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Phytoremediation potential of charophytes: Bioaccumulation and toxicity studies of cadmium, lead and zinc

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Abstract

The ability for usage of common freshwater charophytes, *Chara aculeolata* and *Nitella opaca* in removal of cadmium (Cd), lead (Pb) and zinc (Zn) from wastewater was examined. *C. aculeolata* and *N. opaca* were exposed to various concentrations of Cd (0.25 and 0.5 mg/L), Pb (5 and 10 mg/L) and Zn (5 and 10 mg/L) solutions under hydroponic conditions for 6 days. *C. aculeolata* was more tolerant of Cd and Pb than *N. opaca*. The relative growth rate of *N. opaca* was drastically reduced at high concentrations of Cd and Pb although both were tolerant of Zn. Both macroalgae showed a reduction in chloroplast, chlorophyll and carotenoid content after Cd and Pb exposure, while Zn exposure had little effects. The bioaccumulation of both Cd and Pb was higher in *N. opaca* (1544.3 μg/g at 0.5 mg/L Cd, 21657.0 μg/g at 10 mg/L Pb) whereas higher Zn accumulation was observed in *C. aculeolata* (6703.5 μg/g at 10 mg/L Zn). In addition, high bioconcentration factor values (> 1000) for Cd and Pb were observed in both species. *C. aculeolata* showed higher percentage of Cd and Pb removal (> 95%) than *N. opaca* and seemed to be a better choice for Cd and Pb removal from wastewater due to its tolerance to these metals.

Key words: charophyte; *Chara aculeolata*; *Nitella opaca*; cadmium; lead; zinc; phytoremediation

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Introduction

Contamination of water by heavy metals can occur naturally during soil erosion and flooding (Song et al., 2010; Zimmer et al., 2010) or anthropogenically by leaching of municipal wastes, agricultural pesticides and effluent and wastewater from industrial manufacturing and mining (Krishna and Govil, 2004; Sanayei et al., 2009; Ato et al., 2010). The contamination of aquatic habitats and domestic water sources by heavy metals such as Cd, Pb, Cr and, Zn may lead to ecotoxicological problems in flora and fauna and damage to human health (Kalay et al., 1999; Järup, 2003). Many countries have set quality criteria for toxic levels of metals in freshwater. The United States Environmental Protection Agency (EPA, 2003) recommends that the concentrations for Cd, Pb and Zn in freshwater should be lower than 0.005, 0.015 and 5 mg/L, respectively. In Thailand, the total permissible amounts of these metals are set higher, 0.03, 0.20 and 5 mg/L for Cd, Pb and Zn, respectively (Homchean, 1972). Cd and Pb are non-essential elements that are highly toxic for most living organisms (Setia et al., 2008) and affect the primary productivity of aquatic plants (John et al., 2008; Piotrowska et al., 2010). On the other hand, Zn is an essential micronutrient (Setia et al., 2008), which acts as a cofactor in enzymes and proteins of cellular metabolism (Lu et al., 2005). However, excessive levels of Zn induce toxicity (Paschke et al., 2006) and plant chlorosis by interfering with Fe metabolism (Rosen et al., 1977).

Numerous macroalgae, both marine and freshwater, have been used as biomonitors or bioindicators of water pollution (Kuyucak and Volesky, 1990; Gosavi et al., 2004; Al-Homaidan et al., 2011). In addition, some species have been used for the removal of heavy metals from wastewater or contaminated water bodies either by biosorption or by bioaccumulation (Hu et al., 1996; Amado Ailho et al., 1997; Wang and Dei, 1999; Axtell et al., 2003; Lamai et al., 2005; Baumann et al., 2009; Gomes and Asaeda, 2009; Bibi et al., 2010; Gao and Yan, 2012). Biosorption and bioaccumulation involve interactions and concentration of toxic metals or organic pollutants in the biomass, either living (bioaccumulation) or non-living (biosorption)

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(Chojnacka, 2010). In bioaccumulation, the first stage is biosorption or a fast metabolism independent surface reaction; the second is a slow metabolism-dependent cellular uptake (Cho et al., 1994).

The capacities of bioaccumulation and biosorption vary among different species of algae and several studies revealed that both marine and freshwater macroalgae can accumulate metals (Lamai et al., 2005; Deng et al., 2007; Gupta and Rastogi, 2008; Gao and Yan, 2012). An advantage of using living organisms over dried biomass is that they have a fast growth rate and hence produce a regenerating supply of metal-removal materials (Sobhan and Sternberg, 1999). Based on their bioaccumulation capacity, several macro- and microalgae have been selected for their potential for phycoremediation, with the aim of finding a more efficient and cost-effective metal removal biosorbents (Kumar et al., 2007; Gomes and Asaeda, 2009; Gao and Yan, 2012).

Charophytes (*Chara*, *Nitella*, *Tolypella* and *Nitellopsis*) occur in a wide range of water bodies: both fresh and brackish and temporary to permanent (Coops, 2002). They are submerged macrophytes providing shelter and food for macro-invertebrates, fish, and waterbirds, and are well-known pioneer colonizers of water bodies (John, 2003). They have a very fast growth rate and are distributed worldwide. Hyperaccumulation of metals by charophytes has first been reported in 1975 for Mn accumulation (Ambaht and Ambasht, 2003). Few laboratory studies have recently demonstrated the phytoremediation potential of charophytes (Gomes and Asaeda, 2009; Bibi et al., 2010; Gao and Yan, 2011). However, to our knowledge, literature pertaining to the remediation of toxic heavy metals by charophytes is relatively scarce. The present study, therefore, aimed to assess the phytoremediation potential of two charophytes, *Chara* and *Nitella*, by determining the bioaccumulation capacity of, and toxicity of heavy metals (Cd, Pb and Zn) to, these two species.

1 Materials and methods

1.1 Macroalgae

Samples of *Chara aculeolata* Kutzing and *Nitella opaca* C. Agardh (ex Bruzelius) were collected prior to the experiment. *C. aculeolata* was found in Bueng Boraphet Reservoir, Nakhon Sawan Province, Thailand, while *N. opaca* was commonly found in natural ponds in Bangkok. Water samples at the collecting sites were analyzed for water quality standards (AOAC, 2006).

Both species of macroalgae were grown in 20 L glass aquaria (23 cm × 45 cm × 28 cm) containing 10% Hoagland's nutrient solution (EPA, 1975) under controlled conditions (25 ± 2 °C, 2200 lux, 12 hr/12 hr light and dark cycle). The pH of the solution was 5.5. After one-week acclimatization, algae were exposed to various concentrations of heavy metals (Cd, Pb, Zn).

1.2 Chemicals

The 10% Hoagland's nutrient solution was supplemented with two nominal concentrations of Cd (0.25 and 0.5 mg/L of Cd prepared from $Cd(NO_3)_2$ standard solution), Pb (5 and 10 mg/L of Pb prepared from $Pb(NO_3)_2$ standard solution), and Zn (5 and 10 mg/L of Zn prepared from $Zn(NO_3)_2$ standard solution). The initial pH of all solutions was 5.5. The tested concentrations of Cd, Pb and Zn were different according to different levels of their toxicity and water quality standard.

1.3 Heavy metal toxicity

Apical tips or thalli of the stock *C. aculeolata* and *N. opaca* (4–5 internodes, 8–10 cm length, 5 g fresh weight) with uniform morphology were harvested and each transferred into 1 L Erlenmeyer's flasks containing various concentrations of Cd, Pb or Zn. Algae cultured in the medium without heavy metals served as controls. There were 3–6 replicates for each treatment. The experiment was carried out for 6 days. The cultured metal solutions were changed on day 3.

1.3.1 Relative growth rate

Algae were harvested after 6 days of exposure to determine the growth rates. Control and treated algae were blotted gently to drain excess water and the fresh weights were recorded. The relative growth rate (RGR) of macroalgae was calculated following the equation:

$$\mathrm{RGR} = [(w_1 - w_0)/w_0]/[(W_1 - W_0)/W_0] \tag{1}$$

where, w_1 (g), W_1 (g) represent the fresh weight at time T_1; w_0 (g), W_0 (g) represent the fresh weight at time T_0; w, W represent different metal treatment and the control, respectively (Gao and Yan, 2012).

1.3.2 Pigment contents

Total chlorophyll, chlorophyll *a*, *b*, and carotenoid contents in algae from each treatment were determined after 6 days of exposure by the absorption spectra of algal extract in a spectrophotometer according to the methods described by Arnon (1949), MacKinney (1941) and Jeffrey et al. (1997). The absorbance of the extract was measured at 663, 645 and 480 nm.

1.3.3 Toxicity symptoms

Toxicity symptoms caused by heavy metals were searched for in both algal species after 3 and 6 days of exposure under a digital compound transmission light microscope (Olympus CH40, Olympus optical Co. Ltd., Japan). The criteria such as reduction of chloroplast, softening of thallus, and detachment of corticating cells were used to evaluate the severity of toxicity symptoms (mild, moderate, severe).

1.4 Heavy metal removal and accumulation

The percentage metal removal was calculated from uptake (U, %):

$$U = [(C_0 - C_1)/C_0] \times 100\% \qquad (2)$$

where, C_0 (mg/L) and C_1 (mg/L) are initial and remaining concentrations of metal in the medium, respectively (Abdel-Halim et al., 2003).

After 6 days, algae from each flask were harvested separately and analyzed for their accumulation of Cd, Pb and Zn. The procedures of digestion of algal materials was performed according to Anderson (1991) and Katz and Jennis (1983). Algae were washed with 5 mmol/L EDTA for 10 min to remove heavy metals bound to the external cell surface (Vasconcelos and Leal, 2001), dried at 60°C until a constant weight was obtained, digested in conc HNO_3 at 200°C prior to conc HCl at 150°C, and impurities removed by filtration (APHA et al., 1998). After digestion, metal concentrations in algae and solutions were determined by a flame atomic absorption spectrophotometer (Variance SpectrAA 55B, Varian Australia Pty Ltd., Australia).

The bioconcentration factor (BCF) is used to determine the ability of algae in concentrating heavy metals or in acting as hyperaccumulator. The value is defined as the ratio of metal concentration in dry biomass to the initial metal concentration in the external medium (Raskin et al., 1994). The BCF values for Cd, Pb and Zn were determined.

1.5 Statistical analysis

The mean relative growth rate, pigment contents and metal concentrations were calculated and subjected to analysis of variance with differences determined using Tukey HSD's multiple comparisons test on the SPSS for Windows program. The 0.05 level of probability was used as the criterion of significance.

2 Results

2.1 Relative growth rate

C. aculeolata and *N. opaca* showed similar growth responses to Cd and Pb. A concentration-dependent decrease in RGR of both algal species was observed (**Table 1**). Significant reduction in RGR was observed when the Cd and Pb concentrations were increased ($P \leqslant 0.05$). *N. opaca* showed greater sensitivity to these metals and the RGRs of both species were severely inhibited, especially at high Cd concentration (0.5 mg/L). However, *C. aculeolata* showed more sensitivity to Zn than *N. opaca* since there was a significant decrease in RGR of *C. aculeolata* ($P \leqslant 0.05$) when exposed to high concentration of Zn.

2.2 Toxicity symptoms

In general, high concentrations of metals caused several toxicity symptoms including reduction of chloroplast resulting in chlorosis, softening of the algal thallus, and detachment of corticating cells in the cortex around central

Table 1 Relative growth rates (RGR) of the *C. aculeolata* and *N. opaca* exposed to different levels of Cd, Pb or Zn after 6 days

Algal species	RGR (% of control)						
	Control	Cd		Pb		Zn	
	0	0.25 mg/L	0.5 mg/L	5 mg/L	10 mg/L	5 mg/L	10 mg/L
C. aculeolata	100.0	19.2^{a}	17.4^{a}	35.1	-15.5^{a}	73.0	28.1^{a}
N. opaca	100.0	-44.9^{ab}	-86.1^{ab}	-23.1^{a}	-59.9^{a}	90.2	76.9

Results are means ± SD of 4–6 independent thalli.
Significant differences are indicated: ^{a} $P \leqslant 0.05$ versus control without heavy metal; ^{b} $P \leqslant 0.05$ versus treated *C. aculeolata*.

Table 2 Toxicity symptoms observed in *C. aculeolata* and *N. opaca* exposed to Cd, Pb and Zn for 6 days*

Algal species	Metal	Concentration (mg/L)	Toxicity symptoms		
			Reduction of chloroplast	Softening of thallus	Detachment of corticating cells
C. aculeolata	Cd	0.25	+++	++	–
		0.5	+++	++	–
	Pb	5	+	++	–
		10	++	++	–
	Zn	5	+	–	–
		10	+	–	+
N. opaca	Cd	0.25	+	+++	–
		0.5	++	+++	–
	Pb	5	+	++	–
		10	+++	+++	–
	Zn	5	+	–	–
		10	+	–	–

* Effects of heavy metals were compared on the stem of the thalli.
+: mild effect; ++: moderate effect; +++: severe effect; –: no effect.

cells of *C. aculeolata* (**Fig. 1**). Effects of heavy metals were characterized as mild, moderate, and severe (**Table 2**). *C. aculeolata* exhibited severe reduction of chloroplast when exposed to Cd while *N. opaca* was more sensitive to Pb

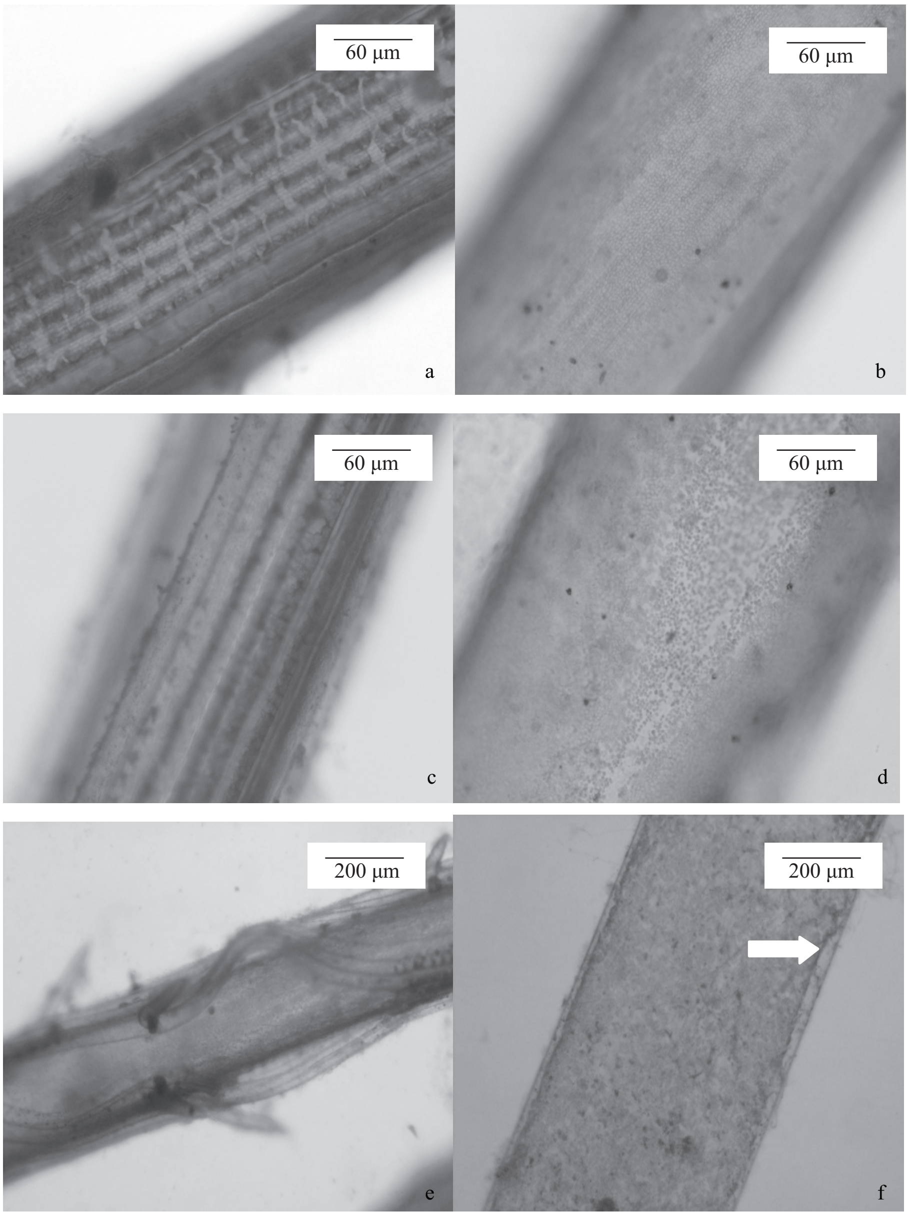

Fig. 1 Light micrographs of untreated *C. aculeolata* (a), untreated *N. opaca* (b). Treated macroalgae showing reduction of chloroplast in *C. aculeolata* (c), reduction of chloroplast in *N. opaca* (d), detachment of corticating cells (e) and softening of thallus (f).

Table 3 Metal removal from the culture media by *C. aculeolata* and *N. opaca* exposed to different metal concentrations after 3 and 6 days

Metal	Concentration (mg/L)	Metal removal (%)			
		Day 3		Day 6	
		C. aculeolata	*N. opaca*	*C. aculeolata*	*N. opaca*
Cd	0.25	100.0 ± 0	60.7 ± 6.5^{a}	98.5 ± 2.5	5.4 ± 2.1^{a,b}
	0.5	91.4 ± 4.8^{c}	23.4 ± 2.4^{a,c}	77.7 ± 7.8^{b,c}	0^{a,b}
Pb	5	96.8 ± 1.3	93.2 ± 1.0	90.6 ± 0.9	76.5 ± 3.2^{a,b}
	10	94.1 ± 0.6	88.5 ± 3.0	95.0 ± 1.6	43.2 ± 7.7^{a,b,c}
Zn	5	17.7 ± 4.2	0^{a}	3.7 ± 1.5	0
	10	0^{c}	0	0	0

Results are means ± SD of 4–6 independent thalli. ^{a} $P \leqslant 0.05$ versus *C. aculeolata*; ^{b} $P \leqslant 0.05$ versus day 3; ^{c} $P \leqslant 0.05$ versus lower concentration of the same metal.

at high concentration. Both Cd and Pb caused softening of thallus in both algal species. Zn appeared to have a very mild effect on reduction of chloroplast but caused a detachment of corticating cells in *C. aculeolata* at high concentration (10 mg/L).

2.3 Pigment contents

Both *C. aculeolata* and *N. opaca* showed significant reductions in total chlorophyll, chlorophyll *a* and *b*, and carotenoid contents with the increased metal concentrations ($P \leqslant 0.05$; **Fig. 2**). In general, *C. aculeolata* was more sensitive to Cd at high concentration (0.5 mg/L) while *N. opaca* was more sensitive to Pb. Zn had no effect on pigment contents of *C. aculeolata* ($P > 0.05$) but caused a significant decrease in carotenoid content of *N. opaca* ($P \leqslant 0.05$). *C. aculeolata* showed the lowest pigment contents (19.9 and 3.8 mg/g for total chlorophyll and carotenoid, respectively) when exposed to high Cd concentration. *N. opaca* showed the lowest pigment contents (13.9 and 2.5 mg/g for total chlorophyll and carotenoid, respectively) when exposed to 10 mg/L Pb.

2.4 Percentage of metal removal

High percentages of Cd and Pb removal were observed in *C. aculeolata* and *N. opaca* (**Table 3**). A total removal of Cd (100%) was found in *C. aculeolata* at 0.25 mg/L Cd on day 3 but the proportion of Cd removed decreased with increasing concentration ($P \leqslant 0.05$). As shown with the results on growth, *N. opaca* was more sensitive to both Cd and Pb than was *C. aculeolata*. All *N. opaca* thalli died at 0.5 mg/L Cd and 10 mg/L Pb. In contrast, a very low percentage of Zn removal was observed in both species of charophytes.

2.5 Bioaccumulation

The present study showed a concentration-dependent accumulation of Cd, Pb and Zn in *C. aculeolata* and *N. opaca* tissues (**Fig. 3**). The metal accumulation in algae increased linearly with increasing concentration of metals in the medium. In *C. aculeolata*, the coefficient of correlation, R^2 values between metal concentration in solution and that accumulated by algae for Cd, Pb and Zn were 0.9821, 0.9462 and 0.9522, respectively. In *N. opaca*, the R^2 values were 0.8196, 0.9681 and 0.7173 for Cd, Pb and Zn, respectively.

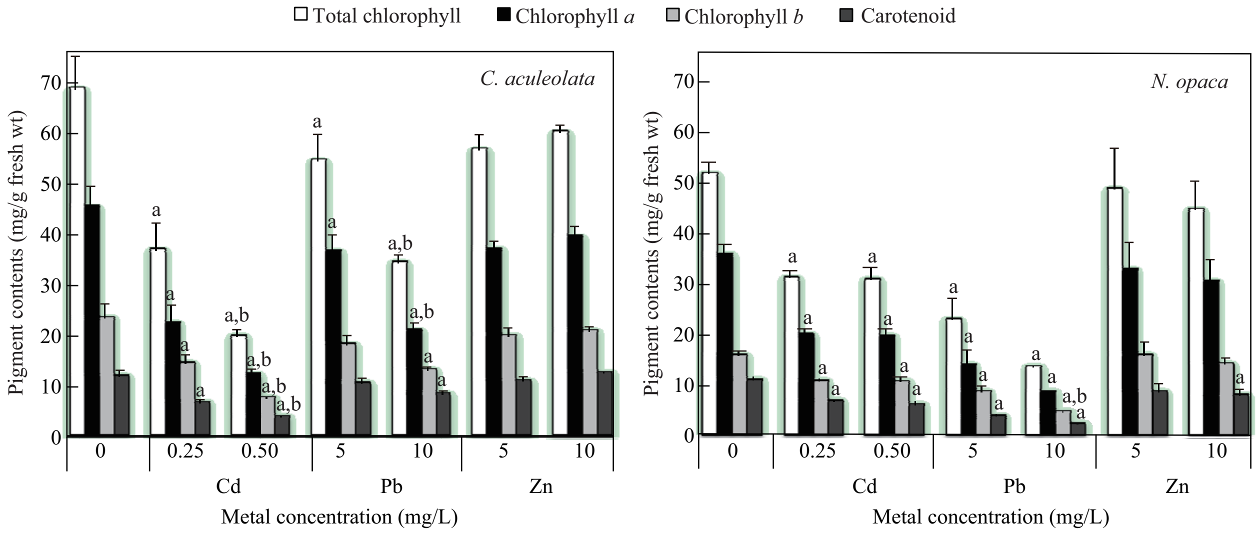

Fig. 2 Effects of Cd, Pb and Zn on contents of total chlorophyll, chlorophyll *a*, chlorophyll *b* and carotenoid of *C. aculeolata* and *N. opaca* at different concentrations after 6-day exposure. Results are means ± SD of 3 independent thalli. Significant differences are indicated: a, $P \leqslant 0.05$ versus control without Cd, Pb or Zn; b, $P \leqslant 0.05$ versus lower concentration of same metal.

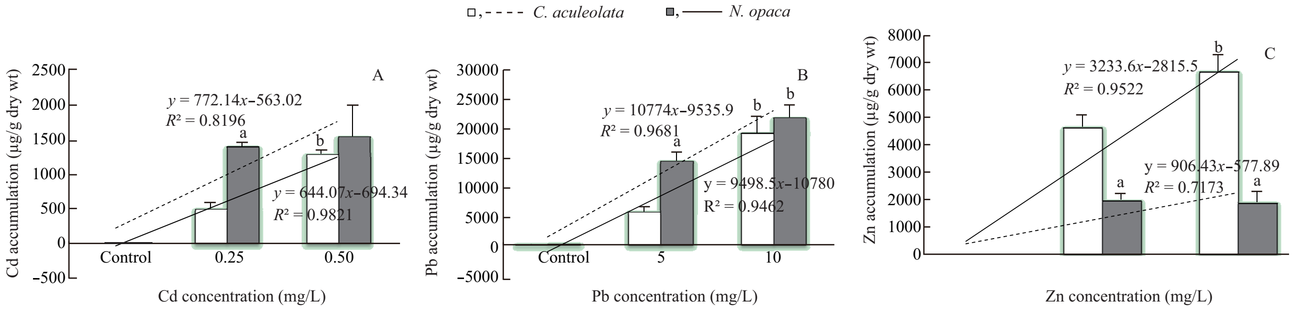

Fig. 3 Accumulation of Cd (A), Pb (B) and Zn (C) by *C. aculeolata* and *N. opaca* treated with different metal concentrations for 6 days. Results are means ± SD of 3 independent thalli. Significant differences are indicated: a, $P \leqslant 0.05$ versus *C. aculeolata*; b, $P \leqslant 0.05$ versus lower concentration.

At low concentrations in the medium, *N. opaca* accumulated significantly more Cd and Pb than *C. aculeolata* ($P \leqslant 0.05$) (**Fig. 3a, b**). However, neither algal species showed any significant difference in Cd and Pb accumulation at high concentration of metals ($P > 0.05$). In contrast, *C. aculeolata* accumulated significantly more Zn than *N. opaca* both at high and low Zn concentration (**Fig. 3c**). *N. opaca* displayed the highest accumulation of Cd at 0.5 mg/L (1544.3 μg/g) and Pb at 10 mg/L (21,657.0 μg/g), while *C. aculeolata* displayed the highest accumulation of Zn at 10 mg/L (6467.2 μg/g) after 6 days of exposure (**Fig. 3**).

2.6 Bioconcentration factor

C. aculeolata and *N. opaca* showed a similar trend of bioconcentration of Cd, Pb and Zn to that observed in the growth and toxicity studies. *N. opaca* was more sensitive to Cd and Pb than *C. aculeolata* but both algal species showed similar response to Zn (BCF values < 1000; **Fig. 4**). Even though *N. opaca* displayed significantly high BCFs for Cd (8577.2) and Pb (3347.5) at low concentrations ($P \leqslant 0.05$), these were not significantly different from those of *C. aculeolata* at high Cd concentration. There was no significant decrease in BCFs with increasing concentrations of Cd, Pb and Zn in *C. aculeolata*, indicating that the alga maintained its ability to accumulate these metals even when metal concentrations were increased.

3 Discussion

3.1 Heavy metal toxicity

Both species of charophytes, *C. aculeolata* and *N. opaca* showed different responses to heavy metals in terms of their growth performance, pigment contents and toxicity symptoms. *C. aculeolata* was more tolerant of Cd and Pb than *N. opaca*. However, similar RGRs were observed when the algae were exposed to Zn. The toxicity of metals in term of growth reduction was in the order of Zn < Pb < Cd. Similar trends have been observed by Rai et al. (1981) in other species of macroalgae, and by Lamai et al. (2005) in *Cladophora fracta*, a freshwater chlorophyte. Both Cd and Pb at high levels cause decreased growth and induce oxidative stress in marine macroalgae (Collén et al., 2003) and in the freshwater species *Chara globularis* and *Hydrodictyon reticulatum* (Gao and Yan, 2012). Oxidative stress can induce cytotoxicity and cell death, as reported for tobacco cells by Garnier et al. (2006). Although Zn at high concentration (5 mg/L) inhibits growth of several marine macroalgae (Amado et al., 1997), *C. aculeolata* and *N. opaca* were not affected, and could be considered as Zn-tolerant. The ecotypes of both algal species might influence their difference in tolerance to metals and therefore other aspects of their metal metabolism, such as accumulation. Both algal species grew in clean freshwater with low BOD values (**Table 4**). *C. aculeolata* found in Bueng Boraphet reservoir required less nutrients than *N. opaca*. Therefore, in nutrient-limiting medium contaminated with heavy metals, *C. aculeolata* survived better than *N. opaca*.

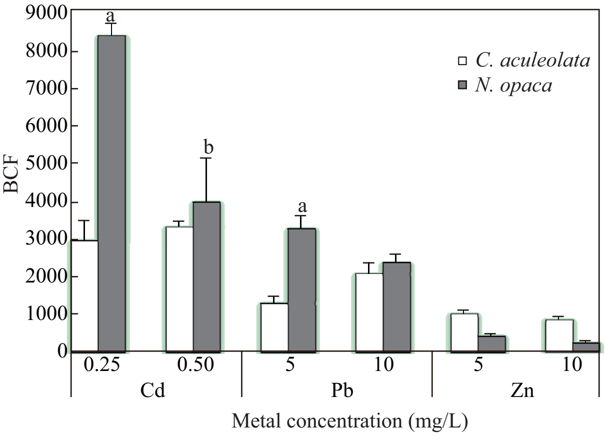

Fig. 4 Bioconcentration factors (BCF) for Cd, Pb and Zn in *C. aculeolata* and *N. opaca* treated with different metal concentrations for 6 days. Results are means ± SD of 3 independent thalli. Significant differences are indicated: a, $P \leqslant 0.05$ versus *C. aculeolata*; b, $P \leqslant 0.05$ versus lower concentration of same metal.

Table 4 Environmental status of the stations from where *C. aculeolata* and *N. opaca* were collected

Species	*C. aculeolata*	*N. opaca*
Temperature (°C)	29.5 ± 0.5	28.1 ± 0.1
pH	6.9 ± 0.4	6.9 ± 0.2
DO (mg/L)	5.7 ± 1.4	2.1 ± 1.6
BOD (mg/L)	2.9 ± 0.4	1.9 ± 0.7
Electrical conductivity (μS/cm)	289.8 ± 17.6	423.7 ± 44.0
Alkalinity (mg/L as $CaCO_3$)	103.8 ± 2.0	142.6 ± 14. 7
TDS (g/L)	0.2 ± 0.0	0.2 ± 0.0
NO_3^--N (mg/L)	0.1 ± 0.0	2.9 ± 2.1
NO_2^--N (mg/L)	0.006 ± 0.001	0.002 ± 0.001
NH_4^+-N (mg/L)	0.034 ± 0.014	1.083 ± 1.684
Ortho-phosphate (mg/L)	0.025 ± 0.003	0.571 ± 0.184

Results are means ± SD of 3–4 stations. TDS: total dissolved solid.

Metal tolerance may be assessed by the degree of toxicity symptoms such as reduction in photosynthetic pigments (chlorophyll and carotenoids) leading to chlorosis, and by softening and detachment of algal cells. *C. aculeolata* and *N. opaca* exposed to Cd, Pb, Zn for 6 days showed significant decreases in chlorophyll *a*, *b*, total chlorophyll and carotenoid contents with increases in metal accumulations. The decrease in chlorophyll concentration was possibly due to increased chlorophyllase activity, disorganization of chloroplast membranes and thylakoids, and inactivation of electron transport of PSII (Boonyapookana et al., 2002). As in the present study, both Cd and Pb have been reported to lower chlorophyll contents of *C. fracta* and chlorophyll fluorescence in several marine macroalgae (Lamai et al., 2005; Baumann et al., 2009). Cd and Pb do also cause the disintegration and disorganization of thylakoid membranes and chloroplast membranes in photosynthetic organisms such as plants, cyanobacteria, micro- and macroalgae (Visviki and Rachlin, 1994; Rangsayatorn et al., 2002). No decline in chlorophyll contents was observed in charophytes treated with Zn, except for a carotenoid reduction in *N. opaca* exposed to high Zn concentration in this study. Baumann et al. (2009) have reported that high Zn concentration does affect the PSII of several marine macroalgae.

3.2 Heavy metal accumulation

Macroalgae with good phytoremediation potential should display an ability to accumulate heavy metals at high concentration, a fast metal removal rate, and also high BCF values. More studies have been focused on marine macroalgae due to their identification as easily available, abundant and effective biosorbent biomass (Kuyucak and Volesky, 1990). Recent studies on the metal bioaccumulation in freshwater macroalgae such as *Chara*, *Nitella*, *Cladophora*, *Enteromorpha* and *Microspora* have revealed the higher accumulation of metals in *Cladophora* and *Chara* (Axtell et al., 2003; Lamai et al., 2005; Gomes and Asaeda, 2009; Bibi et al., 2010; Al-Homaidan et al., 2011; Gao and Yan, 2012). Different species of macroalgae have been found to have varying metal accumulation capacity and removal rates. Our study revealed that *C. aculeolata* and *N. opaca* possessed the potential to accumulate heavy metals in their thalli with bioaccumulation increasing with increase in external concentration in the order of Pb > Zn > Cd. *N. opaca* and *C. aculeolata* accumulated higher concentration of these metals at all concentrations tested, i.e., 21,657 μg/g for Pb (at 10 mg/L), 6467 μg/g for Zn (at 10 mg/L), and 1544 μg/g for Cd (at 0.5 mg/L). These were much higher than those reported for freshwater charophytes in other studies: 2540 μg/g Zn at 1 mg/L after 35 days for *Nitella graciliformis* (Bibi et al., 2010), 3650 μg/g Pb at 160 mg/L after 15 days for *Chara globularis*, and 1660 μg/g Pb at 1.61 mg/L after 15 days for *Chara corallina* (Gao and Yan, 2012). Harvesting habitat, experimental conditions, and major nutrients used may have affected patterns of metal accumulation in these members of the same family (Charophyceae) in addition to differences among species (Kinkade and Erdman, 1975; Lee and Wang, 2001). The high concentrations of metals accumulated by charophytes in this study were by far the highest concentrations encountered.

A BCF value of > 1000 can be used to indicate hyperaccumulating capacity (Boonyapookana et al., 2002). Based on the BCF values, *C. aculeolata* and *N. opaca* could be considered as Cd hyperaccumulator (BCF 3000–8000), and moderate Pb accumulators (1300–3300). In comparison, *C. fracta* shows BCF values of 1190–1230 for Pb and 1160–1200 for Cd (Lamai et al., 2005). As in other studies (Wang and Dei, 1999; Lamai et al., 2005), BCF decreased with increasing metal concentrations, probably due to growth reduction induced by higher metal concentration. The accumulation potential of *C. aculeolata* and *N. opaca* based on the BCF values was in the order of Cd > Pb > Zn.

Different aquatic plants and macroalgae display varying metal removal rates. The present results revealed that the metal removal was up to 100% for Cd, 96.8% for Pb, and 17.7% for Zn in *C. aculeolata* at low metal concentration and 3 days of exposure. In *N. opaca*, they were 60.7%, 93.2%, and 0%, respectively. *C. aculeolata* was a Cd and Pb remover while *N. opaca* was only a Pb remover and neither species was a Zn remover. In comparison, another macroalga, *Microspora* shows a total removal rate of over 95% for Pb after 10 days of exposure (Axtell et al., 2003). *Salvinia minima* (aquatic fern) and *Spirodela punctata* (aquatic plant) remove 70%–90% of Pb and Zn in 2 days of exposure to concentrations of 1–8 mg/L (Srivastav et al., 1993). High percentage of Cd and Pb removal in this study was probably the result of high metal adsorption at outer cells and high uptake into intracellular cytoplasm.

In aquatic ecosystem, many metal-bearing waste streams contain substances such as organic matter, alkaline earth metals, and dissolved organic carbon (DOC) that may decrease the removal capability of the metal ions by algae (Brauckmann, 1990; Eilbeck and Mattock, 1987). DOC (such as humic acid, fulvic acid) stays dissolved in aquatic system or in soil solution under natural conditions (Harter and Naidu, 1995). DOC reduces metal adsorption onto soil surfaces or algal cell surface by competing more effectively for the free metal ion and forming soluble organo-metallic complexes (Giusquiani et al., 1998). Macroalgae such as charophytes are usually used in the secondary wastewater treatment for removal of residual free metal ions (Stottmeister et al., 2006). Hence, the practical removal of metals by these algae requires additional analysis of degree of DOC that affects metal removal since the concentration of DOC varies with season, type of water, and chemical and biological processes such as microbial decomposition (Karlik and Szpakowska, 2001).

4 Conclusions

Both species of charophytes, *C. aculeolata* and *N. opaca* showed a preference for bioaccumulation in the order of Cd > Pb > Zn. While *C. aculeolata* removed both Cd and Pb at relatively similar rates, *N. opaca* showed a preference for Pb removal. The primary limitation on this bioaccumulation and removal potential was the lethal dosages apparent at 0.5 mg/L Cd and 10 mg/L Pb in both species. *N. opaca* was more sensitive to low dose of Cd and Pb.

Phytoremediation, the use of tolerant macroalgae that accumulate metals at high rates may offer an effective, inexpensive and environmental friendly option of heavy metal remediation. *C. aculeolata* should be a better choice than *N. opaca* due to its tolerance to many metals, and ideally should be used to treat large volume of wastewater with low concentration of metals.

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