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Accumulation of Copper and Zinc and their Effects on Growth and Maximum Quantum Yield of the Brown Macroalga *Padina Gymnospora*

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Abstract— Accumulation and the effects of copper and zinc on the macroalga *Padina gymnospora* were assessed in the laboratory after artificial exposure to copper and zinc (separately or in combination). Exposure concentrations of copper used were 0, 25, 50, 100, 500 and 1,000 μg l^{-1}. Concentrations of zinc exposure were 0, 50, 100, 250, 1,000 and 5,000 μg l^{-1}. The simultaneous (or combined) addition of copper and zinc (Cu+Zn) involved 25+50, 50+100, 100+250, and 500+1,000 μg l^{-1}. Accumulation patterns were determined by measuring tissue contents of copper and zinc, while the tolerance was assessed by measuring growth rate and photosynthetic performance, as maximum quantum yield (Fv/Fm). Furthermore, the influence of major nutrients (phosphates and nitrates) on algal-metal-accumulation was estimated after additions of nitrate (1, 10 and 20 mg l^{-1}) and phosphate (0.1, 1 and 2 mg l^{-1}). The data obtained showed that the accumulation increased linearly with the increase in metal concentration in the growth media. However, the presence of additional nitrate and phosphate reduced both the metal accumulation and their toxic effects. Both algal growth rate and maximum quantum yields were negatively affected in proportion to the increase in metal concentrations and exposure time. At exposures to 50, 100 and 500 μg l^{-1} copper, inhibition in growth was greater than 50%, while an exposure to 1,000 μg $l^{-1}$of zinc was needed to cause the same effect on growth and Fv/Fm. Growth rate was a more sensitive indicator of stress than maximum quantum yield in response to exposure to copper and zinc.

INTRODUCTION

Heavy metals are toxic to both plants and animals and since they are not biodegradable they can accumulate through the food chain eventually posing a serious health risk to humans and other top predators (Dallinger and Kautsky, 1985; Forstner, 1990; Kaewsarn and Yu, 2001). On the Tanzanian coast, the levels of many metals, especially copper and zinc; have been locally elevated during recent years (Ferletta *et al*., 1996; Engdahl *et al*., 1998). This is possibly a result of the increased discharge of untreated industrial wastewaters and solid wastes into the marine environment in urban areas (Daffa, 1996; Kangwe, 1999; Engdahl *et al*., 1998). Consequently, monitoring of these metals is of great importance, and there is a need to find good bio-indicators.

Some marine macroalgae are reported to tolerate and to accumulate high levels of heavy metals from the surrounding seawater (e.g. Bryan, 1983; Say *et al*., 1990; Karez *et al*., 1994; Amado Filho *et al*., 1996; Ferletta *et al*., 1996; Engdahl *et al*., 1998),

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and *Padina gymnospora* has a high capability of retaining heavy metals in cell walls (Salgado *et al*., 2005). *P. gymnospora* is a brown macroalga widely distributed in the Zanzibar Channel and it has been used as a bio-indicator of heavy metal pollution (Ferletta *et al*., 1996; Engdahl *et al*., 1998). However, to our knowledge, no laboratory studies have previously investigated the metal accumulation pattern by *P. gymnospora* collected from the Western Indian Ocean, and neither has the influence of nutrients on its metal uptake, or growth and photosynthetic responses to heavy metal exposure, been studied. The few laboratory studies of toxicity of heavy metals to macroalgae are from temperate regions and limited information is available from the tropical environment.

In this study, the aim was to investigate the response of the tropical *P. gymnospora* exposed to different levels of individual and combination of copper and zinc, using experimental metal concentrations comparable to those found naturally in the field including more extreme concentrations representative of polluted areas. As the presence of major nutrients in the seawater could reduce metal accumulation and its impact on macroalgae (Haglund *et al*., 1996), the influence of nitrates and phosphates on metal accumulation and toxicity were also investigated.

MATERIALS AND METHODS

Culture conditions

Healthy, young (~5 cm long and 3 cm wide) thalli of *Padina gymnospora* were collected near Bawe Island, Zanzibar, a location relatively free from land based pollution (Kangwe, 1999). They were then kept in temperature-controlled laboratory facilities of the Institute of Marine Sciences (IMS), Zanzibar and cultivated in 4 litre glass flasks containing 0.2 μm filtered natural seawater with pH 8.0, temperature 26 ºC, salinity 36 psu, nitrate 4.7 µg-at, NO_3-N l^{-1}, phosphate 0.26 µg-at PO_4^{-3}-P l^{-1}, zinc 2.9 µg.l^{-1} and copper 1.1 µg.l^{-1}. The algal stocking density was about 4±0.5 g.l^{-1}. The selected algae were acclimated to culture conditions for 24 hours before being exposed to copper or zinc. Each flask was aerated to provide water movement. The experimental flasks were subjected to 12 hrs light and 12 hrs dark cycles with fluorescent tubes providing a photosynthetic photon flux density of about 120 μmol photons $m^{-2}s^{-1}$.

Experimental design for heavy metal exposure

The macroalgae were exposed to copper in the form of $CuCl_2$ (Merck). Concentrations used in μg Cu l^{-1} were 0, 25, 50, 100, 500 and 1,000 μg l^{-1}. Similarly, zinc was added in the form of $ZnCl_2$ (Merck) and zinc concentrations used were 0, 50, 100, 250, 1000 and 5000 μg l^{-1}. Exposure to a simultaneous addition of copper and zinc in the growth media involved 25+50, 50+100, 100+250, and 500+1,000 μg (Cu+Zn) l^{-1}, respectively. Six replicate flasks were run for each treatment. The seawater was changed twice a week with the metal concentration as previously added.

The exposure experiments lasted 21 days, after which samples were collected from each flask and washed with de-ionised water to remove salts. Newly developed, young algal thalli tips (~2 cm from the growing ends of the blades) were separated from the old parts and oven dried at 60°C until attaining a constant weight. Such prepared samples were analysed for copper and zinc as reported in Engdahl *et al*., (1998) using Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, Spectro, Marlborough, MA, USA). The analytical quality assurance was evaluated using the Certified Standard Reference Material (Ulva lactuca, BCR 276), Community Bureau of Reference.

Effect of nitrate and phosphate on accumulation of copper and zinc

Macroalgae growing in a media containing either 500 μg Cu l^{-1} or 1000 μg Zn l^{-1} were exposed to three different concentrations of nitrate and phosphate. Sodium nitrate ($NaNO_3$, Merck, Darmstadt, F.R. Germany) and hydrous sodium hydrogen phosphate ($NaHPO_4.12H_2O$, Merck, Darmstadt, F.R. Germany) were used as sources of N and P, respectively. Three different nitrate and phosphate concentrations were tested, namely high nutrient (HN), with 20 mg l^{-1} nitrate and 2 mg l^{-1} phosphate; intermediate nutrient (IN), with 10 mg l^{-1} nitrate and 1 mg l^{-1}

of phosphate and low nutrient (LN), with 1 mg l^{-1} nitrate and 0.1 mg l^{-1} phosphate. Six replicates of each treatment were used to asses the effect of nitrate and phosphate on metal accumulation. Control flask C1 had no metal addition while C2 had 500 μg Cu l^{-1} or 1,000 μg Zn l^{-1} but without additional nitrate or phosphate.

Growth rate (DGR)

The daily growth rate (DGR) was estimated using the formula DGR%= $(Wt/Wo)^{1/t}$-1) * 100, where DGR% represents daily growth rate as a percentage, Wt represents fresh weight at time t, Wo is initial fresh weight and t is time in days (see Haglund *et al.*, 1996). Wt was estimated after 4, 7, 14 and 21 days of exposure. Weights were measured within an ample time to avoid drying of the thalli using a standard weighing balance. Before measuring the weight of the plant, excess water was removed by blotting off using soft tissue paper.

Maximum quantum yield (Fv/Fm)

The maximum quantum yield was measured on day 4, 7, 14 and 21 using a Plant Efficiency Analyser (PEA, Hansatech Instruments Ltd, Lynn, Norfolk, UK). Thalli were dark-adapted for about 15 minutes before measurements were taken and on completion of the measurements, Fv/Fm were automatically calculated from the recorded Fo and Fm data (for details see e.g. Beer and Björk 2000; Beer *et al.*, 2001).

Effective concentration at 50% inhibition (EC_{50}) and no observable effect concentrations (NOEC)

The DGR and Fv/Fm values expressed as percentage of the control were plotted against the logarithm of the exposure concentrations A line was fitted to the plotted individual values using linear regression analysis. EC50, is the effective concentration of the toxicant causing 50% inhibition and NOEC is the highest concentration of toxicant with no observable effect (Modified from Haglund *et al.*, 1996). EC50 and NOEC values were determined for 7 and 21days for copper and zinc exposure using the dose response diagram. As an example Fig. 1 is a growth dose response diagram used for determination of EC50 and NOEC values for individual exposure to copper for 7 days.

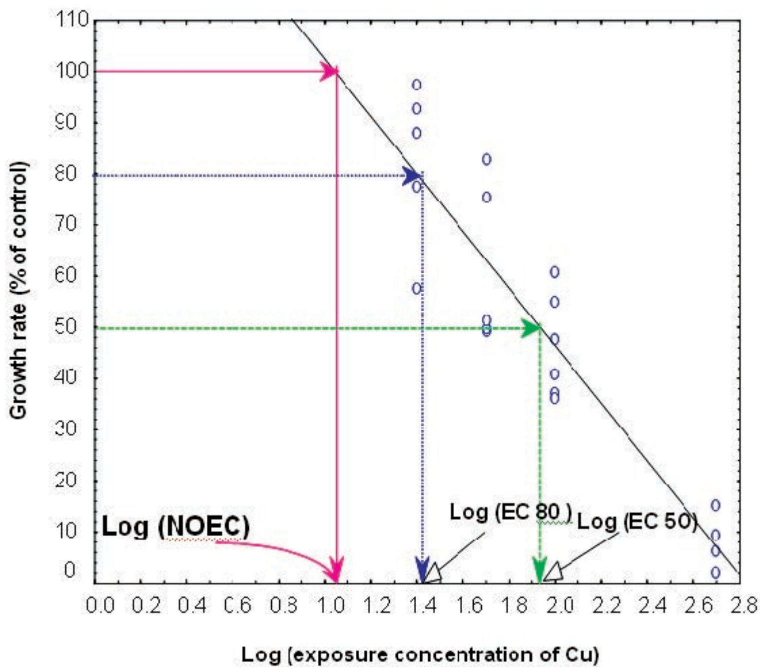

Fig. 1. An example of a dose response diagram for determination of EC_{50}, and NOEC values. This dose response diagram is for daily growth rate of *Padina gymnospora* subjected to different concentrations of copper

Statistical Analysis

The significance of the effects of heavy metal concentrations and exposure time on daily growth rate and maximal quantum yield were tested by two way analysis of variances (ANOVA). Before performing ANOVA, the homogeneity of the variance was assessed by Cochrain's test. If a significant effect of concentrations of heavy metal on DGR and Fv/Fm were found, a post hoc Turkey's test was performed to determine at which concentration the difference existed. The difference between metal accumulated in young and old tissue were determined using a Student's t-test for independent variables. The statistical significance was accepted for $P < 0.05$.

RESULTS

Copper and zinc accumulation

The accumulation of copper and zinc in algal thalli increased linearly with the metal concentration when added separately and added together in the culture media (Fig. 2 and 3). Accumulation appeared slightly higher in the young parts of the algal thalli than in the older parts, but the difference was not significant (t-test, $p>0.05$). Accumulations of copper or zinc in the algal thalli exposed to media with only one of the metals were mostly higher than when both metals were added simultaneously. For example, when *Padina* were exposed to 25+50 μg (Cu+Zn) l^{-1} the copper accumulation decreased to 23% as compared to accumulation occurred at exposure to 25 μg Cu l^{-1}. With the 500+1,000 μg (Cu+Zn) l^{-1} exposure, the accumulation of copper was 56% lower compared to individual exposure to 500 μg Cu l^{-1}. Compared to accumulation of zinc when added individually, exposure to copper+zinc 100+250 and 500+1,000 μg l^{-1}, lead to a decrease of zinc uptake to 67% and 65% respectively. However, the total addition of both metals resulted in the accumulated copper and zinc being nearly equal to the level of accumulation when exposed to separate treatments. Total mean copper and zinc uptake during exposure to 100+250 μg (Cu+Zn)l^{-1}

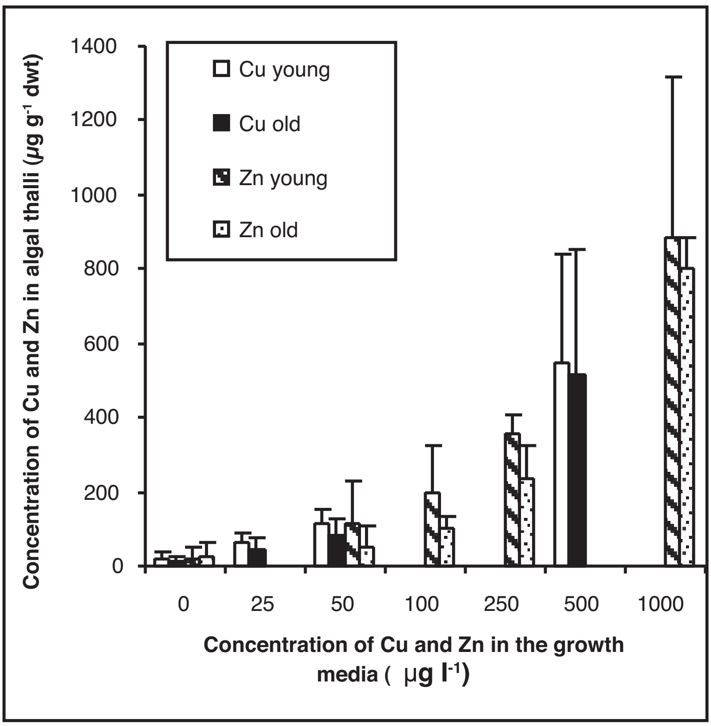

Fig. 2. Accumulation of copper or zinc in young and old parts of thalli of *Padina gymnospora* exposed to different individual concentrations of copper and zinc for 21 days (n=5)

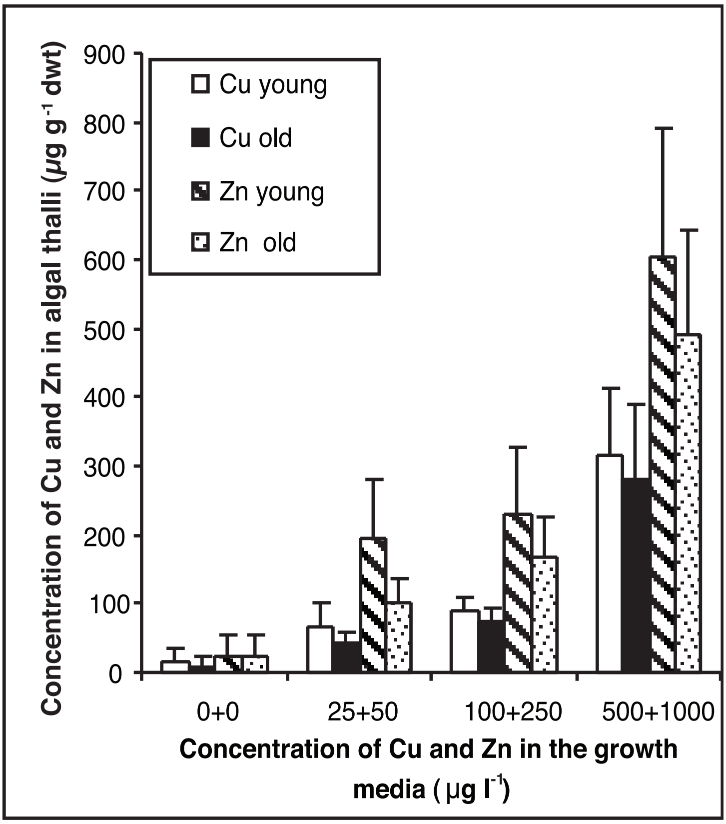

Fig. 3. Accumulation of copper and zinc in young and old parts of thalli of *Padina gymnospora* exposed to different concentrations of a mixture of copper and zinc for 21 days (n=5)

in young tissue was 253 and 172 μg (Cu+Zn)g^{-1}. At 500+1,000 μg (Cu+Zn)l^{-1} total mean uptake of copper and zinc were about 816 in the young part and 771 μg (Cu+Zn)g^{-1} in the old part of the thalli.

Influence of nutrients on metal accumulation

Accumulation of both copper and zinc in thalli was always reduced when the metals were added together with nitrate and phosphate (Fig 4 and 5). With increase of nutrient concentration in the growth media, the accumulation of metals decreased. For example, in algae grown in media containing 500 μg Cu l^{-1}, 20 mg l^{-1} nitrate and 2 mg l^{-1} phosphate, the accumulation of copper was reduced by 71% compared to when the thalli was exposed to the same copper levels without nutrients. Accumulation of zinc while exposed to 1000 μg l^{-1} Zn, 20 mg l^{-1} nitrate and 2 mg l^{-1} phosphate was about 72% lower than without the nutrients.

Effect of copper and zinc on daily growth rates and maximum quantum yields

Both the daily growth rate (DGR) and maximal quantum yield (Fv/Fm) decreased significantly with both metal concentration and exposure time (ANOVA, $p<0.01$) (Tables 1 & 2). For example, the DGR of thalli exposed to 100 μg Cu l^{-1}, decreased rapidly to about half at day 4, and then continued to drop so that only about 17% remained after 21 days. With 500 μg Cu l^{-1} exposure, the DGR was totally inhibited. However, in the nutrient experiment the algae in the same set-up still managed to produce some growth, though lower than in the other combinations (Table 1). Also exposure to zinc caused a reduction in DGR and Fv/Fm although to a lower degree. A decline in DGR and Fv/Fm

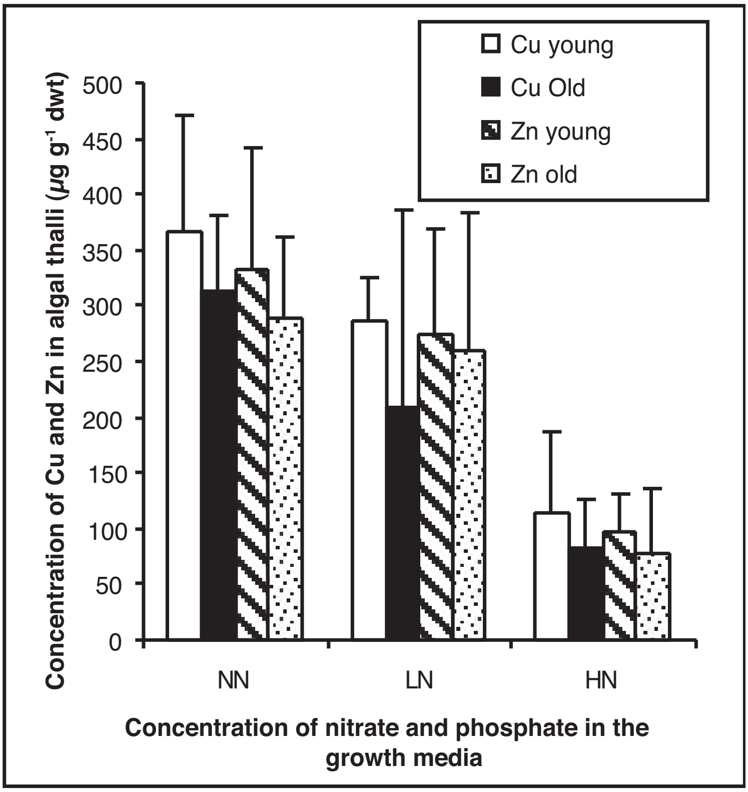

Fig. 4. Accumulation of copper and zinc in young and old parts of thalli of *Padina gymnospora* exposed to individual 500 µg Cu/l, and 1,000 µg Zn/l at different concentrations of nutrients ($NO_3 + PO_4$) for 21 days. HN, LN, and NN stands for the highest, the lowest and no added nutrient concentrations respectively (n=5)

were also observed in the algae exposed to zinc and copper added simultaneously. Addition of nitrate or phosphate to the growth media together with copper and/or zinc reduced their inhibitory effects on both growth and photosynthetic capacity (Tables 1 & 2).

NOEC and EC_{50}

The results for EC_{50} and NOEC values are presented in Table 3. Generally, the EC_{50} and NOEC values for Fv/Fm are higher compared to those of DGR. In addition, values of EC_{50} for zinc exposure were much higher compared to EC_{50} for copper experiments.

DISCUSSION

Accumulation of copper and zinc

The accumulation of copper and zinc in the algal thalli increased with the concentration of the metal added to the growth media in a near linear fashion, both when added singly and when added simultaneously. However, the accumulation of each metal was lowered when the alga was exposed to copper and zinc simultaneously, while the total accumulation of metal remained the same. A similar effect has been reported in the brown macroalga *Ascophyllum nodosum* exposed to copper and zinc (Strömgren, 1980). It is, however, not known if the two metals in *P. gymnospora* compete for the common uptake mechanism and/or binding site. In contrast to what has been observed here and by Strömgren (1980), a synergistic interaction was

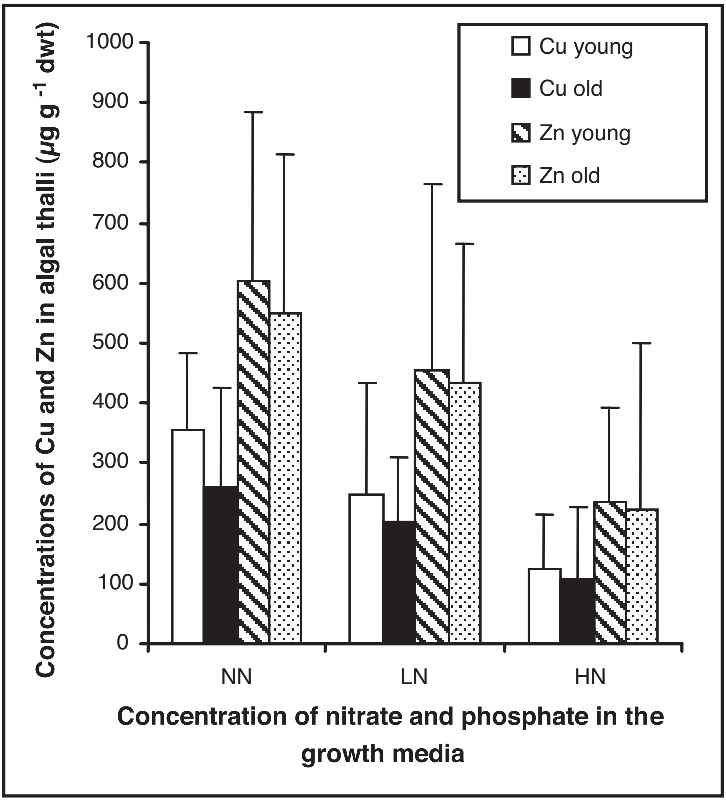

Fig. 5. Accumulation of copper and zinc in young and old parts of thalli of *Padina gymnospora* exposed to a mixture of copper and zinc at 500 μg Cu/l + 1,000 μg Zn/l at high, and low concentrations of nutrients (NO_3 + PO_4) for 21 days. HN, LN, and NN stands for the highest, the lowest and no added nutrient concentrations respectively (n=5)

found in algal uptake of copper, Fe, and zinc in Cd exposed *Enteromorpha* (now Ulva) prolifera (Haritonidis *et al.* 1994). However, even closely related species have been reported to exhibit different accumulation strategies for different heavy metals (Rainbow & Phillips 1993; Rainbow 1995).

The accumulation of metals occurred at a range of concentrations, from 17 to 544 μg g^{-1}dwt of copper and about 21 to 887 μg g^{-1}dwt of zinc. The lower concentrations are comparable to those reported in previous studies of naturally occurring macroalgae in Tanzania's relatively unpolluted coastal regions, where the concentrations of copper in *Padina* tissue have been reported to range from 2–16 μg g^{-1}dwt, and for zinc, ranged from 3–47 μg g^{-1} dwt (Engdahl *et al.*, 1998) and also 3-17 μg g^{-1}dwt for copper, 6-104 μg g^{-1} dwt for zinc (Ferletta *et al.*, 1996). Reports of metal concentrations in marine algae in more polluted areas of the world vary considerably with copper levels over 100 μg g^{-1} dwt in *Laminaria* and *Fucus* for example, and up to 335 μg g^{-1}dwt in *Liagora* (see review by Correa *et al.*, 1996). The concentrations of metals in natural seawater also varies considerably, and might reach as high as 600 μg l^{-1} of copper as recorded in a copper tailing area (Correa *et al.*, 1996) and even levels as high as 1,800 μg l^{-1} have been reported for zinc (Amado Filho *et al.*, 1997).

The capability of *Padina gymnospora* to accumulate heavy metals is related to the presence of charged polysaccharides in the cell wall matrix, the sulphated fucans and alginates, which act as an ionic filter protecting cells from high concentrations of heavy metals (Amado Filho *et al.*, 1996; Andrade *et al.*, 2002; Farina *et al.*, 2003). In addition, the physode found in the algal cellwall has also been reported as an organelle involved in heavy-metal accumulation by brown algae (Ragan and Glombitza, 1986; Karez and Pereira

Table 1. Mean daily growth rates (DGR%) of *Padina gymnospora* exposed to different concentrations of copper, zinc and copper+zinc (values expressed as % of the controls ± Standard error, N=12)

Conc. (μg/l)	Metal	Exposure Days 4	7	14	21
25	Cu	119.3±19.2	104.3±3.2	93.7±3.8	79.3±15.3
50	Cu	76.9±10.8	68.8±9.4	53.7±7.2	24.2±7.3
100	Cu	53.1±6.5	45.1±3.6	31.9±6.8	16.6±2.8
500	Cu	-5.4±3.2	-9.11±1.9	-17.7±12.9	-30.7±20.8
500	Cu(HN)	80.1±7.4	78.8±4.8	68.9±5.0	56.3±5.6
500	Cu(IN)	69.4±7.3	63.0±2.9	57.0±4.6	54.9±4.1
500	Cu(LN)	41.9±7.9	36.9±6.8	34.3±5.0	28.9±4.8
500	Cu(NN)	34.2±2.4	29.5±2.4	28.6±1.6	23.4±0.7
50	Zn	90.3±8.6	83.2±5.7	80.7±4.0	78.2±3.6
100	Zn	84.9±10.1	84.4±8.6	79.1±7.5	78.2±5.7
250	Zn	80.9±6.3	81.7±7.8	73.6±6.3	67.4±5.2
1000	Zn	66.3±8.1	53.3±5.1	48.9±7.3	41.6±6.1
1000	Zn(HN)	94.0±8.5	85.1±6.6	77.9±5.4	74.5±3.3
1000	Zn(IN)	84.1±5.5	73.4±4.3	68.8±4.5	68.0±2.4
1000	Zn(LN)	83.0±7.3	72.0±7.0	68.7±4.3	63.1±1.6
1000	Zn(NN)	60.7±10.4	55.3±8.6	48.0±3.8	44.5±4.9
25+50	Cu+Zn	64.3±1.1	48.7±15.1	45.3±9.6	46.8±6.4
50+100	Cu+Zn	60.1±10.9	46.6±12.5	50.8±6.2	36.9±4.2
100+250	Cu+Zn	60.1±6.8	44.7±12.0	39.7±4.7	35.7±2.8
500+1000	Cu+Zn	55.7±4.7	35.9±1.6	29.2±6.2	25.1±5.2
500+1000	Cu+Zn(HN)	58.3±7.3	48.0±5.3	43.8±4.6	40.0±4.8
500+1000	Cu+Zn(IN)	41.9±6.4	38.9±3.0	38.3±3.0	32.2±2.6
500+1000	Cu+Zn(LN)	33.9±6.1	32.4±3.8	30.1±3.1	24.8±3.7
500+1000	Cu+Zn(NN)	33.2±3.3	34.5±7.9	24.4±4.4	16.7±2.7

HN,=High nutrient, IN= Intermediate nutrient, LN=Low nutrient, NN=No nutrient.

1995; Schoenwaelder, 2002). Furthermore, the presence of protonated and deprotonated phenolic groups with ester-linked carbonyl oxygen could be involved in heavy metal sequestration (Tretyn *et al*., 1996).

The addition of key nutrients (nitrate and phosphate) to the growth medium reduced both metal accumulation and its negative effect on growth and photosynthesis. Whereas a similar observation has been reported for a red alga, *Gracilaria tenuistipitata* (Haglund *et al*., 1996), the reverse effect was found when the green alga *Ulva fasciata* was exposed to zinc with elevated nitrate levels (Lee and Wang, 2001). These effects of nitrate and phosphate on the accumulation of the two metals are interesting from both a physiological and ecological view and should be considered when seaweed samples have been collected at sites with different nutrient regimes. However, the nitrate and phosphate concentrations required to cause a significant effect on metal accumulation in *P. gymnospora*, were substantially higher than natural concentrations detected at different sites along the Tanzanian coast (Lugomela *et al*., 2002; Hamisi *et al*., 2004). However, the lowest concentration of nutrients used in our experiment may be found close to sewer pipes or areas where discharges of untreated sewage occur.

Aquatic organisms have an efficient uptake of free metal ions; however, many factors seem to strongly influence bioaccumulation of metals and may complicate predictions of metal bioavailability (Luoma 1983, 1989). This is a function of factors including total concentration and speciation

Table 2. Mean maximum quantum yield (Fv/Fm) of *Padina gymnospora* exposed to different concentrations of copper, zinc and copper+zinc (values expressed as mean % of the controls ± Standard error, N=12)

Concentration (μg/l)	Metal	Days 4	7	14	21
25	Cu	105.0±1.5	97.9±2.2	90.9±2.2	86.4±1.8
50	Cu	93.7±1.6	93.6±1.6	86.7±2.9	83.1±2.9
100	Cu	90.3±2.9	85.2±2.4	77.0±2.6	69.2±2.7
500	Cu	64.5±4.0	61.6±2.0	56.7±1.3	46.5±2.1
500	Cu(HN)	100.3±1.0	96.0±2.8	82.6±5.3	78.8±3.2
500	Cu(IN)	99.3±5.7	96.2±0.9	90.3±2.2	80.6±2.0
500	Cu(LN)	78.1±4.5	70.5±7.8	61.5±6.7	55.4±5.8
500	Cu(NN)	69.1±5.9	63.7±7.3	51.9±4.7	37.9±4.1
50	Zn	105.4±2.5	104.2±1.2	97.1±2.8	98.4±1.6
100	Zn	102.9±3.0	103.9±2.0	95.6±3.9	91.7±4.3
250	Zn	96.6±3.3	94.6±5.1	90.7±3.5	82.2±2.8
1000	Zn	86.3±5.6	84.2±5.2	79.1±6.1	72.3±5.2
1000	Zn(HN)	98.5±3.4	98.2±2.4	95.6±2.5	90.4±2.3
1000	Zn(IN)	101.1±1.3	97.7±1.0	88.2±4.6	83.9±4.2
1000	Zn(LN)	97.9±2.0	93.3±3.7	83.2±6.6	80.6±8.4
1000	Zn(NN)	90.9±2.9	80.9±5.4	71.3±5.0	66.8±5.6
25+50	Cu+Zn	98.6±2.3	95.1±0.0	91.0±3.5	79.6±4.5
50+100	Cu+Zn	98.4±3.4	92.2±2.1	83.3±6.1	71.8±13.0
100+250	Cu+Zn	93.9±4.6	87.4±5.2	74.9±14.2	64.7±18.8
500+1000	Cu+Zn	89.9±3.7	80.6±6.2	68.7±16.5	48.0±12.5
500+1000	Cu+Zn(HN)	94.9±2.4	97.1±2.3	94.9±2.5	88.8±2.5
500+1000	Cu+Zn(IN)	87.9±2.6	85.0±2.3	79.1±2.2	73.3±2.6
500+1000	Cu+Zn(LN)	83.6±5.5	81.3±7.1	74.6±8.1	66.1±6.7
500+1000	Cu+Zn(NN)	67.7±4.5	60.4±5.0	51.0±3.1	49.7±1.9

HN,=High nutrient, IN= Intermediate nutrient, LN=Low nutrient, NN=No nutrient

Table 3. Concentration range of Cu and Zn (mg/l) corresponding to EC50, and NOECs values for DGR and Fv/Fm of *P. gymnospora* exposed to Cu and Zn for 7 and 21 days of exposure. The values were determined through extrapolation using dose response diagrams

Toxicant	No of Exposure Days	Response measured	EC50 (mg/l)	NOEC (mg/l)
Cu	7	*DGR*	0.076-0.096	0.013-0.015
		Fv/Fm	1.288-2.049	0.022-0.024
	21	*DGR*	0.029-0.039	0.005-0.008
		Fv/Fm	0.396-0.436	0.012-0.002
Zn	7	*DGR*	2.182-2.818	0.002-0.033
		Fv/Fm	n.a	0.13-0.141
	21	*DGR*	0.629-0.719	0.008-0.020
		Fv/Fm	8.035-46.309	0.017-0.026

n.a not affected

(physical-chemical forms) of metals, mineralogy, pH, redox potential, temperature, total organic content and particulate matter, as well as water velocity, and turbulence (Luoma, 1983). Therefore, caution is needed when comparing the status of different heavy metal concentrations from field sites having significant differences in these parameters.

Effects of copper and zinc

Copper and zinc inhibited both growth and photosynthetic efficiency of *P. gymnospora* and their inhibitory effect increased with increase in the concentration of metals. Several possible cellular toxicity targets for copper and zinc have been suggested in algae (see Jensen *et al*., 1974; Sorentino, 1979; Lobban and Harrison, 1994; Lewis *et al*., 1998). Heavy metal toxicity may be linked to oxidative stress (Livingstone, 2001, Pinto *et al*., 2003; Collén *et al*., 2003) as copper or zinc cause the production of hydroxyl radicals leading to oxidative cellular damage (Hammond and Jones, 1996; Collén *et al*., 2003; Andrade *et al*., 2006) or by inhibiting cellular antioxidant capacities (Sies, 1999; Rijstenbil *et al*., 1998). Furthermore, transport of copper ions to chloroplasts via the cytoplasm could inhibit photosynthesis by uncoupling electron transport to Nicotinamide Adenosine Diphosphate ion ($NADP^+$) and at higher concentration lead to death, by irreversibly binding to chloroplast membranes and preventing photosynthesis (Sorentino, 1979).

Our data showed that a higher copper concentration was needed to cause a 50% inhibition of -maximum quantum yields than of daily growth rates (Table 3), possibly indicating that the adverse effects were not related primarily to photosynthesis. The same effect was previously shown for the green macroalga *Enteromorpha intestinalis* (Lewis *et al*., 1998) where a significant growth inhibition occurred at 50 μg Cu/l while maximal quantum yield was not significantly inhibited until copper concentrations were ten times higher. The EC_{50} and NOEC assessments (Table 3) showed that copper was more toxic to *P. gymnospora* than zinc, as previously reported for other macroalgae (e.g. Rai and Kumar, 1981; Kangwe, 1999).

In this study, *Padina* was able to tolerate the highest concentrations of copper (500 μg l^{-1}) and zinc (1000 μg l^{-1}) used. The highest concentrations tested such as 500 μg l^{-1} (copper) and 1000 μg l^{-1} (zinc) are higher than those reported in the natural environment, hence in a polluted environment with similar levels of copper and zinc *P. gymnospora* probably will be able to tolerate.

In summary, it is apparent that *Padina gymnospora* has shown an ability to accumulate and tolerate high concentrations of copper and zinc. *P. gymnospora* is sessile, easy to identify, available in high abundance in the region, wher it is commonly found in the intertidal areas and is easily collected. Thus, it will probably serve as an indicator of heavy metal contamination in the marine environment in the region. However, when using *Padina*, or any seaweed as a bio-indicator, it is important to bear in mind that some environmental factors, such as nutrient loading could affect the bioaccumulation of metals. Hence, in areas with heavy metal contamination together with increased nutrient levels, the concentrations of different heavy metals might be underestimated.

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