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Removal of Heavy Metals by *Cladophora* sp. in Batch Culture: The Effect of Wet-mixed Solidified Soil (loess) on Bioremoval Capacities

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The heavy metal removal capacity of filamentous green alga *Cladophora* sp. cultured together with wet-mixed solidified soil (loess) was tested. A *Cladophora* sp. was cultured for 5d, with added Chu No. 10 medium, in stream water contaminated by high concentration of heavy metals from a closed mine effluent. Heavy metal ion concentrations of the medium and in algal tissue were measured every day during the experiment. Dissolved metals (Al, Cd, Cu, Fe, Mn, Zn) in medium were rapidly removed (over 90% elimination) within 1-2d when alga and loess were added. Dissolved heavy metals dropped by only 10% when algae were cultured without loess. The *Cladophora* sp. accumulated much more heavy metals when cultured with loess than when the alga was cultured alone. *Cladophora* sp. exhibited a maximum uptake capacity for Al (17,000 μg g^{-1} algal dry weight). The metal bioremoval capacities of the algae were in the order Al, Fe, Cu, Mn, Zn and Cd. The heavy metal removal capacity of *Cladophora* sp. showed significant increases when wet-mixed solidified soil was added to culture media.

Key words : filamentous algae, *Cladophora*, bioremoval capacity, heavy metal, loess

INTRODUCTION

The major sources of heavy metal pollution in the streams are industrial waste and mine effluent, including that from closed mines. Heavy metal pollution of water creates a serious hazard to aquatic organisms as well as human health (Letterman and Mitsch, 1978; Volesky and Holan, 1995; Sahu *et al.*, 2007). Heavy metals cannot be easily eliminated from a water body because they do not breakdown. Aquatic organisms can absorb heavy metals directly by assimilation or indirectly through the food chain. Non-degradable inorganic pollutants like heavy metals ultimately disturb fresh water ecosystems because these pollutants accumulate at the higher trophic levels of the food chain.

Chemical precipitation, ion exchange, reverse osmosis and solvent extraction are most commonly using procedures for heavy metal removal (Sternberg and Dorn, 2002). Although these technologies tend to be efficient, they are economically viable only for very large industrial units and they are expensive when used to handle large volumes of wastewater with low levels of metal contamination (Bux *et al.*, 1997). New technologies for heavy metal removal are therefore required. Although bioremoval is potentially problematic in that growth and clearance efficiency of the biological agent may be limited by toxic effects of the heavy metals in solution, this approach offers a potential alternative technology for heavy metal removal, as recently shown (Roy *et al.*, 1992; Wilde and Benemann, 1993; Singh *et al.*, 2007). Bioremoval is the accumulation of heavy metals

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by biological materials such as algae and aquatic plants. Bioremoval can be very effective in shallow water bodies (1-5 m) with low concentrations (1-20 mg L^{-1}) of heavy metal ions (Roy *et al.*, 1992). The advantages of bioremoval are effective reduction of heavy metal concentrations to very low levels, the environmentally friendly nature of the technology, and that the bioremoval agent may be relatively easy to grow in large quantities (Wilde and Benemann, 1993; Yu *et al.*, 1999). Various algae are used to remove heavy metals from contaminated water because algae are ubiquitous.

Filamentous green algae of the genus *Cladophora* are possibly the most widespread fresh water macroalgae. They are common and abundant in flowing streams, lake shores and irrigation channels. Previous works have shown that *Cladophora* species possess excellent potential for removal of heavy metals (Sobhan and Sternberg, 1999; Ozer *et al.*, 2000; Sternberg and Dorn, 2002; Chantana *et al.*, 2005).

Biosorption occurs in a manner similar to that of ion exchange in inactivated cells (Nakajima and Sakaguchi, 1986; Aksu *et al.*, 1998). In living cells, the ion exchange step may be followed by a metabolism-dependent uptake step in which the metal is transported into the cells. In recent years, there has been increasing interest in using ecologically inert clays and loess as a means of mitigating harmful algal blooms (Na *et al.*, 1996; Anderson, 1997; Bae *et al.* 1998; Sengco *et al.*, 2001). Clays are relatively inexpensive and are presumed to have little or no direct toxic effects on aquatic organisms (Howell and Shelton, 1970; Portman, 1970). Clay minerals are neutralized by an excess of heavy metal ions, due to high anionic strength on clay surface, which may, in turn, be effected by the growth and metabolism of algae cultured with the clays (Sengco *et al.*, 2001). Algae may benefit from digestion of organic substances adsorbed to clay particles (Avnimelech *et al.*, 1982).

The main objectives of this study include 1) validation of possibility of using the *Cladophora* on heavy metal removal of mine effluent and 2) examination of the effect of loess on algal bioremoval capacity of heavy metals.

MATERIALS AND METHODS

1. Algal preparation

The *Cladophora* sp. used for this study was collected from Sinchun stream located in Daegu city in southeast South Korea. The algae were washed with flowing water at sampling sites to remove dirt and debris, and were re-washed several times with distilled water in the laboratory.

2. Culture and test conditions

Two experiments were performed. In one test, the culture medium was adjusted to pH 7 prior to algal inoculation. In the other test, the pH of the medium was not adjusted. The culture experiments were conducted in five 500 mL Erlenmeyer flasks for 5 d. The culture medium tested for algal bioremoval capacity was raw stream water with high levels of heavy metals because of effluent from the closed tungsten mine located at the upper part of the Sinchun stream passing through Daegu city. The raw water was filtered using Whatman no. 4 filter paper to remove dirt and debris. A fertilizer solution (Chu No. 10 medium of 5 mL) was added to each culture flask containing filtered raw water (350 mL). All cultures received approximately 1 g wet weight of algae, with or without wet-mixed solidified soil (loess, 15 g) developed for pavement. The pH of some media was adjusted to pH 7 with NaOH before culture commenced. Metal concentrations in culture media and algal tissue measured prior to culture served as controls.

3. Measurements and procedures

Dissolved heavy metal concentrations in culture media, and concentrations of heavy metals in algae tissue were measured before algal inoculation, and at 1 d, 2 d, 3 d, 4 d and 5 d of culture. On each of the five days, all algal materials from a single flask were harvested for heavy metal analysis. The pH levels were monitored throughout the experiments. All culture supernatants received 2 drops of 1.0 M nitric acid to prevent metal precipitation before analysis. Heavy metal ion concentrations in culture solutions and algal tissue were measured by the procedure recommended in Standard Methods (APHA, 1995) using an inductively coupled plasma optical emission spectrometer (ICP-OE, Perkin-Elmer 4300DV). Before digestion, algae was separated by filtration using membrane filter of pore size 0.45 μm, and collected algae were placed in a drying oven at 60°C for 2 d for dry weight determination. The samples were cooled to room temperature and

weighed on a digital balance until a constant weights was obtained. The samples were then placed in a muffle furnace at 560°C for 24 hr to remove volatile components and to induce oxidization of adsorbed heavy metals. The ashes were dissolved in 15 mL of 10% HNO_3 for 2 days, and filtrated using a 0.2 μm Milipore filter. The concentrations of heavy metals in algal tissue were measured using a using inductively coupled plasma optical emission spectrometer (ICP-OE, Perkin-Elmer 4300DV). Bioconcentration factor (BCF) was calculated, to quantify algal metal removal potential. The BCF is the ratio of the metal concentration in the dry algal biomass to the initial concentration of metal in the feed solution (Raskin *et al.*, 1994).

RESULTS AND DISCUSSION

Through two experiments, we examined the heavy metal (Al, Cd, Cu, Fe, Mn and Zn) biosorption capacities of a *Cladophora* sp. alga from the stream water influx of closed mine effluent. Loess strongly increased the heavy metal biosorption capacity of *Cladophora* sp. When the culture media was adjusted to pH 7 before the culture, the pH of the culture media without loess ranged from 7 to 7.4 throughout the test. The pH of the culture media to which loess had been added increased to pH 8.0 after 1 d and then remained in the range of 7.5-8.0 (Fig. 1A). When the pH was not adjusted before culture, the pH values of culture media without loess were strongly acidic (3.1-3.3) throughout the experiment. The pH values of culture media receiving loess rapidly increased to pH 6.5 after 2 d, however remained almost constant level at pH 6.5 to 6.7 thereafter (Fig. 1B).

The effectiveness of heavy metals removal by *Cladophora* sp. in batch culture, with or without loess, is shown in Figs. 2 and 3. Heavy metal removal was more effective when the algae were cultured with loess than was the case when the soil was absent. In experiments where pH values were not adjusted before culture, the removal rates of dissolved heavy metals by the last day of culture were between 9.2% (Al) and 20.2% (Fe) when the *Cladophora* sp. was cultured without loess. When the algae were cultured in the medium with loess, however, heavy metals removal rates of 56-100% were noted (Fig. 2). The heavy

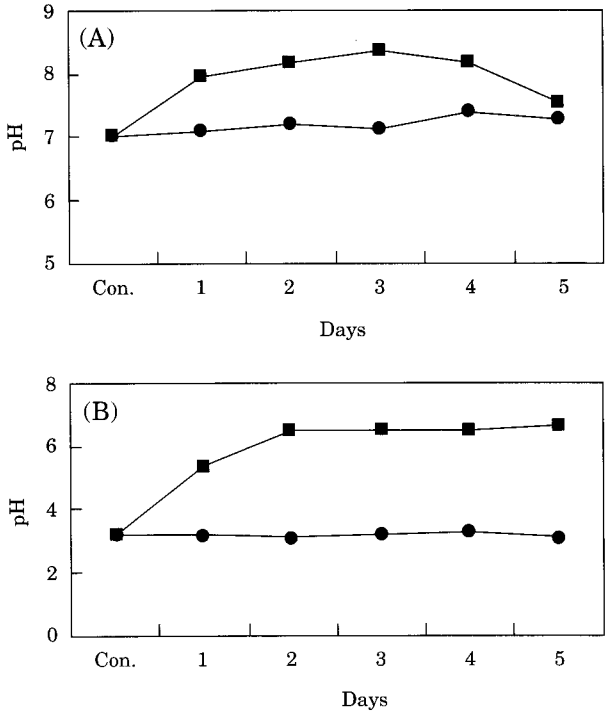

Fig. 1. The pH variations of culture media during the experimental periods. (A) The media were adjusted to pH 7 before culture began, (B) The pH values were not adjusted.

metals were rapidly removed (within the 1-2 d of culture) when *Cladophora* sp. was cultured with loess. Over 96% of Al and Fe were removed within the first day. Over 71% of Cd, Cu and Zn were removed within 2 d, and 84% of Cd, 79% of Cu and 92% of Zn were removed by 5 d. Only 56% of Mn was removed after 5 d (Fig. 2). In experiments where the pH was adjusted to pH 7 before culture, algae in media both with and without loess exhibited metal removal rates of almost 100% for all metals, except that the Mn removal rate was 26.5% when the *Cladophora* sp. was cultured without soil (Fig. 4). The removal rates of Cd, Cu and Mn were faster in media adjusted to pH 7.

The heavy metal accumulations in the *Cladophora* sp. tissue at different culture time are shown in Fig. 3 and 5. The heavy metal concentrations in algal tissue varied according to culture time and medium composition. In the experiments where media were not adjusted to pH 7 before culture, maximal accumulations of heavy metals in algal tissue were, in order: Fe (5,618 μg g^{-1}), Al (3,188 μg g^{-1}), Mn (335 μg g^{-1}), Zn (127 μg

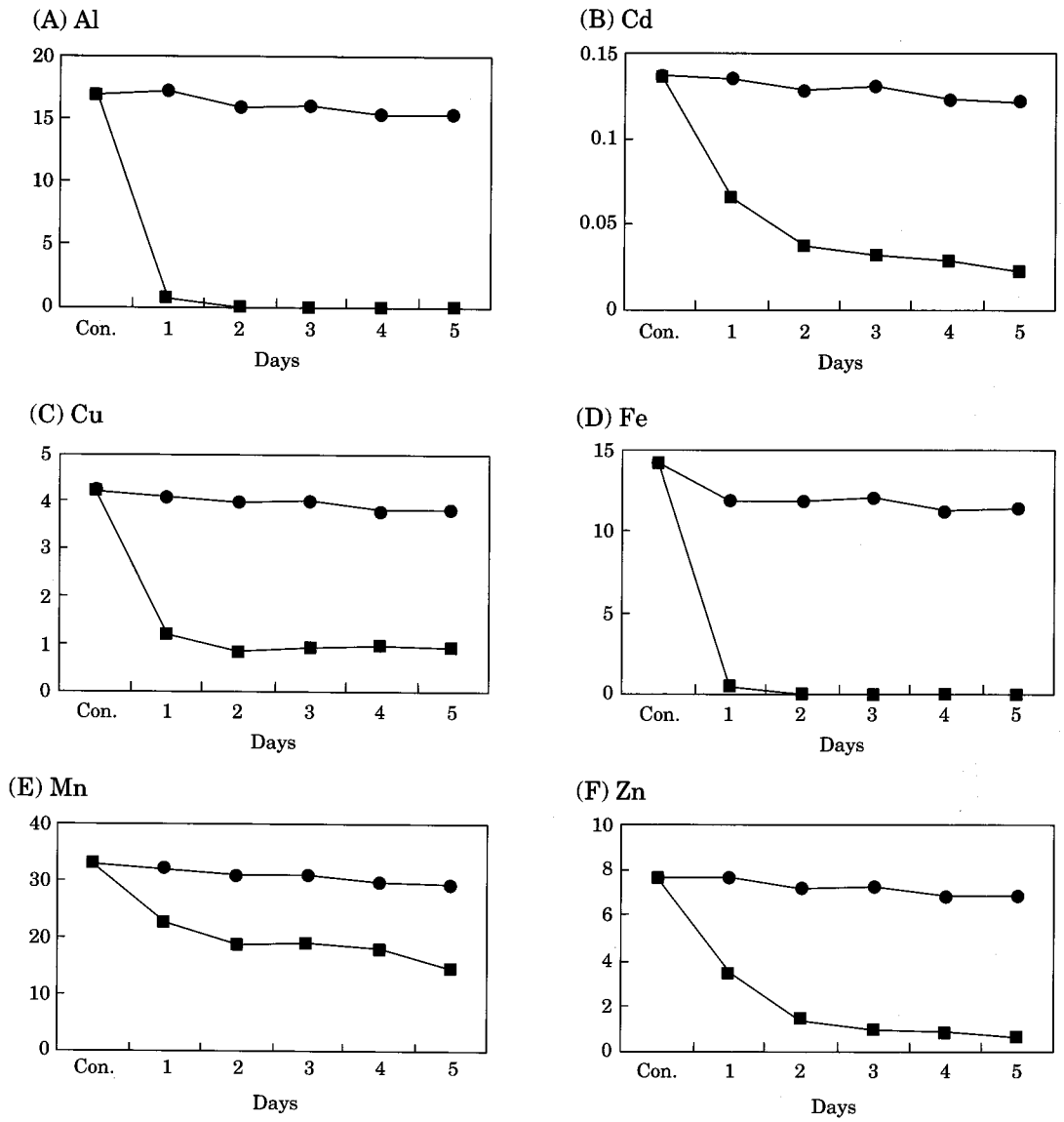

Fig. 2. The concentrations (mg L^{-1}) of dissolved heavy metals in the culture media of *Cladophora* sp. Cultured with and without loess. (●: no loess ■: with loess).

g^{-1}), Cu (97 μg g^{-1}) and Cd (0.8 μg g^{-1}) when *Cladophora* sp. was cultured in media without loess (Fig. 3). When the algae were cultured in media with loess, the maximal concentrations of heavy metals accumulated in algal tissue were, in order: Al (17,707 μg g^{-1}), Fe (5,185 μg g^{-1}), Cu (1,299 μg g^{-1}), Mn (595 μg g^{-1}), Zn (416 μg g^{-1}) and Cd (5.7 μg g^{-1}). The algae were especially effective in the accumulation of Al and Fe. All of these metals attained maximal concentrations in algal tissue after 3 d of culture. The accumulation of all metals except Fe was faster when algae were cultured in loess-containing media compared to accumulation rates in media without loess. In experiments where pH was adjusted before culture, algae in media with and without loess exhibited maximum metal concentrations in the order: Mn (5,906-16,453 μg g^{-1}), Al (5,775-12,807 μg g^{-1}), Fe (3,092-11,948 μg g^{-1}), Zn (1,423-1,901 μg g^{-1}), Cu (531-1,491 μg g^{-1}) and Cd (36.4-53.3 μg g^{-1}) (Fig. 5). Present study, a *Cladophora* sp. cultured in medium without loess, and without pH adjustment prior to culture, showed considerable chlorosis caused by toxic effects of heavy metal levels.

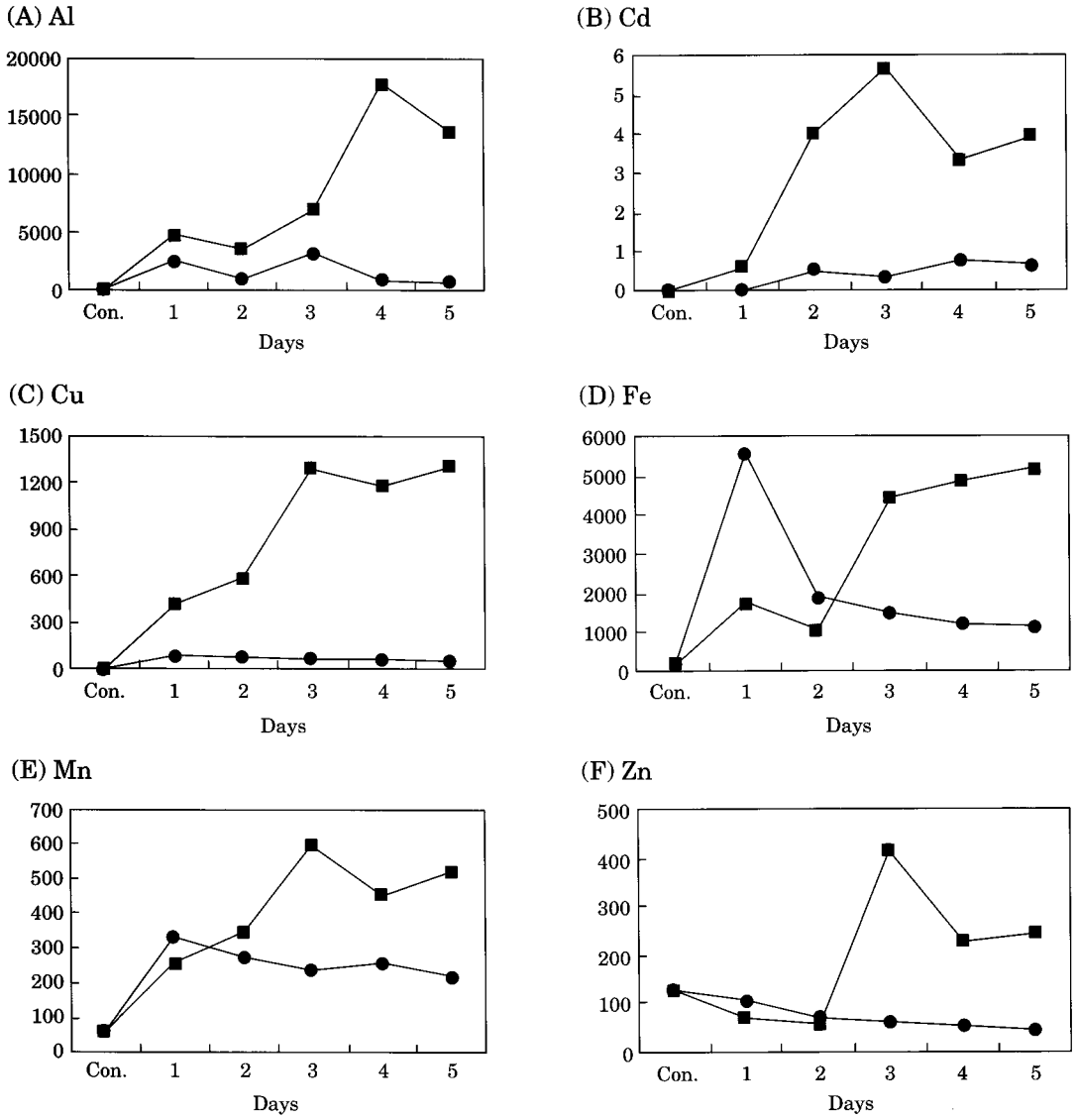

Fig. 3. The accumulation (μg g^{-1}) of heavy metals by *Cladophora* sp. from culture media with and without loess. (●: no loess, ■: with loess).

Under these conditions, heavy metal ion concentrations in the culture media declined 9.2%(Al)-20.2% (Fe) after 5 d culture. Heavy metal absorption occurred largely in the first 1-2 d of culture, and, after this time, little further absorption occurred. Accumulation of heavy metals by algal tissue was much greater when the algae were cultured with loess. A possible explanation about these results may involve an ion exchange mechanism proposed for inactivated cell, which features a definite and constant number of adsorption sites on the non-living biomass (Nakajima and Sakaguchi, 1986). After these sites become saturated with metal ions, an adsorption equilibrium is established, resulting in no net concentration change. This model may explain previous reports that *Cladophora fracta* exposed to heavy metals (Cd and Pb) showed a reduction in chloroplast numbers, cell wall crumbling, and chloroplasts destruction at high metal concentrations (Chantana *et al.*, 2005). Similar results were observed in a toxicity symptom study, in which different algal species were exposed to heavy metals (Lasheen *et al.*, 1990; Leborans and Novillo, 1996).

The heavy metal biosorption capacities of the

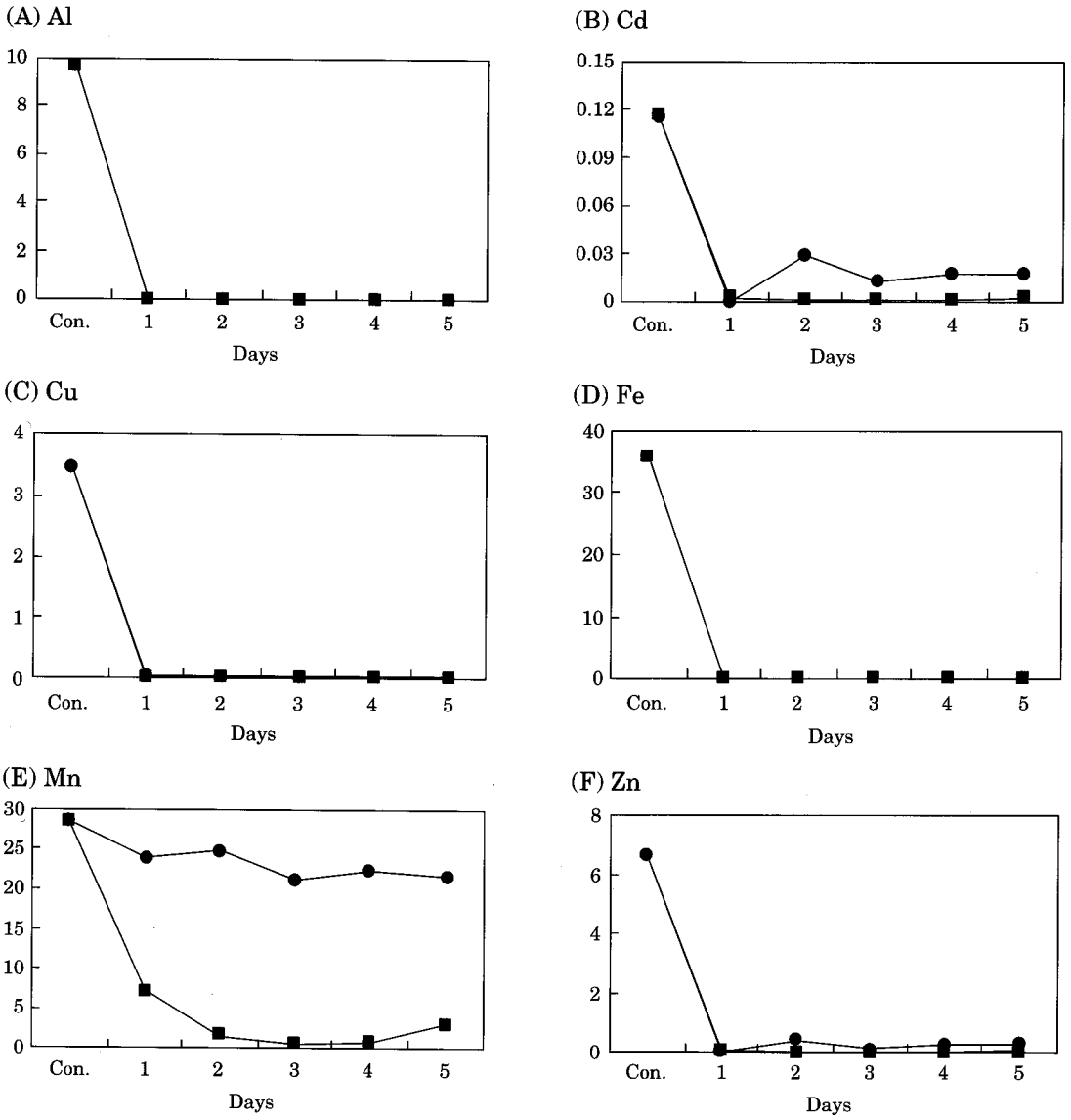

Fig. 4. The concentrations (mg L^{-1}) of dissolved heavy metals in culture media with and without loess. The pH was adjusted to pH 7 before culture in all cases. (●: no loess, ■: with loess).

Cladophora sp. cultured in media with loess increased 1.8-fold (Mn) to 13.4-fold (Cu) when media were pH not adjusted prior to culture (Fig. 3), and 1.3-fold (Zn) to 3.9-fold (Fe) in tests where the media were adjusted to pH 7 before culture (Fig. 5). The maximum *Cladophora* sp. BCFs for six metals (Al, Cd, Cu, Fe, Mn and Zn) are shown in Fig. 6. The maximum BCFs were observed on day 3-4 of culture. The BCF of Al was highest (1,048-1,307), and the BCFs for Cd, Cu, Fe, Mn and Zn were 41-444, 308-426, 331-365, 18-575 and 54-284, respectively, when the algae were cultured with loess. The BCFs for all heavy metals increased significantly when the algae were cultured in loess-containing medium. In the experiments where media were not adjusted to pH 7 before culture, this was particularly evident with Cu, Cd and Al accumulation. The BCFs increased dramatically (5.6-13.4-fold) when algae were cultured in loess-containing medium (Fig. 6A).

The present study, *Cladophora* sp. BCF values for six metals in loess-containing media were significantly higher than those in loess-free media.

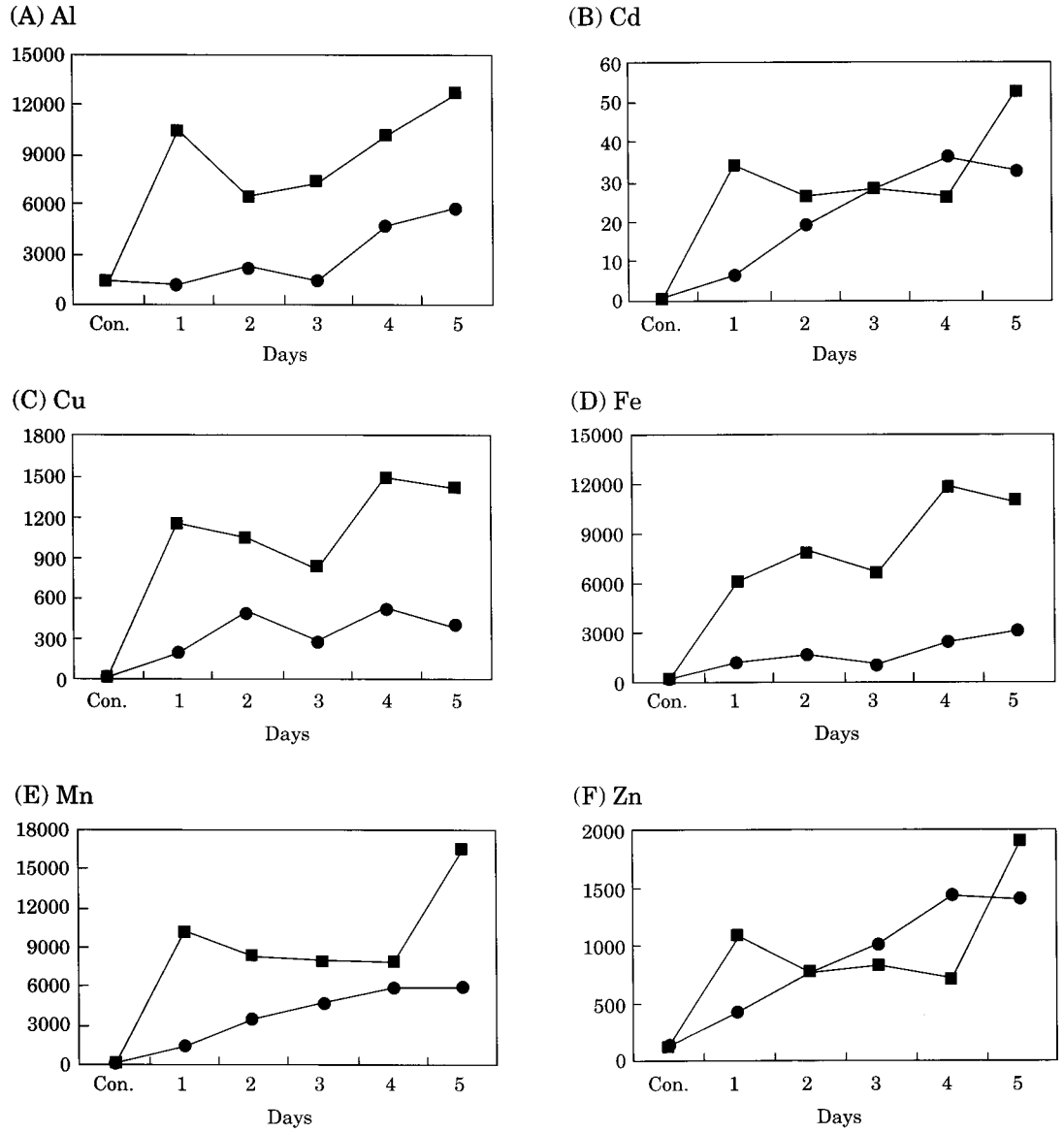

Fig. 5. The accumulation (μg g^{-1}) of heavy metals by *Cladophora* sp. from culture media with and without loess. The pH was adjusted to pH 7 before culture in all cases. (●: no loess, ■: with loess).

The pH of culture media without loess was strongly acidic (pH 3.1-3.3 throughout the experiment), but the pH of culture media with loess was sustained at pH 6.5 to 6.7. These results may indicate that loess rapidly adsorbs heavy metal cations because of high anionic strength on the surfaces of soil particles. Heavy metal toxicity for the *Cladophora* sp. is thus neutralized, and biosorption capacities and physiological activities may be accelerated. Similar results were reported in previous work. Sternberg and Dorn (2002) showed that viable algae were more effective in Cd removal when compared to non-viable algae. Gupta *et al.* (2001) reported that the heavy metal adsorption capacity of Spirogyra depends strongly on equilibrium pH.

In conclusion, our results supports previous findings that *Cladophora* possesses excellent heavy metal bioremoval potential (Ozer *et al.*, 2000; Sternberg and Dorn, 2002; Chantana *et al.*, 2005), and suggest that loess both effectively removes heavy metals and accelerates the heavy

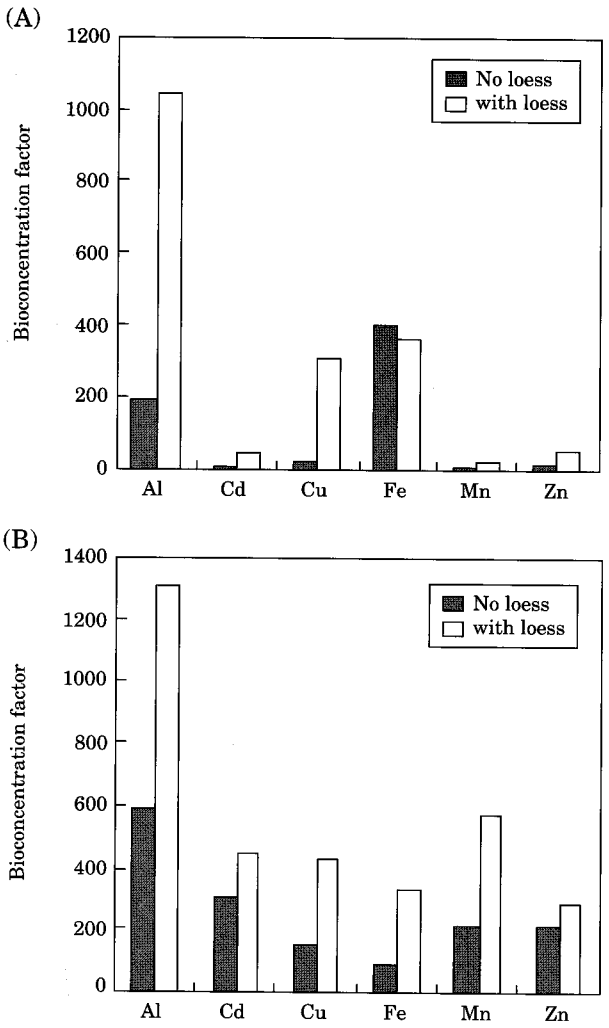

Fig. 6. Maximal *Cladophora* sp. BCFs heavy metals. A: The media were adjusted to pH 7 before culture began. B: The pH values were not adjusted.

metal absorption capacity of the algae. This study also indicates that bioremoval using *Cladophora* sp. and loess together may be a highly effective and cost-efficient method for heavy metal removal from industrial wastewater or closed mine effluent. Further studies on algal optimum growth conditions, and work on the design and management of an aquatic plant, are needed.

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