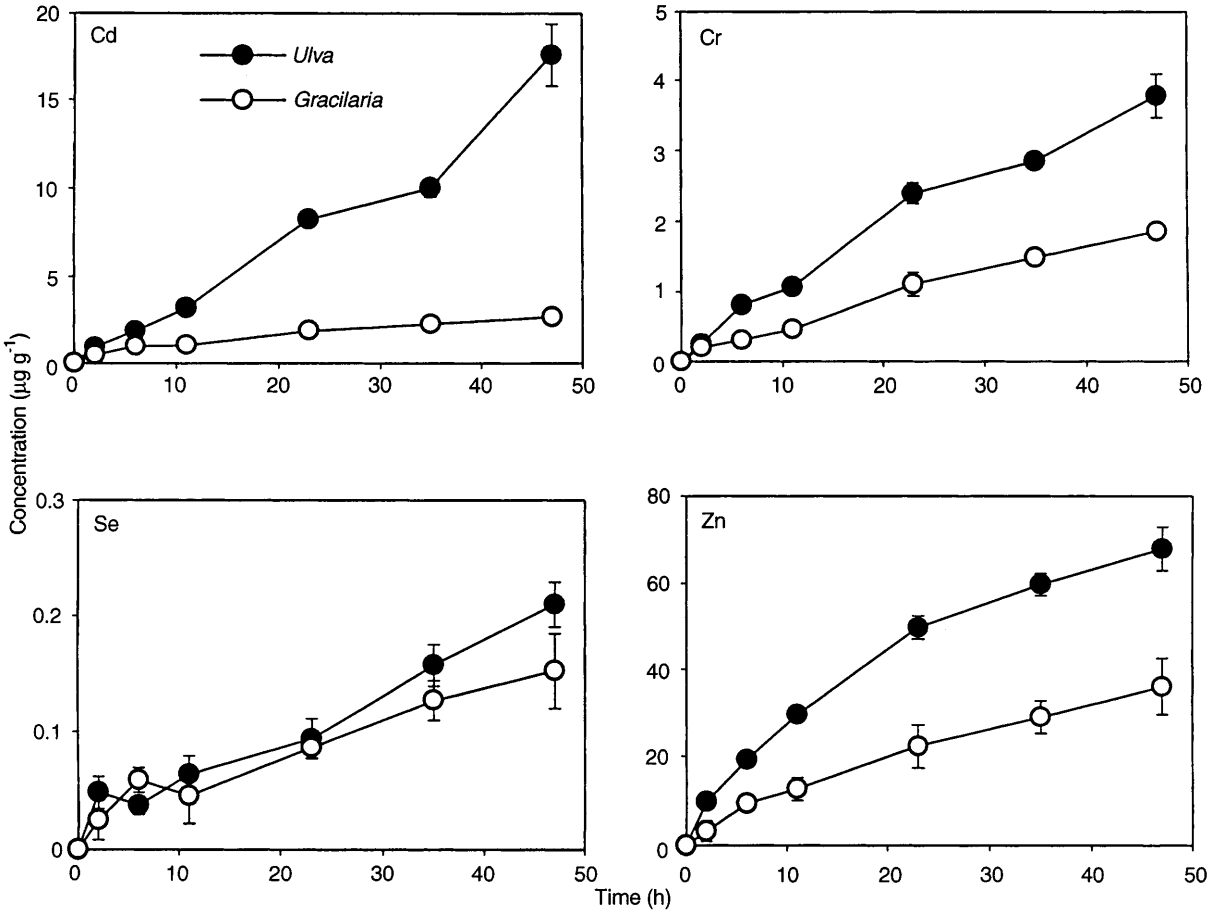


Fig. 2 *Ulva lactuca*, *Gracilaria blodgettii*. Metal accumulation in seaweeds over a 2 d exposure period; mean ± SD ($n = 3$). The concentration of metals in the dissolved phase was 89 n*M* for Cd, 192 n*M* for Cr, 126 n*M* for Se and 308 n*M* for Zn

Uptake rates were therefore calculated as the slopes of the linear regression between metal concentration in seaweeds and duration of exposure between 2 and 47 h, and then correlated with metal concentrations in the dissolved phase (Fig. 3). A log–log linear relationship between metal uptake rate and metal concentration in the dissolved phase was consistently observed for all four trace elements. The coefficients describing the log–log relationship were close to 1 for Cr in both species, and their uptake rates were thus directly proportional to Cr concentration in the dissolved phase. For other metals (except Zn in *Gracilaria blodgettii*), the coefficients were typically <1, indicating that metal uptake rate constants were higher at lower metal concentrations. For example, the uptake rate for Cd in *Ulva lactuca* varied by 13-fold with two orders of magnitude variation in Cd concentration in the dissolved phase. The uptake rates were 3 to 14 times higher for Cd and 1.8 to 2.2 times higher for Zn in *U. lactuca* than in *G. blodgettii*, but were comparable for Cr and Se between these two species (Fig. 3). We did not measure the surface area of the algae, thus comparison of their uptake rates in terms of surface area was not possible.

Metal uptake during light and dark exposure

Metal uptake during light or dark exposure proceeded linearly over time between 2 and 23 h (Figs. 4, 5). Uptake rates were calculated as the slopes of the linear regression between metal concentration in seaweeds and exposure time (Table 1). In *Ulva lactuca*, dark exposure significantly inhibited the uptake of Cd (by 5.2-fold) and Zn (by 1.4-fold) ($P < 0.05$), but the uptake of Cr and Se was relatively unaffected by dark exposure. Metal uptake in *Gracilaria blodgettii* was not significantly different between light and dark exposure ($P > 0.05$), suggesting that uptake in this species was comparatively independent of the light or dark conditions. We also measured metal accumulation in heat-killed (by boiling) *U. lactuca*. In contrast to live seaweeds, uptake rates were much higher in killed seaweeds for all four trace elements. By the end of 23 h exposure, concentrations were 1.5-fold for Cd, 1.4-fold for Cr, 3-fold for Se, and 2-fold for Zn higher than in light-exposed live seaweeds. A linear pattern of uptake was found for Cr and Zn, whereas uptake of Cd and Se appeared to level off at 11 and 2 h, respectively.

Metal uptake at different salinities

A linear pattern of metal uptake was also found for both seaweed species between 2 and 23 h (Figs. 6, 7). How-

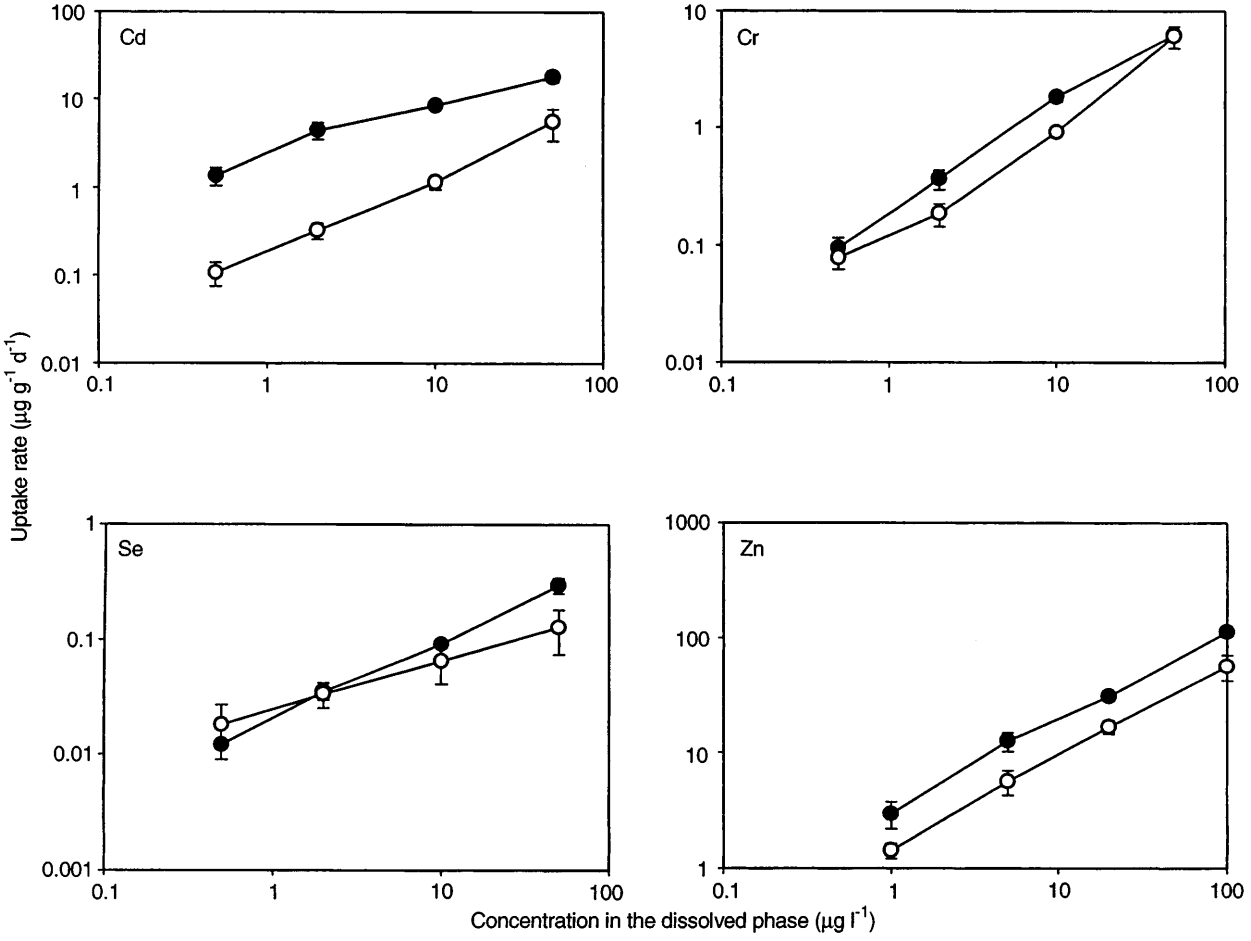


Fig. 3 *Ulva lactuca*, *Gracilaria blodgettii*. The uptake rates of metals in seaweeds, *U. lactuca* (●) and *G. blodgetti* (○), at different metal concentrations in the dissolved phase; mean ± SD ($n = 3$). Equations describing the relationships between uptake rates (I, µg g^{-1} d^{-1}) and metal concentrations in the dissolved phase (C_w, µg l^{-1}) follow. *U. lactuca*: for Cd, $I = 2.362\ [C_w]^{0.542 \pm 0.065}$ ($r^2 = 0.972$); for Cr, $I = 0.190\ [C_w]^{0.918 \pm 0.039}$ ($r^2 = 0.996$); for Se, $I = 0.020\ [C_w]^{0.686 \pm 0.024}$ ($r^2 = 0.997$); for Zn, $I = 3.145\ [C_w]^{0.779 \pm 0.031}$ ($r^2 = 0.997$). *G. blodgettii*: for Cd, $I = 0.184\ [C_w]^{0.847 \pm 0.032}$ ($r^2 = 0.997$); for Cr, $I = 0.121\ [C_w]^{0.953 \pm 0.084}$ ($r^2 = 0.985$); for Se, $I = 0.025\ [C_w]^{0.612 \pm 0.039}$ ($r^2 = 0.992$); for Zn, $I = 1.880\ [C_w]^{0.924 \pm 0.107}$ ($r^2 = 0.974$)

ever, Se showed an abnormally high uptake between 12 and 23 h, i.e., its concentration in *Ulva lactuca* increased by 5- to 8.4-fold at 10 and 15‰, compared to a 1.7- to 3.4-fold increase at 20 and 28‰ during this period. In *Gracilaria blodgettii*, concentration increased by 2.6-fold at 10‰, compared to a 1.7- to 2.2-fold increase at higher salinities. In a replicate experiment with *U. lactuca*, the results were also comparable, e.g., 5.4- to 14.8-fold increase at 10 and 15‰, compared to 2.2–5.5-fold increase at 20 and 28‰. The uptake rates of Se were therefore calculated only between 2 and 12 h. Salinity had a significant influence on metal uptake in *U. lactuca* (Table 2, ANOVA, single-factor analysis, Zar 1996). Lowered salinity enhanced metal uptake considerably. For example, the uptake rate increased by 2- to 3-fold when the salinity decreased from 28 to 10‰. For *G. blodgettii*, no significant influence of salinity on metal uptake was found for Cd, Cr or Zn, whereas Se uptake was significantly affected by salinity ($P < 0.05$, ANOVA). However, the Cd uptake rate at 10‰ was two times higher than at 28‰.

Metal depuration in *Ulva lactuca*

A two-compartmental loss was found for all four metals (Fig. 8). There was an initial metal loss when the seaweeds were transferred to non-radioactive waters within the first 2 d, followed by a slower loss between 2 and 10 d. To calculate the depuration (=efflux) rate constant from the slower exchanging compartment, we regressed the percentage of metals retained in *U. lactuca* over time (between 2 and 10 d, Table 3). In general, metal loss was independent of the metal exposure concentration (and thus metal body burden in the tissues). There was no major influence of metal exposure concentration on the depuration rate constant or the percentage of metals distributing in the slower exchanging compartment. Among the four metals, Cd was most slowly lost from the seaweeds; its loss rate was not significantly different from zero, suggesting that *U. lactuca* retained Cd very efficiently in their tissues. Both Se and Zn were lost from

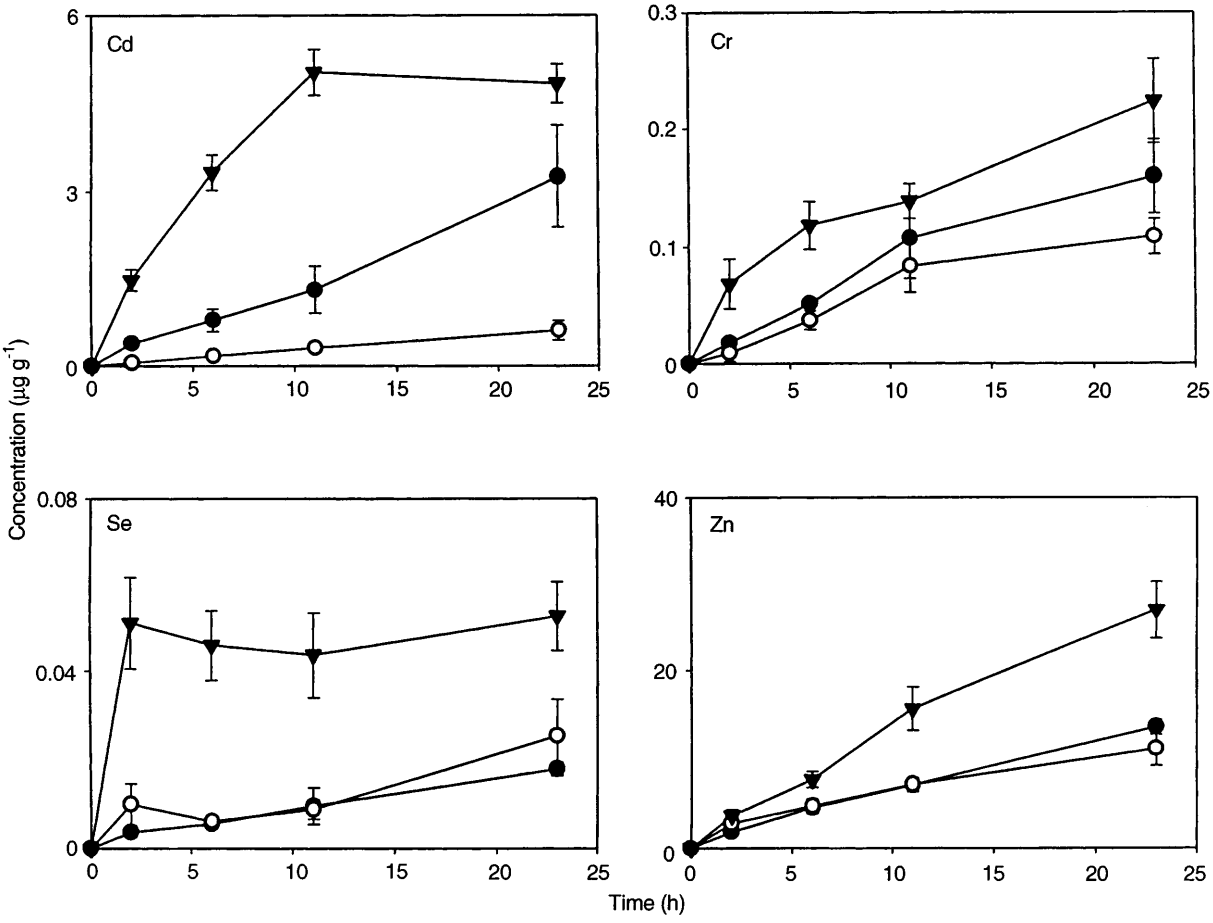


Fig. 4 *Ulva lactuca*. Metal accumulation in seaweed exposed to light (●) and dark (○). The uptake of heat-killed seaweed (▼) is also presented. Mean ± SD (n = 3)

the tissues rapidly, and their calculated efflux rate constants were as high as 0.12 to 0.16 d^{-1}. Cr was lost from the seaweeds with an efflux rate constant of 0.05 to 0.08 d^{-1}. The proportion of metals partitioning in the slower exchanging compartment was 79 to 96% for Cd, 69 to 87% for Cr, 64 to 75% for Se and 70 to 81% for Zn.

Discussion

Our results suggest that metal uptake in two marine macroalgae follows a linear pattern over a 2 d exposure period, consistent with previous studies on the marine brown algae *Laminaria digitata* (Bryan 1969) and *Fucus vesiculosus* (Boisson et al. 1997). At higher metal concentrations, a two-phase uptake pattern is observed, suggesting that absorption onto the seaweed surface may contribute to metal accumulation. Similarly, a two-stage metal uptake has been previously proposed in macroalgae, including an initial metabolism-independent absorption onto the outer cell wall and then a slower incorporation into the cell body (Levine 1984). In our study, we did not consider surface absorption within the initial period of exposure.

The linear relationship between metal uptake rate and metal concentration in the dissolved phase indicates that metal uptake proceeded by passive diffusion or a facilitated transport process. Our data also suggest that metal transport is probably controlled by binding with intracellular protein ligands. Metals (Cd and Zn) that tend to associate with proteins have a much higher uptake rate than metals that are not sulfur seeking (e.g. Cr, Se), thus facilitated transport by binding with protein ligands could be important in the transport of these metals. Similarly, Bryan (1969) proposed that Zn in the brown alga *Laminaria digitata* was probably bound to some soluble substances such as proteins inside the cell. In contrast, Haug and Smidsrød (1967) suggested that accumulation of Ca, Sr and Mg in brown algae (*L. digitata*) was largely the result of ion exchange between seawater and the acid polysaccharide alginate (the main biochemical component in brown seaweeds) in the cell walls. The amount of these ions in the cytoplasm and vacuole comprises a relatively small portion of the total in the whole thallus. Ion exchange was also proposed to be the major mechanism for Zn uptake in seaweeds (including *Ulva lactuca*, Gutknecht 1965) and for Sr accumulation in the brown alga *Ascophyllum nodosum* (Skipnes et al. 1975).

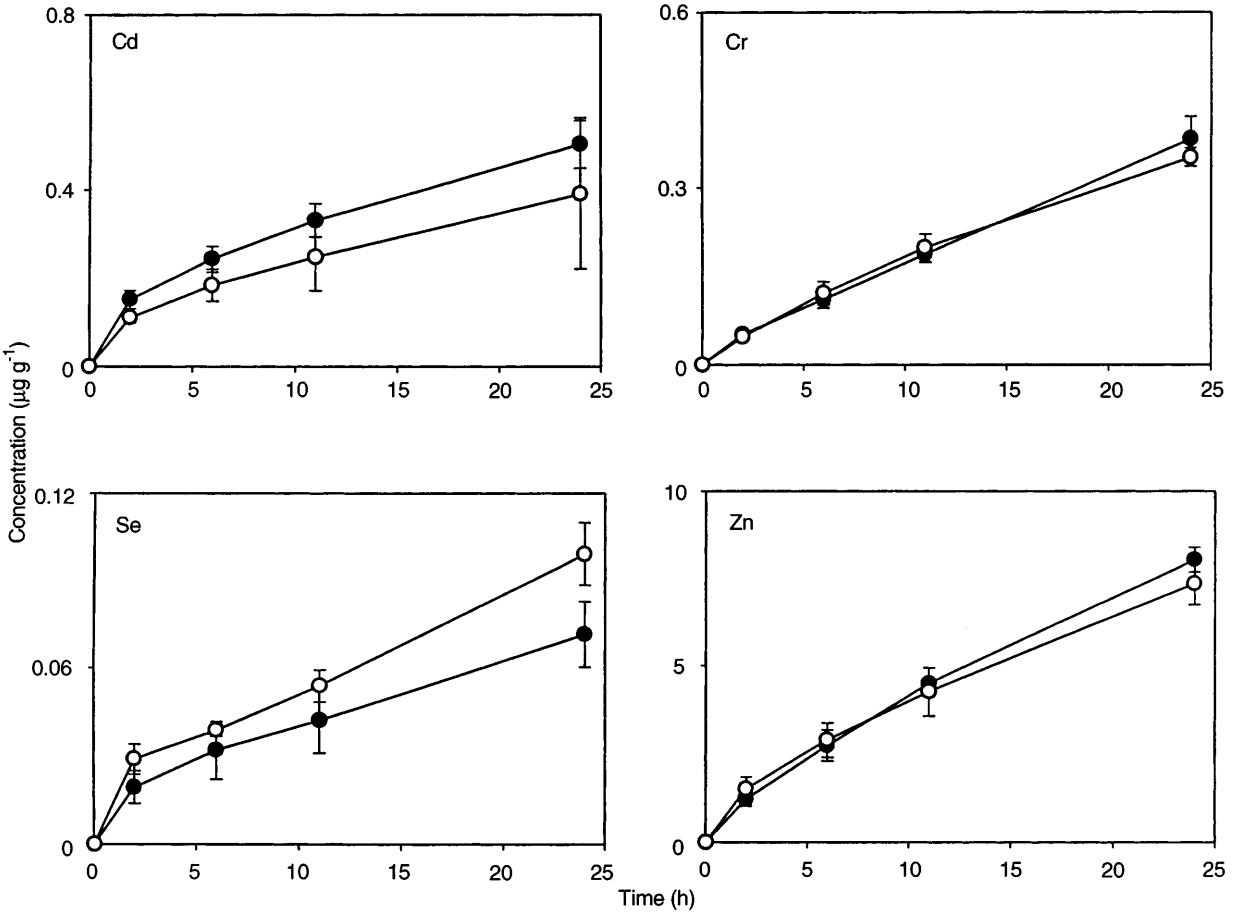


Fig. 5 *Gracilaria blodgettii*. Metal accumulation in seaweed exposed to light (●) and dark (○). Mean ± SD (n = 3)

Compared to Cd and Zn, uptake of both anionic Cr and Se was considerably slower. These anionic elements were presumably taken up through the anionic channels analogous to phosphate and sulfate (Simkiss and Taylor 1995), but this was not specifically investigated in the present study. Se has been shown to behave as a sulfur analog in algae and higher plants, and is metabolized to seleno-amino acids that cannot be incorporated into protein (Wrench 1978; Fisher and Reinfelder 1991). Based on the very low bioconcentration factor of Se (40 to 150), it is unlikely that Se is an essential element for these seaweeds. In marine phytoplankton, Se has been shown to be essential for some species (Harrison et al. 1988), and their bioconcentration factors were in the range of 10^3 to 10^5 (Fisher and Reinfelder 1995).

The increase in metal uptake in heat-killed *Ulva lactuca* is consistent with earlier findings (Gutknecht 1963, 1965; Skipnes et al. 1975). These results suggest that non-metabolic absorption plays an important role in the metal uptake by seaweeds. Consequently, both the cytoplasm and extracellular components have a large quantity of binding sites available for metals. The binding substances in the living plants are contained in a membrane-surrounded structure (probably vacuoles) (Skipnes et al. 1975). Following destruction of the membranes by heat, the binding substances were then directly accessible for metal binding. Furthermore, these binding ligands were probably not released into the dissolved phase when the heat-killed algae were exposed to the metals.

The coefficient describing the log–log relationships between uptake rate and dissolved concentration was <1 for Cd, Se and Zn, but was close to 1 for Cr. Cd was highly regulated by *Ulva lactuca* during its uptake, and its uptake rate increased only 13-fold while the Cd concentration increased 100-fold. Ho (1990) found that Cd concentrations in *U. lactuca* collected at different locations off Hong Kong Island were very comparable, whereas a clear concentration gradient was easily identified for other metals, which is consistent with our experimental results. This species may thus not be the best biomonitor for Cd contamination in coastal waters. Similarly, Se uptake rates in *U. lactuca* and *Gracilaria blodgettii* were partially regulated and their coefficients were only 0.686 and 0.612, respectively. Some regulation of Mn uptake in *Fucus vesiculosus* was also documented by Morris and Bale (1975).

A few field studies have demonstrated that metal concentrations in seaweed reflected metal concentrations in ambient environments (Morris and Bale 1975; Seeliger and Edwards 1977). Laboratory studies also re-

Table 1 *Ulva lactuca*, *Gracilaria blodgettii*. Uptake rates of metals (μg g^{-1} d^{-1}) in two marine macroalgae exposed to light and dark for 23 h. Data are presented as mean ± SD (n = 3). Statistically significant differences between light and dark treatments are indicated by * ($P < 0.05$, t-test)

Illumination	Cd	Cr	Se	Zn
Ulva lactuca				
Light	3.307 ± 0.917	0.160 ± 0.037	0.016 ± 0.002	13.16 ± 1.00
Dark	0.639 ± 0.205 (*)	0.112 ± 0.015	0.020 ± 0.008	9.37 ± 1.27 (*)
Gracilaria blodgettii				
Light	0.369 ± 0.036	0.359 ± 0.033	0.056 ± 0.006	7.38 ± 0.49
Dark	0.295 ± 0.028	0.322 ± 0.012	0.078 ± 0.009	6.30 ± 0.44

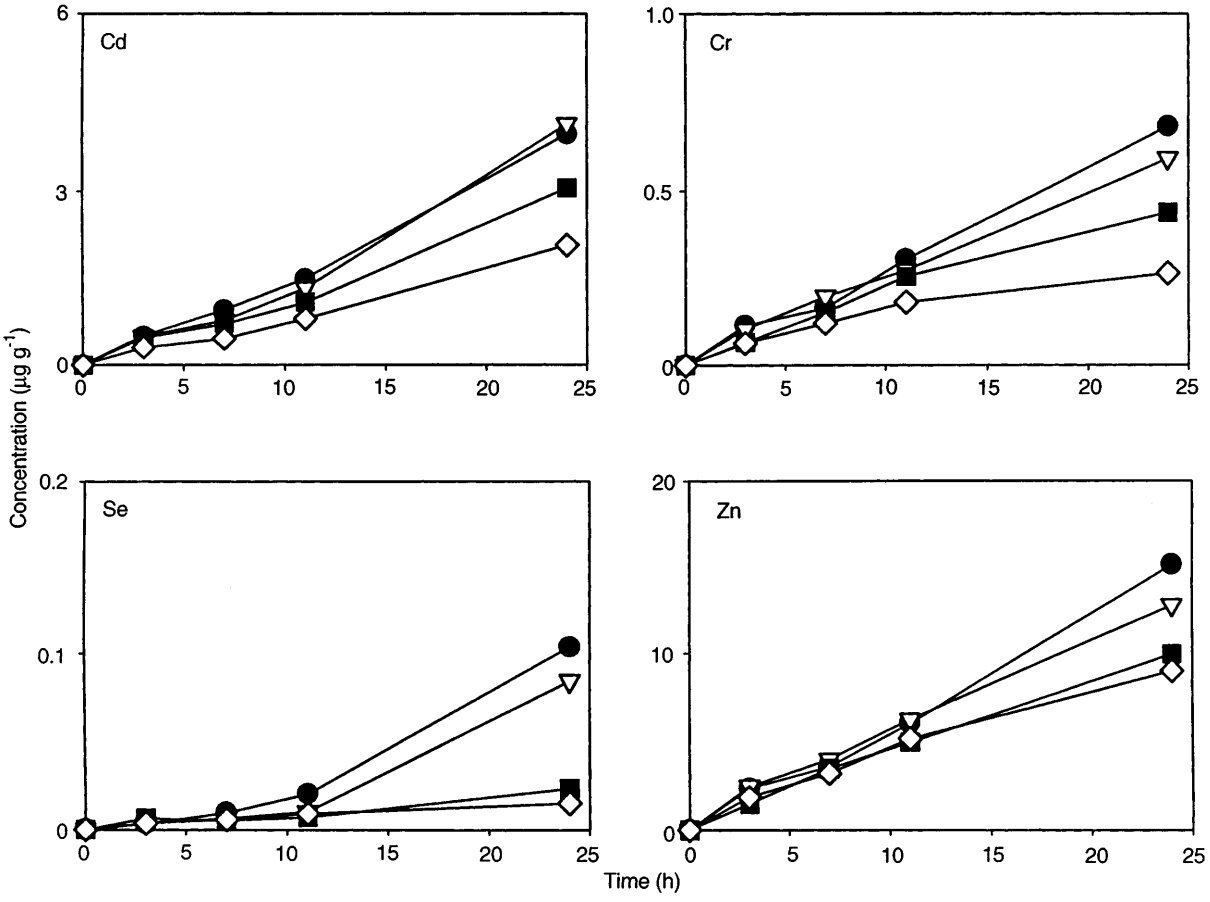


Fig. 6 *Ulva lactuca*. Metal accumulation in seaweed at different salinities: 10‰ (●), 15‰ (▽), 20‰ (■) and 28‰ (◇). Standard deviations of the uptake rates are presented in Table 2

vealed a linear relationship between Zn concentration in the seaweeds and Zn concentration in the water (Gutknecht 1965; Bryan 1969). In *Laminaria digitata*, the coefficient was <1, thus relatively more Zn was absorbed at low rather than at high Zn concentrations, resulting in a lower bioconcentration factor at higher Zn concentration (Bryan 1969). In contrast, Gutknecht (1965) indicated that Zn concentrations in *Ulva lactuca* and *Porphyra umbilicalis* were directly proportional to Zn concentration in the water (i.e. the *b* coefficients were about 1). In freshwater moss, *Sphagnum papillosum*, uptake rates of Cd, Cr and Zn were also directly proportional to the metal concentration in ambient water (Gstoettner and Fisher 1997).

Dark significantly inhibited the uptake of Cd and Zn in *Ulva lactuca*, but not in *Gracilaria blodgettii*. Dark exposure has been shown to inhibit Zn accumulation in *Laminaria digitata* (by 65%, Bryan 1969) and Cd uptake in the red alga *Gracilaria tenuistipitata* (Hu et al. 1996). In an experimental outdoor study, Rice and Lapointe (1981) demonstrated that the uptake of Cd, Fe and Rb in *Ulva fasciata* increased with decreasing light exposure levels, whereas Zn uptake was unaffected by the light level. However, it is difficult to conclude whether their uptake is light or energy dependent (e.g. Bachmann and Odum 1960). Light can affect nutrient uptake indirectly through photosynthesis, which can provide energy for active transport and produce carbon skeletons that are necessary for incorporation of nutrient ions into larger molecules (e.g. amino acids and proteins) (Lobban and Harrison 1994). In addition, a decrease in photosynthetic activity in plants may lower the intracellular pH,

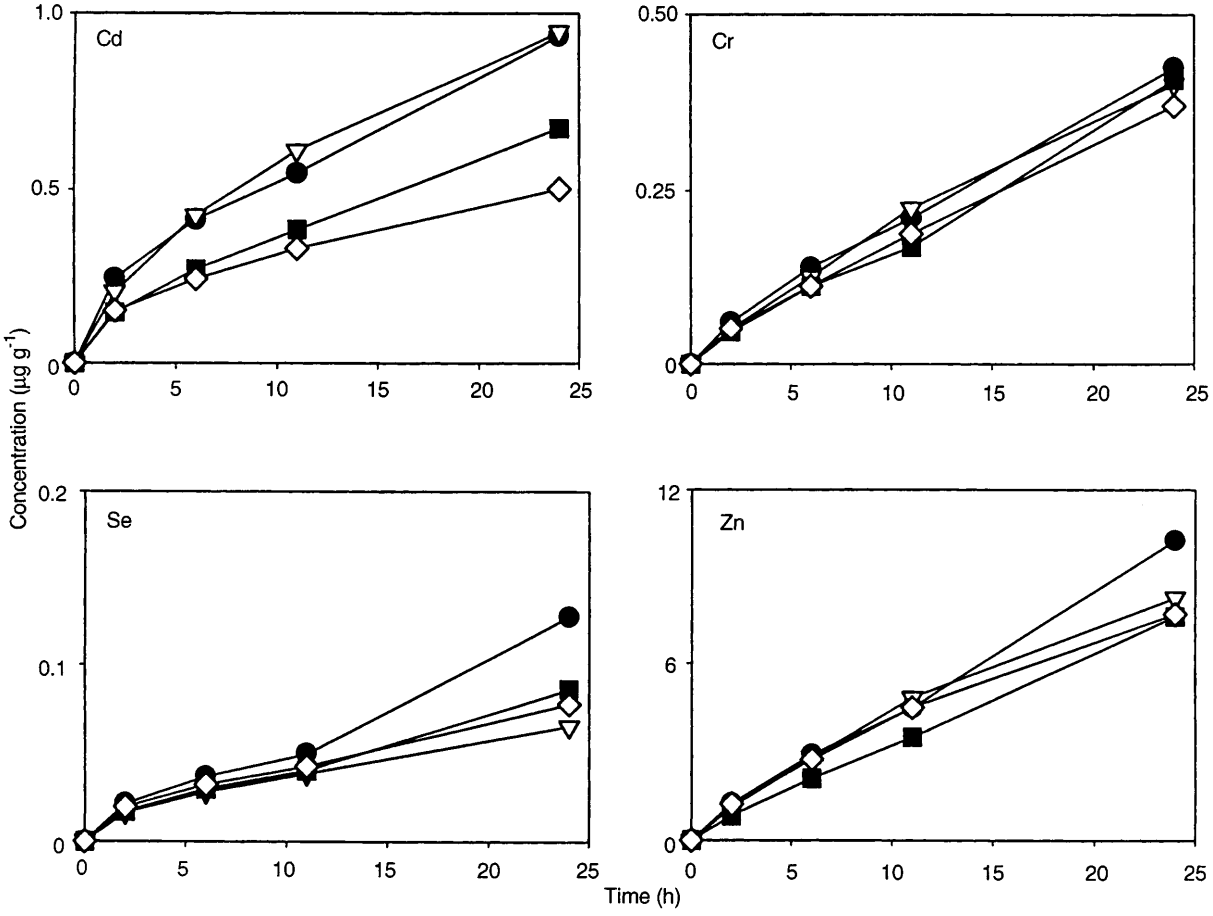


Fig. 7 *Gracilaria blodgettii*. Metal accumulation in seaweed at different salinities: 10‰ (●), 15‰ (▽), 20‰ (■) and 28‰ (◇). Standard deviations of the uptake rates are presented in Table 2

which may decrease the non-metabolic absorption of metals (Gutknecht 1963; Bryan 1969). Among the four trace elements, binding of Cd and Zn may strongly depend on the intracellular pH, and it is likely that a decrease in pH associated with inhibition of photosythetic activity may have contributed to the decrease in their uptake rates.

The effects of salinity on metal uptake varied with the metals, seaweed species and experimental conditions. In our experiments, metal uptake in *Ulva lactuca* was greatly dependent on the salinity, whereas only Se and Cd in *Gracilaria blodgettii* were affected by salinity. In the brown alga *Fucus vesiculosus*, salinity had no apparent effect on Zn uptake but had a negative effect on Cd accumulation (Bryan 1983). Carlson and Erlandsson (1991) however found that variations in salinity (8 to 24‰) did not significantly affect metal (Mn, Zn and Co) uptake in *F. vesiculosus* collected from the same location. In a field study, salinity inversely affected metal concentration in *Ulva rigida* collected from the Lagoon of Venice, Italy, primarily due to the high metal activity and burden of the

Table 2 *Ulva lactuca*, *Gracilaria blodgettii*. Uptake rates of metals (µg g^{-1} d^{-1}) in two marine macroalgae exposed to different salinities for 23 h. Data are presented as mean ± SD (n = 3). Statistically significant effects of salinity on metal uptake are indicated by *($P < 0.05$), **($P < 0.01$), and ***($P < 0.001$) (single-factor ANOVA)

Salinity (‰)	Cd	Cr	Se	Zn
Ulva lactuca				
10	4.038 ± 0.758	0.673 ± 0.045	0.051 ± 0.016	15.25 ± 0.78
15	4.333 ± 0.100	0.558 ± 0.058	0.020 ± 0.014	12.06 ± 1.47
20	3.058 ± 0.241	0.418 ± 0.049	0.071 ± 0.055	9.56 ± 0.52
28	2.087 ± 0.284 (**)	0.220 ± 0.006 (***)	0.014 ± 0.001 (*)	8.16 ± 0.89 (***)
Gracilaria blodgettii				
10	0.746 ± 0.025	0.360 ± 0.054	0.075 ± 0.007	8.86 ± 1.61
15	0.775 ± 0.186	0.378 ± 0.064	0.052 ± 0.008	7.67 ± 0.76
20	0.562 ± 0.235	0.392 ± 0.066	0.076 ± 0.009	7.40 ± 1.51
28	0.369 ± 0.036	0.345 ± 0.051	0.063 ± 0.003 (*)	6.96 ± 0.58

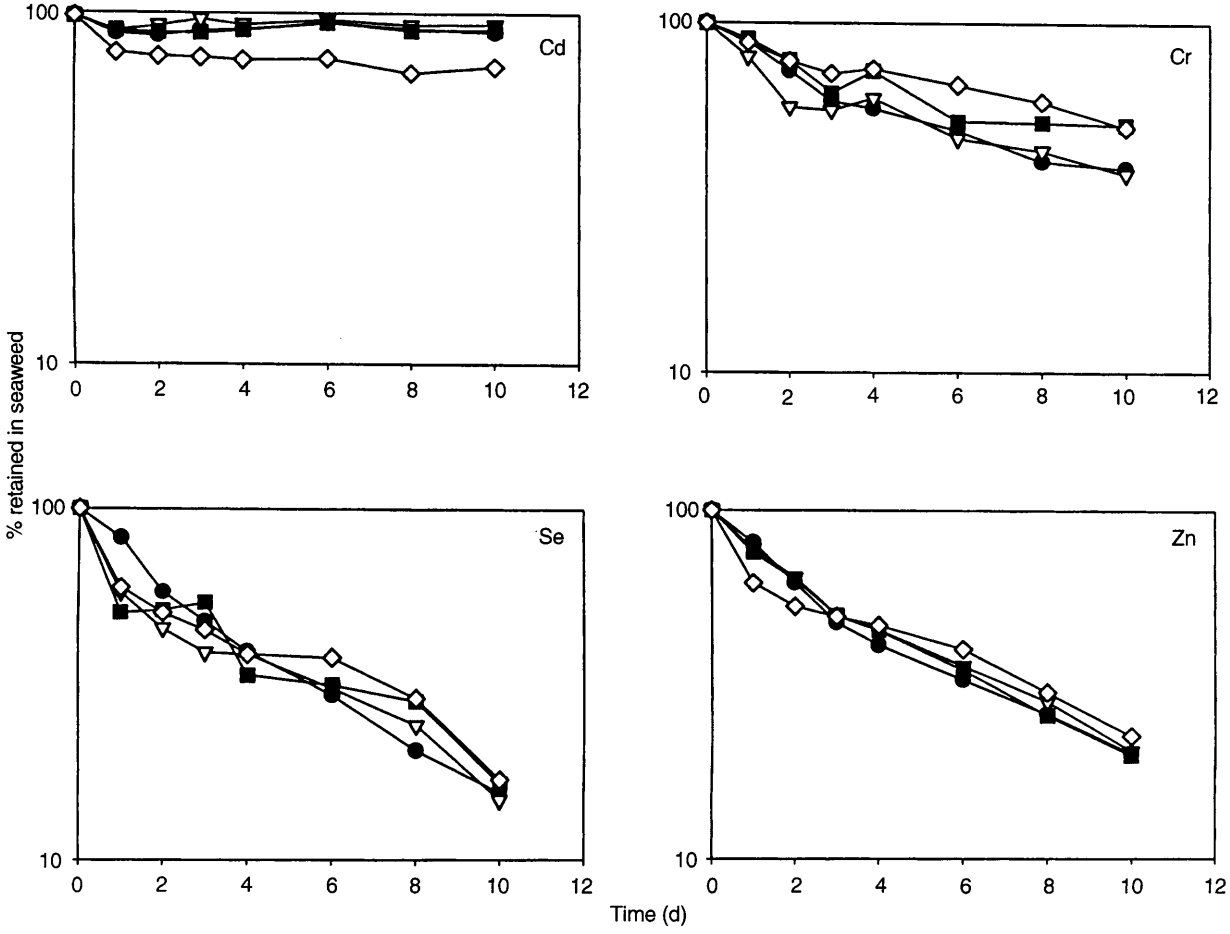


Fig. 8 *Ulva lactuca.* Percentage of metals retained in seaweed following 2 d exposure to different metal concentrations in the dissolved phase. Standard deviations of the depuration rate constant are presented in Table 3 (●: 4.46 n*M* Cd, 9.61 n*M* Cr, 6.33 n*M* Se, 15.4 n*M* Zn; ▽: 17.8 n*M* Cd, 38.5 n*M* Cr, 25.3 n*M* Se, 76.9 n*M* Zn; ■: 89.2 n*M* Cd, 192 n*M* Cr, 126 n*M* Se, 308 n*M* Zn; ◇: 446 n*M* Cd, 961 n*M* Cr, 633 n*M* Se, 1538 n*M* Zn

bioavailable metals in the freshwater flowing into the Venice lagoon (Favero et al. 1996).

Both physico-chemical change (e.g. free ion concentration, activity coefficient or ionic strength) and physiological change (e.g. membrane permeability) are probably responsible for the observed salinity effects. Among the four metals, Cd speciation is mostly influenced by variation in salinity; its free ion concentration may increase by 4-fold with a decrease in salinity from 28 to 10‰ (Mantoura et al. 1978). Speciation of Cr(VI) and Se(IV) and the free ion concentration of Zn are less affected by salinity within this range. When the salinity was lowered from 28 to 10‰, there was a consistent increase (2- to 3-fold) in uptake rates for all metals in *Ulva lactuca*. Thus, it appears that physiological control is more important to the salinity effect than change in metal geochemistry. In *Gracilaria blodgettii*, the uptake rate of Cd increased by 2-fold when the salinity was decreased from 28 to 10‰ (albeit statistically insignificant), whereas no major effect was found for Cr and Zn. Thus, it is likely that an increase in free ion Cd concentration at lowered salinity may be primarily responsible for the observed increase in Cd uptake in this seaweed.

For both seaweed species, the dramatic increase in Se uptake between 12 and 23 h at lowered salinity remained unclear. One possible explanation is the lower concentrations of anionic ions (e.g. sulfate, phosphate) at lower salinity (due to dilution with distilled water). In addition, nutrients may be depleted due to the rapid uptake of seaweeds with increasing duration of exposure. The Se(VI) was then taken up mistakenly as a nutrient. Such a mechanism would necessitate that Cr(VI) (its uptake was constant during this period) and Se(VI) were accumulated by different transport systems. This aspect needs to be examined further. In a previous study, nitrogen availability was found to affect the rate of metal uptake (Fe, Mn, Zn, Cd and Rb) by *Ulva fasciata* (Rice and Lapointe 1981). Such an effect was primarily due to the change in the seaweed growth rate as a response to variation in environmental conditions. For example, concentrations of Cd and Rb decreased, whereas Mn concentration increased with increasing specific growth rate.

Very little Cd was lost from *Ulva lactuca* after being transferred to clean seawater, in contrast to Cr, Se and

Table 3 *Ulva lactuca*. Depuration rate constants of metals (k_e) in seaweed following exposure to different concentrations of dissolved metals for 2 d. Percentage of metals (%) distributed in the slower exchanging compartment and the biological retention half-life ($t_{1/2}$) of metals are also shown. Data are presented as mean ± SD (n = 3)

Metal	Exposure concentration (n*M*)	Percentage	k_e (d^{-1})	$t_{1/2}$ (d)
Cd	4.46	90 ± 1	0	∞
	17.8	96 ± 1	0.001 ± 0	∞
	89.2	90 ± 1	0	∞
	446	79 ± 2	0.012 ± 0.012	438 ± 542
Cr	9.61	80 ± 15	0.076 ± 0.015	9.4 ± 1.8
	38.5	69 ± 1	0.059 ± 0.007	11.8 ± 1.5
	192	82 ± 19	0.082 ± 0	8.4 ± 0
	961	87 ± 11	0.050 ± 0.015	15.2 ± 4.6
Se	6.33	75 ± 23	0.158 ± 0.021	4.4 ± 0.6
	25.3	64 ± 7	0.141 ± 0.036	5.2 ± 1.2
	126	74 ± 18	0.147 ± 0.044	5.3 ± 1.9
	633	64 ± 15	0.136 ± 0.069	8.4 ± 6.6
Zn	15.4	74 ± 13	0.138 ± 0.020	5.1 ± 0.7
	76.9	79 ± 14	0.135 ± 0.019	5.2 ± 0.7
	308	81 ± 7	0.152 ± 0.035	4.8 ± 1.2
	1538	70 ± 6	0.118 ± 0.045	6.9 ± 2.8

Zn. Higgins and Mackey (1987) found that pretreatment of kelp, *Ecklonia radiata*, with EDTA released 90% of the total Zn and Cd, 25% of Cu and 7% of Fe, suggesting that a large proportion of Cd and Zn was probably associated with the apparent free space. In addition, Zn was found to be associated with extracellular polymers produced by epiphytic bacteria in the red alga *Gracilaria sordida* (Holmes et al. 1991). By contrast, Bryan (1969) demonstrated the irreversibility of Zn binding in *Laminaria digitata*, i.e. there was negligible loss of bound Zn to the ambient environments. A recent study by Boisson et al. (1997) also confirmed the very efficient retention of metals by the brown alga *Fucus vesiculosus*. For example, the efflux rate constant for Cd was not significantly different from zero. The efflux rate constant of Zn was 0.0001 to 0.0003 d^{-1}, and the corresponding biological half-lives were 239 to 756 d (compared to 100 d, Gutknecht 1965). Our calculated biological half-lives of Zn in *U. lactuca* (4 to 7 d) were comparable to previous measurements by Gutknecht (1965) (4 d). Gutknecht (1965) also showed that the rate constant of metal loss varied greatly among different seaweed species (ranging from 4 to 100 d for Zn).

The uptake rates of trace metals in the seaweeds (J, μg g^{-1} d^{-1}) can be described by the following equation (Landrum et al. 1992; Luoma et al. 1992; Luoma and Fisher 1997):

$$J = k_u \times C_w \quad , \qquad (1)$$

where k_u is the uptake rate constant from the solute phase (l g^{-1} d^{-1}) and C_w is the metal concentration in the dissolved phase (μg l^{-1}). This simple equation assumes that a pseudo-equilibrium is established between the binding ligands and the metals in solution and that the ligands available for metal binding are not saturated. Because k_u was dependent on the metal concentration, we calculated the k_u at each metal concentration by dividing J with C_w (Table 4). The k_u in *Ulva lactuca* was comparable between Cd and Zn, followed by Cr > Se. In *Gracilaria blodgettii*, Zn exhibited the highest uptake rate constant, followed by Cd > Cr > Se (data not shown).

Table 4 *Ulva lactuca*. The calculated uptake rate constant (k_u) and the bioconcentration factor (*BCF*) of metals. Data are presented as mean ± SD (n = 3). Concentration factors of Cd at low concentration were not calculated due to their negligible loss

Metal	Concentration (n*M*)	k_u (l g^{-1} d^{-1})	BCF (l kg^{-1})
Cd	4.46	2.709 ± 0.598	–
	17.8	2.195 ± 0.478	–
	89.2	0.844 ± 0.076	–
	446	0.359 ± 0.044	3.0×10^4
Cr	9.61	0.189 ± 0.044	2.5×10^3
	38.5	0.184 ± 0.034	3.1×10^3
	192	0.183 ± 0.015	2.2×10^3
	961	0.125 ± 0.010	2.5×10^3
Se	6.33	0.024 ± 0	150
	25.3	0.018 ± 0.003	130
	126	0.009 ± 0.001	60
	633	0.006 ± 0.001	40
Zn	15.4	2.956 ± 0.762	2.1×10^4
	76.9	2.494 ± 0.480	1.8×10^4
	308	1.541 ± 0.113	1.0×10^4
	1538	1.128 ± 0.045	1.0×10^4

Bioconcentration factor (BCF) with an equilibrium assumption is therefore calculated as the k_u divided by the efflux rate constant (Table 4). It is important to note that no steady state was reached within the 47 h of exposure in our experiments. BCFs for Cd were not calculated at the low Cd concentrations because their efflux rates were not significantly different from zero. Among the four trace elements, the BCFs were the highest for Cd and Zn (1 to 3×10^4) and the lowest for Se (40 to 150). BCFs were also inversely related to the ambient concentration for Se and Zn, whereas the BCFs of Cr remained rather constant over the concentrations examined. Our calculated BCFs in *Ulva lactuca* were comparable to the field measurements. For example, BCFs in field-collected samples were 1.4 to 2.6×10^4 for

Cd and 1.7 to 2.5×10^4 for Zn in the brown alga *Fucus vesiculosus* (Morris and Bale 1975; Foster 1976). In the green alga *Ulva rigida*, BCFs in field-collected samples were 1.6×10^4 for Cd and 2.0 to 2.6×10^4 for Zn. BCFs of Cd and Zn in a freshwater moss, *Sphagnum papillosum*, determined by radiotracer techniques were 4×10^3 and 2×10^4, respectively (Gstoettner and Fisher 1997). In this study, the BCF of Cr(VI) was 2 to 3×10^3 in *U. lactuca*, in contrast to Cr(III), which had a BCF of 300 to 800 in the moss (Gstoettner and Fisher 1997). The higher uptake of Cr(VI) in comparison to Cr(III) has been contributed to its greater permeability of the cell membrane (Nieboer and Jusys 1988; Wang et al. 1997).

In many earlier field studies, the BCF was calculated directly as the ratio of metal concentrations in the organisms to metal concentration in the ambient water. Dissolved metal concentration was not measured using the trace metal clean technique; thus the reliability of the field-measured BCFs for these metals is not yet clear. In addition, we found that BCF decreased with increasing metal concentrations for Se and Zn, whereas the BCF for Cr was relatively independent of the ambient concentration. These experimental results suggest that BCFs determined under the laboratory conditions, which typically employ unrealistically high metal concentrations, should be applied to field studies with caution.

It is possible to calculate the likely metal concentration in ambient seawater if the metal concentrations in the seaweeds and BCF are known. The ranges of metal concentration in *Ulva lactuca* collected from Hong Kong waters were 15 to 102 $\mu g\ g^{-1}$ for Zn and 0.3 to 0.9 $\mu g\ g^{-1}$ for Cd (Ho 1990). Using the BCFs determined at the lowest metal concentration, we calculated that the dissolved concentration of Cd and Zn in Hong Kong coastal waters would be as low as 89 to 270 p*M* for Cd and 11 to 73 n*M* for Zn. The highest Zn concentration (73 n*M*) was located in the Victoria Harbor, presumably due to the input from industrial water, particularly the metal products industry as well as untreated domestic water from a population of more than 3.5 million. The model-derived Cd and Zn concentrations in Hong Kong coastal water however did not show appreciable elevation compared to many other coastal and estuarine systems (e.g. San Francisco Bay, 0.62 to 1.5 n*M* Cd, 8 to 26 n*M* Zn, Flegal et al. 1991).

Metal concentrations in *Ulva lactuca* (Mn, Fe, Cu, Zn and Pb) were several times higher in samples collected from the urban sites than in those from the rural sites around the Island of Hong Kong, implying that *U. lactuca* is a good indicator of contamination by these metals (Ho 1990). Talbot and Chegwidden (1982) also suggested that *U. lactuca* can be a good indicator for metals including Cd, Fe, Mn and Pb. Our kinetic study indicated that *U. lactuca* would be an excellent indicator for Cr and Zn contamination. Because *U. lactuca* showed partial regulation of Cd uptake, it is probably not a good indicator for Cd contamination across a concentration gradient (Ho 1990).

Employment of seaweeds as biomonitors has been cautioned by Phillips (1990, 1993), largely because of the dependence of metal concentration on the growth rate of the plants. Thus, any biological and environmental conditions (tissue age, season, degree of exposure to air, salinity, nutrient, temperature) that can potentially influence the growth rate may affect metal concentration in the seaweeds (Bryan and Hummerstone 1973; Eide et al. 1980; Rice and Lapointe 1981; Woolston et al. 1982; Forsberg et al. 1988; Haritonidis and Malea 1995; Favero et al. 1996). Rice and Lapointe (1981) emphasized that the growth characteristics of macroalgae should be considered when they are used as pollution biomonitors for trace metals. It is also likely that metal uptake can be affected by the extracellular polysaccharide of epiphytic bacteria (Holmes et al. 1991). Nevertheless, *Ulva lactuca* may serve as an good biomonitor of metal contamination in the subtropical waters because of its cosmopolitan distribution, simple morphology and tolerance to metals and eutrophication. The influence of other environmental conditions (e.g. nutrient level) and biological conditions (e.g. growth) on metal uptake in these seaweeds must also be studied.

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