Cadmium binding ability of the blue-green alga *Hapalosiphon welwitschii* Nägel under controlled conditions

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The ability of the blue-green alga *Hapalosiphon welwitschii* Nägel (a cyanobacterium) to bind cadmium from its medium was tested in cultures exposed to different metal concentrations (0.5, 1.5, 2.5, 3.5, 4.5, 5, 6, 8, 10 and 12 ppm). The amount of cadmium removed was shown to be time and concentration dependent. With increasing cadmium concentrations of 0.5 to 10 ppm, significant levels of the heavy metal were detected on the cell wall. However, at 12.0 ppm, a higher amount of the heavy metal was detected inside the cytoplasm. The cells also exhibited efflux of cadmium after 24 and 48 h of incubation. The presence of Cu^{2+} significantly increased the amount of bound cadmium. On the other hand, the ions Co^{2+}, Mg^{2+}, and Ni^{2+} significantly decreased the amount of cadmium bound to the cells most probably because of competition for binding sites resulting in inhibition of cadmium binding. The maximum binding of cadmium by the cells was at pH 9, but was not significantly different from the binding observed in cultures grown at the optimum pH of 7.5. However, there was a significant decrease in cadmium binding when the pH of the medium was acidic (pH 3 and pH 5). The results obtained from this study may provide baseline information for further studies on the potential of the blue-green alga *H. welwitschii* to clean up cadmium contaminated areas.

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INTRODUCTION

The contamination of aquatic systems by heavy metals is becoming a serious problem worldwide. This is a consequence of global industrialization, which has become a major environmental concern due to hazardous effects on human and environmental health (Xiong et al. 2008). Heavy metals, such as cadmium, are non-biodegradable and are, therefore, mobilized and carried into the food web as a result of leaching from waste dumps, polluted soils and water (Allluri et al. 2007). The search for more microorganisms that can treat and clean up contaminated areas has become a widening endeavor among environmentalists.

Several microorganisms such as bacteria, e.g., *Sedum alfredii* Hance (Xiong et al. 2008), *Stenotrophomonas maltophilia* (Parungao et al. 2007), *Bacillus circulans* strain EB1 (Yilmaz and Ensari 2005), and *Corynebacterium glutamicum* (Choi and Yun 2004); blue-green algae, e.g., *Nostoc calcicola* (Pant 2000), *Synechococcus aquatilis* (Reynaud) strain SY 101 (Vallarta, et al. 1998), and *Anacystis nidulans* (Singh 1985); and microalgae, e.g., *Tetraselmis suecica* (Perez-Rama et al. 2002) and *Chlorella vulgaris* (Carr et al. 1998); have been reported to possess the ability to take up and accumulate heavy metals.

Blue-green algae are considered excellent microorganisms to serve as models for the investigation of environmental problems because they can serve as pollution indicators (Lee et al. 1999). These microorganisms can also be used to remove toxic heavy metals like cadmium, since they are normally present in many aquatic ecosystems. The relative ease in culturing and maintaining these photosynthetic prokaryotes makes them suitable agents for bioremediation.

H. welwitschii is a free-living, filamentous nitrogen-fixing blue-green alga that was isolated from Uprolo River, which is near a mining plant in Mogpog, Marinduque. Its ability to survive in an area known to be contaminated with various industrial effluents may indicate that this microorganism exhibits some tolerance for heavy metal pollutants. To our knowledge, there has not been much study on this blue-green alga, particularly on its binding of heavy metals. This study aimed to provide an initial investigation on the cadmium-binding ability of *H. welwitschii*.

EXPERIMENTAL

Microorganism and culture conditions

H. welwitschii was provided by the Microalgal and Cyanobacterial Laboratory of the Natural Sciences and Research Institute (NSRI), University of the Philippines, Diliman, Quezon City. *H. welwitschii* was cultured in sterilized 400 ml bottles containing 150 ml BG-11 medium at pH 7.5 (Rippka 1988). The cultures were grown at room temperature ranging from 25°C – 30°C and with constant illumination using a 40 W white fluorescent lamp. The cultures were placed on a shaker set at 100 rpm to ensure the even distribution of nutrients.

All glassware and polycarbonate containers used in the study were washed with detergent and water, followed by acid-washing with 5% nitric acid (HNO_3). The glassware and polycarbonate containers were then rinsed twice with de-ionized distilled water (ddH_2O) and autoclaved at 15 psi for 15 min at 121°C.

A stock solution of 50 mg/L cadmium chloride ($CdCl_2$), Merck 95%, was prepared. In a range-finding test to determine the tolerance levels of the organism below LD50, the stock solution was diluted to achieve the desired final concentrations of cadmium of 0.5, 1.5, 2.5, 3.5, 4.5, 5.0 6.0, 8.0, 10.0, and 12.0 ppm of $CdCl_2$. Control cultures without cadmium were also included in the test. The methods of Vallarta et al. (1998) were adapted in the conduct of this study. All cadmium binding experiments were done in triplicate and were conducted twice.

Effect of varying cadmium concentrations

Thirteen-day old cultures, which correspond to the mid-logarithmic growth phase of *H. welwitschii*, were harvested and pelleted at 10,000 rpm for 25 minutes. The pellets were then washed with ultra-pure water (triple distilled, deionised). A total of 0.12 g wet weight of cells was used in each of the cadmium exposure treatments because this weight represented the minimum biomass that showed cadmium binding in a preliminary experiment. The samples were maintained at pH 7.5 at room temperature for 2 h with constant illumination. After 2 h, the samples were transferred into 30 ml centrifuge tubes and spun at 10,000 rpm for 20 min. The supernatants were collected and the pellets were washed with ultra-pure water to remove all unbound cadmium ions from the cell. After washing, the pellet was re-suspended twice in 10 ml 0.01 M Ethylenediaminetetraacetic Acid (EDTA) to further remove the cell wall-bound cadmium (Perez-Rama et al. 2002). The suspension was centrifuged for 20 min at 10,000 rpm and the supernatants were collected. The remaining pellets were digested overnight with 1:1 volume ratio of concentrated HNO_3 and the digested pellets were filtered the following day using a Whatman #42 filter paper. The collected supernatants and the acid-digest filtrates were prepared for metal analysis.

Time-dependency for cadmium binding

Cultures of *H. welwitschii* cells at the mid-logarithmic phase of growth were harvested and then washed with ultrapure water. The same amount of biomass of 0.12 g wet weight of cells was added individually to 10 test tubes with 10 ml BG-11 medium (pH 7.5) treated with 5 ppm Cd. The cultures were agitated continuously under lighted conditions (40 W fluorescent lamps) at room temperature. After 2 h of incubation, the cultures were collected at these time intervals: 2.6, 3.2, 3.8, 4.3, 4.9, and 5.6 h. The collected samples were spun and the supernatants and pellets were collected as described above.

Efflux Mechanism

To determine whether an efflux mechanism can also take place in the organism, the 40 ml culture medium left in the time course set-up was collected after one day of incubation. The samples were then centrifuged at 10,000 rpm for 20 min. The supernatant from each sample was collected to represent the amount of cadmium that was effluxed into the medium. The pellets were washed twice with, and resuspended in, 20-ml ultrapure water. Each sample was incubated for 24 h under lighted conditions at room temperature. After 24 h, the samples were centrifuged at 10,000 rpm for 20 min. The collected supernatant represents the excluded or effluxed intracellular cadmium. All collected samples were prepared for metal analysis.

Effect of divalent cations

Fifty ml Erlenmeyer flasks with 20 ml of BG-11 medium

were separately treated with equal amounts of 5 ppm cadmium and the test metal ions. The four cations used were Co^{2+}(AR, $Co(NO_3)_2.6H_2O$), Ni^{2+}(AR, $Ni(NO_3)_2.6H_2O$) , Cu^{2+}(AR, $Cu(NO_3)_2.6H_2O$) and Mg^{2+}(AR, $MgCl_2.6H_2O$). The same amount of biomass of 0.12 g wet weight of cells of *H. welwitschii*, as used in the other set-ups, was inoculated in each treatment under lighted conditions and at room temperature for 2 h. After 2 h, the samples were collected and centrifuged as described earlier.

Effect of pH

Test tubes with 10 ml of BG-11 medium (pH 7.5) and 5 ppm cadmium were prepared with the pH adjusted to 3, 5, 9 and 11 using either 1N HCl or 1N NaOH. The same amount of biomass of 0.12 g *H. welwitschii* used in the earlier experiments was added to each medium and incubated at room temperature with constant illumination and agitation. After 2 h, the samples were collected following the procedure described earlier.

Preparation of liquid samples for metal analysis

Supernatants collected from each sample and acid-digest filtrates were placed in separate 50 ml beakers and covered with watch glass. The beakers were placed on a hot plate and were evaporated to about 15-20 ml under the fume hood at just below boiling temperature. The solutions were cooled, after which, 3 ml of 1N HNO_3 and 3 ml of ultra-pure water were added. The samples were again heated to a gentle reflux and were evaporated to near dryness. The addition of HNO_3 and ultra-pure water was repeated until the digests were light in color or did not change in appearance after continued refluxing. The samples were again evaporated to near dryness. Three ml of 1:1 1N HNO_3 and ultra-pure water were added to dissolve any precipitate or residue that may have resulted from evaporation. The beaker walls and the watch glass coverings were also washed with ultra-pure water and the washings were filtered through a Whatman #42 filter paper to remove silicates and other insoluble material. The final volume was adjusted to 5 ml by adding ultra-pure water. All prepared samples were placed in acid-washed polycarbonate vials and submitted to the Institute of Chemistry of the University of the Philippines, Diliman, Quezon City for Flame Atomic Absorption Spectrophotometry (FAAS). The results of the analysis of the supernatants collected after washing with EDTA represent the amount of cadmium bound to the cell wall (Soares et al. 2002). The acid digestion of the pellets with concentrated HNO_3, which disrupts the cell wall and opens up the cytoplasm, provides the amount of cadmium located in the cytoplasm (Perez-Rama et al. 2002).

Statistical Analyses

The data were analyzed using the statistical software program SPSS version 10. Analysis of Variance (ANOVA) was done to compare the treatments performed simultaneously. The Duncan's Multiple Range Test (DMRT) was done for treatments which showed significant difference in the ANOVA. The level of significance was set at $p < 0.05$.

RESULTS AND DISCUSSSION

Effect of varying cadmium concentrations

The metal binding capacity of microorganisms has led to several studies designed to evaluate the potential of microbes in removing toxic metals from contaminated environments. In this study, we show that *H. welwitschii*, a free-living, nitrogen-fixing blue-green alga isolated from a river near a mining plant in Mogpog, Marinduque, displays a high binding ability to remove cadmium from its culture medium.

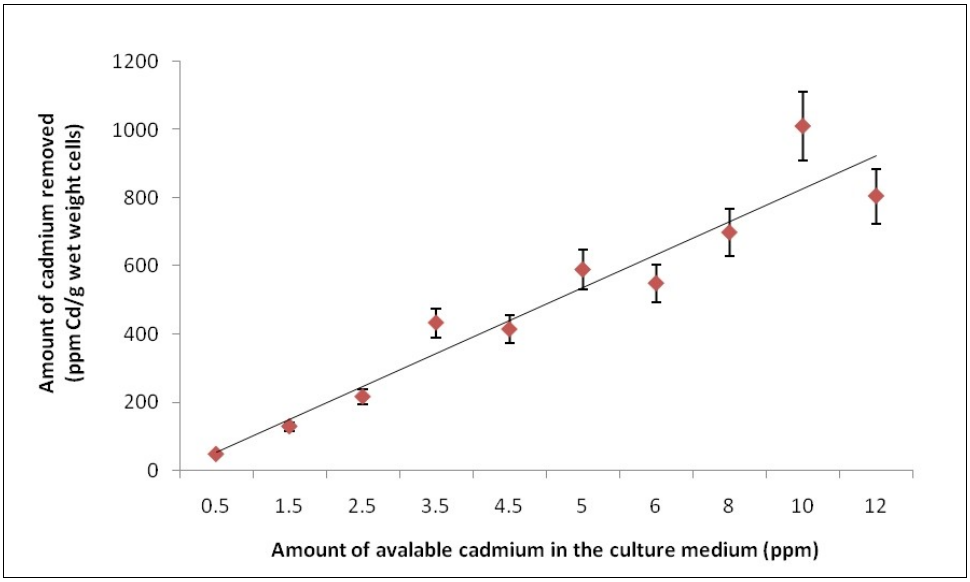

Figure 1. Effect of varying cadmium concentrations (ppm) on the binding by *H. welwitschii* cells. The values represent mean ±SEM of three replicates.

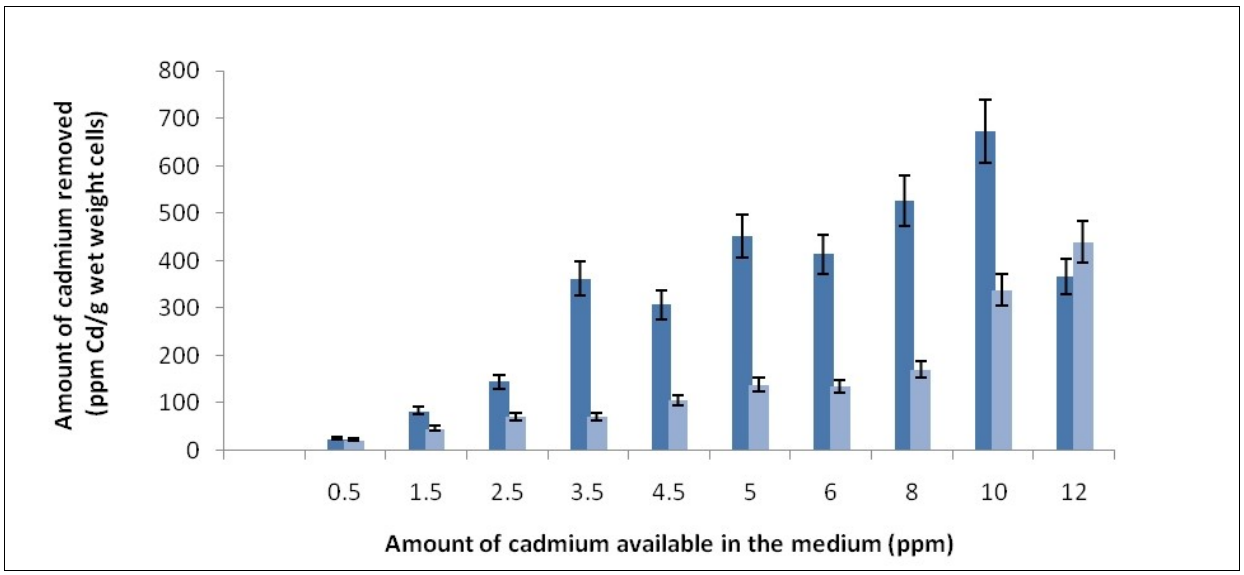

Figure 2. Effect of available cadmium concentrations on the cellular location in *H. welwitschii* cells. The cadmium ions were located either on the cell wall ( ) or inside the cytoplasm ( ) as determined by Flame Atomic Absorption Spectroscopy (FAAS). Error bars represent standard error of the means. The values represent mean ±SEM of three replicates.

After 48 h of incubation, the amount of cadmium bound was shown to be concentration dependent (Fig. 1). Treatment with various concentrations of cadmium solutions showed that, as the concentration of available cadmium was increased, the amount of cadmium bound also increased, up to the highest concentration tested. The lowest available cadmium concentration of 0.5 ppm resulted in the lowest amount of cadmium removed (47.4 ppm Cd/g wet weight cells), while the highest amount of cadmium removed (1010 ppm Cd/g wet weight cells) was observed at 10 ppm. The amounts of cadmium removed from the medium, at other concentrations of cadmium, were not significantly different from each other. However, at the highest available cadmium concentration of 12 ppm, the amount removed decreased significantly (805.0 ppm Cd/g wet weight cells) compared to the amount removed at 10 ppm (1010.0 ppm Cd/g wet weight cells).

It is possible that the binding of cadmium to *H. welwitschii* cells was dependent on the ratio of the external free metal ion concentration (Brady and Duncan 1994). Ybarra and Webb (1998) also noted that the uptake of metal ions by cells depends on the maintenance of a concentration gradient across bacterial cell membranes. With regards to the decline in the amount of cadmium by the blue green algal cells at 10 ppm, this observation might indicate that the 0.12 g of wet cells is not enough to bind more cadmium. It is therefore suggested that a study on the effect of varying weights of *H. welwitschii* cells be done because it is likely that the amount of cadmium removed could also be dependent on the weight of the blue green algal cells. As in the study done by Hamdy (2000), the amount of cadmium removed increased when the biomass of the alga *Laurencia obtuse* was increased. Also, in the study by Carr et al. (1998), increasing the cell number of *Chlorella vulgaris* significantly increased the efficiency of metal removal.

One of the ways microorganisms are used in the bioremediation of industrial wastes and other polluted environments is by their ability to take up heavy metals. Heavy metal uptake processes by biological cells are known under the general term biosorption (Yilmaz and Ensari 2005). Biosorption may include sequestering and binding the heavy metal to the cell wall, thus preventing the metal from reaching the intracellular cytoplasm, or by accumulating the metal inside the cytoplasm by binding to specific components, such as metallothionein (Silver and Misra 1988).

In this initial investigation on the ability of *H. welwitschii* to bind cadmium from its culture medium, it was shown that significantly higher amounts of cadmium were adsorbed on the cell wall than in the cytoplasm when the cultures were exposed to 0.5 to 10 ppm $CdCl_2$ (Fig. 2). However, when the cells were incubated with 12.0 ppm $CdCl_2$, a higher amount of the cadmium was located inside the cytoplasm compared to the amount found on the cell wall.

The binding of metal ions to the extracellular surface is the first mechanism employed by microorganisms in reducing the toxicity of the metal, or in increasing its resistance to the metal (Perez-Rama et al. 2002; Macfie and Welbourn 2000; Ybarra and Webb 1998). The cell wall plays an important role in the binding of metals to microorganisms by sequestering the metals (Carr et al. 1998; Gnassia-Barelli and Romeo 1985).

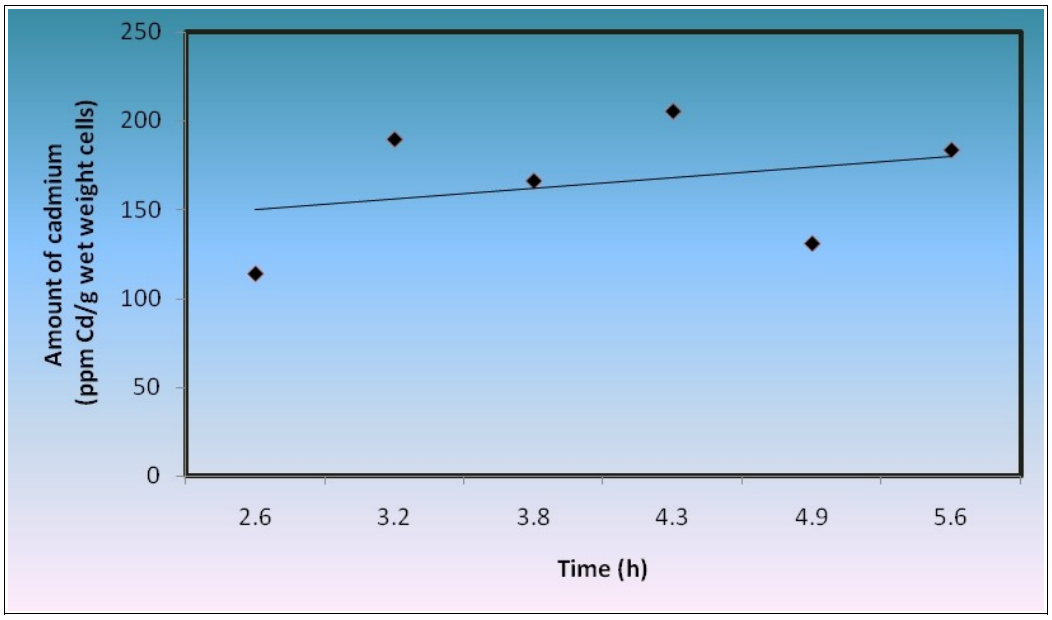

Figure 3. Experimental data (♦) and fitted linear form for the time course of cadmium removal by 0.12 g of *H. welwitschii* cells exposed to 5 ppm of cadmium. The data represent total mean amount of cadmium recovered in both cell wall and in the cytoplasm. The values represent mean ± SEM of three replicate.

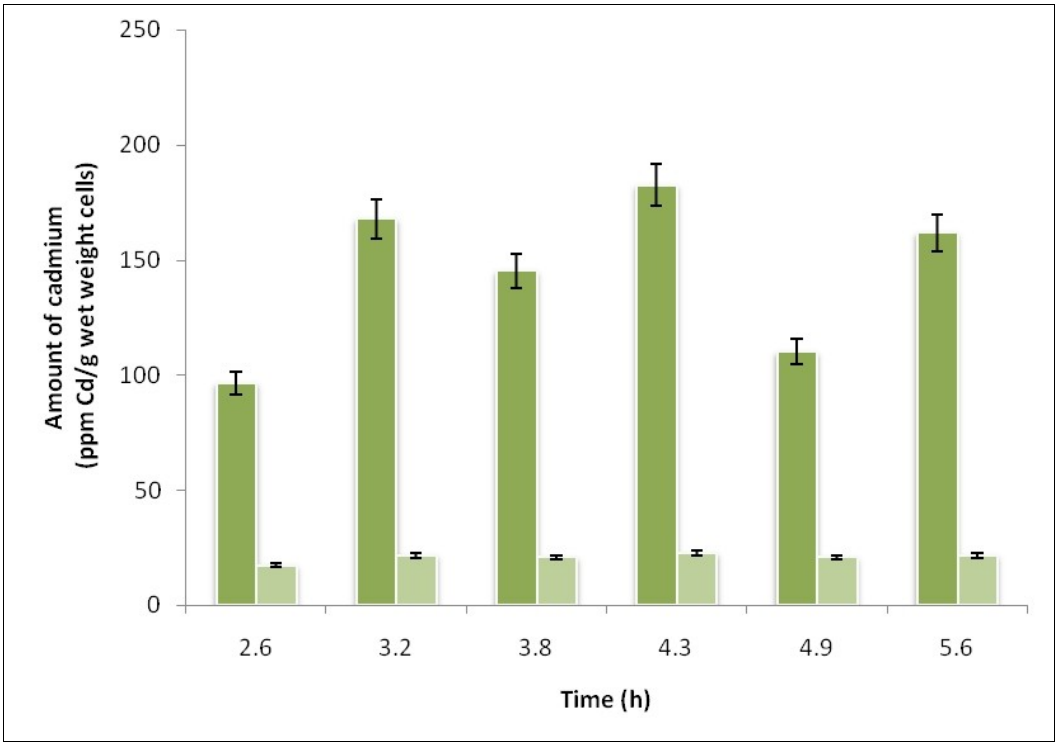

Figure 4. The amount of cadmium bound to the cell wall (■) and to the cytoplasm (■) of *H. welwitschii* as a function of time. Error bars represent standard deviation of the mean. Error bars represent standard error of the means. The values represent mean ±SEM of three replicates.

The cells of blue-green algae are enclosed by a rigid cell wall made up of peptidoglycans, which consist of linear chains of the disaccharide *N*-acetylglucosamine-β 1,4-*N*-acetylmuramic acid with peptide chains (Gupta et al. 2000). In the case of cadmium, it is possible that the metal binds to the cell wall because of electrostatic interaction between the positively-charged metal (Cd^{2+}) and the negatively-charged groups on the cell surface, e.g., the carboxyl groups and the lipopolysaccharide phosphoryl groups (Kapoor and Viraraghavan 1997).

The location of cadmium inside the cytoplasm at the highest cadmium treatment of 12 ppm suggests the possibility of bioaccumulation of metals in *H. welwitschii*. It can be inferred from this result that, at high cadmium treatments, all the binding sites on the cell wall have already been occupied, so that the metal slowly diffuses through the membrane and into the cytoplasm. In the cytoplasm, the cadmium can be linked to ligands, which are known to bind heavy metals. These ligands include sulfhydryl-containing molecules, like amino acids, peptides and proteins (Perez-Rama et al. 2002; Kennish 1982). A common protein known to bind heavy metals within the cytoplasm is metallothionein (Berger and Thomaser 1995; Harr et al. 1990; Hamer 1986; Nagano et al. 1984). In a separate study that the authors have conducted, metal tolerance genes (*smtA* and *smtB*) encoding for a group of metallothioneins, were found in cadmium treated and untreated cells of *H. welwitschii* (de Guzman and Cao, unpublished results).

Time-dependency for cadmium binding

Although it was not the primary aim of this work to study the kinetics of uptake of the metal, a short-term study (i.e., 5.6 hours) of the binding of 5 ppm cadmium by *H. welwitschii* cells was performed. In assays done at intervals of 35 minutes, it was found that certain amounts of cadmium were removed by the cells from the medium (Fig. 3). This suggests that the binding of cadmium by this blue-green alga is a function of exposure time, at least in the early period of metal removal, in agreement with the results of others (Chen and Pan 2005; Choi and Yun 2004; You and Park 1998; Romeo and Gnassia-Barelli 1985; Singh 1985). A short-term metal ion binding study with a different cyanobacterial biomass had been conducted by Gardea-Torresdy et al. (1998), where the time intervals used were 5, 10, 15, 30, 60, 90, and 120 min. In that study, Pb^{2+}, Cd^{2+}, and Ni^{2+} were bound immediately within the time span, while Cu^{2+} and Cr^{2+} required longer times to reach maximum adsorption.

In the 5.6 h of contact time of cadmium to *H. welwitschii* Nägel, almost 1 ppm or about 20 % of the initial 5 ppm of cadmium added to the culture of cadmium was removed by 0.12 g wet weight cells. This result is relatively high compared to the maximum 2.5 % of 10 mg l^{-1} cadmium accumulated in 15 days by *Chorella stignamatophora* (Rebhun and Ben-Amotz 1984). This is an indication that even at a short time of exposure to the metal, *H. welwitschii* Nägel cells are capable of removing the metal from its medium. However, the relatively short period of time of exposure did not reveal the equilibrium or saturation point of cadmium binding by the *H. welwitschii* cells. It is therefore recommended in future studies to extend the exposure and sampling time.

The amount of cadmium bound to the cell wall was significantly higher compared to the amount of cadmium accumulated in the cytoplasm by the *H. welwitschii* Nägel

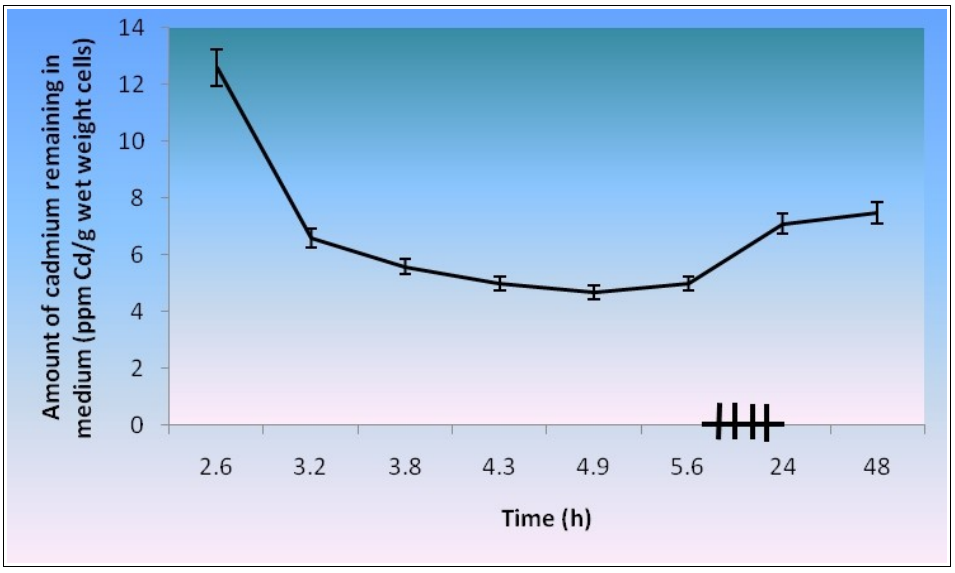

Figure 5. The amount of cadmium remaining in the medium after 2 h of incubation and every 35 mins thereafter. After 24 and 48 h of incubation from the last sampling time, a sigificant increase in the amount of cadmium effluxed into the medium was noted.

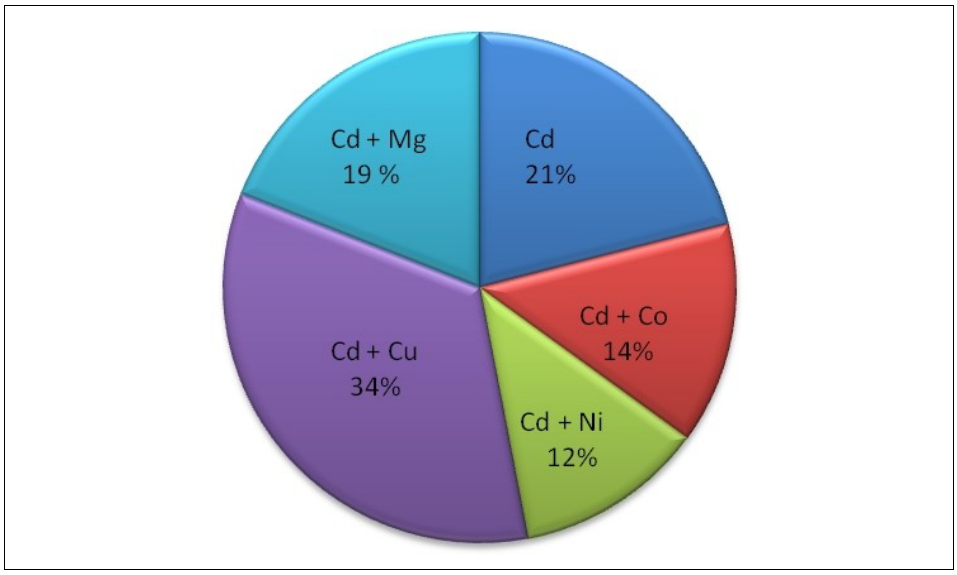

Figure 6. Relative percentage of cadmium bound by *H. welwitschii* cells in the presence of other divalent cations.

cells during the 5.6 h of incubation (Fig. 4).

Efflux mechanism

The process of exclusion or efflux of heavy metals by microorganisms is one way by which they become tolerant to heavy metals (Jain 1990). In this study, *H. welwitschii* cells displayed an efflux or exclusion of the intracellularly-bound cadmium to the external medium after 24 and 48 h of incubation. The remaining culture medium used in the time course experiment was used to determine cadmium efflux by the cells of *H. welwitschii*. A significant decrease in the amount of residual cadmium was noted at 3.5 h and up to the last sampling time of 5.6 h (Fig. 5). However, 24 and 48 h of further incubation revealed that the amount of cadmium in the medium significantly increased. Silver and Phung (1996) noted that one of the mechanisms by which microorganisms reduce their toxicity to heavy metals in their systems is through energy-dependent efflux of the toxic ions. Some strains of *Staphylococcus aureus* (Tynecka et al. 1981) and *Synechococcus aquatilis* SY01 (Vallarta et al. 1981) also displayed an efflux mechanism. The efflux mechanism was reported to be regulated by numerous plasmid-encoded genes. The cyanobacterium *Synechocystis* PCC6803 responds to cadmium stress by negative and positive regulation of genes that operate in metal uptake and export, respectively (Houot et al. 2007). Also in another cyanobacterium, *Anabaena* PCC 7120, a novel $Zn^{2+}/Pb^{2+}/Cd^{2+}$-responsive operon that consists of genes which encode for a Zn^{2+}/Pb^{2+} CPx – ATPase efflux pump (*aztA*) was identified and characterized by Liu et al. (2005).

Since an efflux of the intracellularly bound cadmium was established in this study, it is suggested that the exact time when the metal ions starts to be excluded into the medium and becomes significant in level be determined. This is to ensure that the potential of *H. welwitschii* to remove cadmium from the medium will not be jeopardized by its own cellular mechanism of protecting itself from the toxic effects of the heavy metal. Comparing the amount of cadmium effluxed by *H. welwitschii* cells to the amount effluxed by *Synechococcus aquatilis* SY01 (Vallarta, et al. 1998), the amount effluxed by *H. welwitschii* is significantly lower (0.0002 mg Cd).

Effect of divalent cations

The presence of other divalent cations had a significant effect on the removal of cadmium by *H. welwitschii.* A significantly high percentage of cadmium was removed in the presence of Cu^{2+} compared with cadmium alone, 34% and 21%, respectively (Fig. 6). On the other hand, the percentage of cadmium removed by the cells in the presence of Mg^{2+} was not significantly different when compared to cultures with cadmium alone. The binding of cadmium in the presence of the divalent cations cobalt and nickel had the least percentage of cadmium detected in the cells (14% and 12%, respectively).

The presence of some competing ions generally affects the adsorption of heavy metal ions and can reduce the efficiency of metal removal (Sampedro et al. 1995). In this study, the divalent cations Co^{2+}, Mg^{2+}, and Ni^{2+}, were found to reduce the amount of cadmium that can be bound by the blue-green algal cells. These ions could have affected the binding of cadmium by direct competition for negatively-charged binding sites (Yun and Volesky 2003). In the presence of Cu^{2+}, significantly high amounts of cadmium were removed by *H. welwitschii* cells. There seems to be no competition for binding sites between these

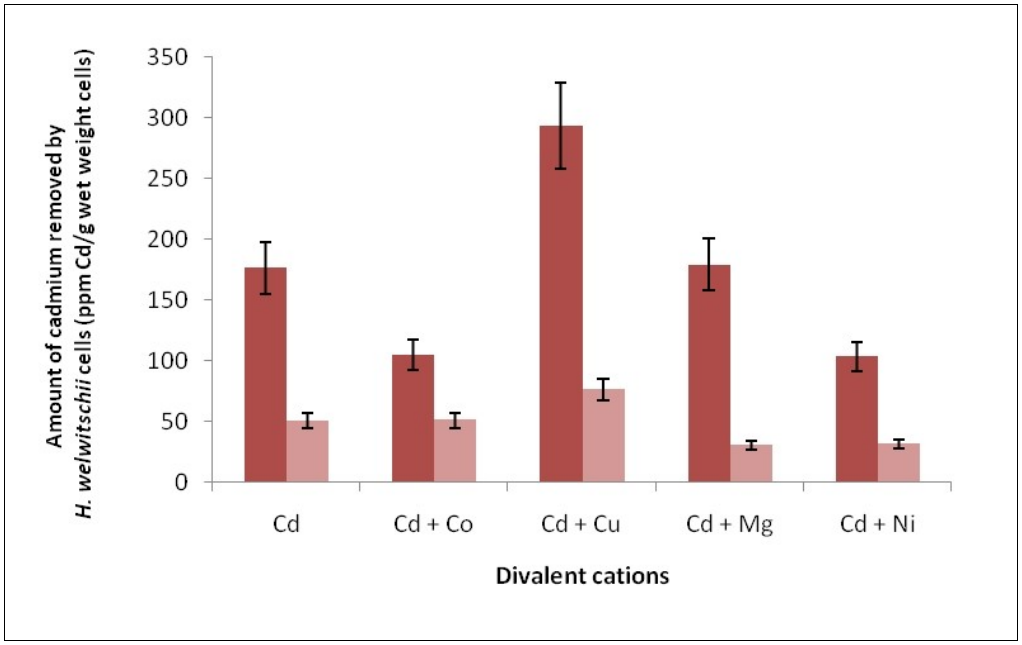

Figure 7. The competitive effect of other divalent cations (5 ppm) on the cellular locations of the cadmium bound by *H. welwitschii* cells. The cadmium ions were detected on the cell wall (■) and in the cytoplasm (▢). Error bars represent standard error of the means. The values represent mean ±SEM of three replicates.

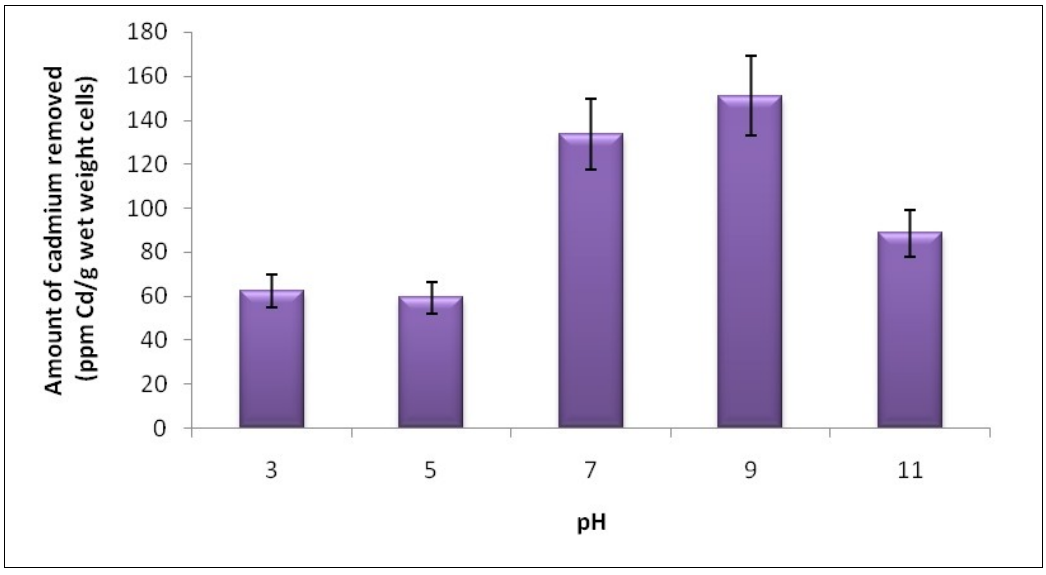

Figure 8. The effect of pH of the culture medium on the removal of cadmium ions from the culture medium by *H. welwitschii*. The optimum pH for growth of the culture is 7.5. Error bars represent standard error of the means. The values represent mean ±SEM of three replicates.

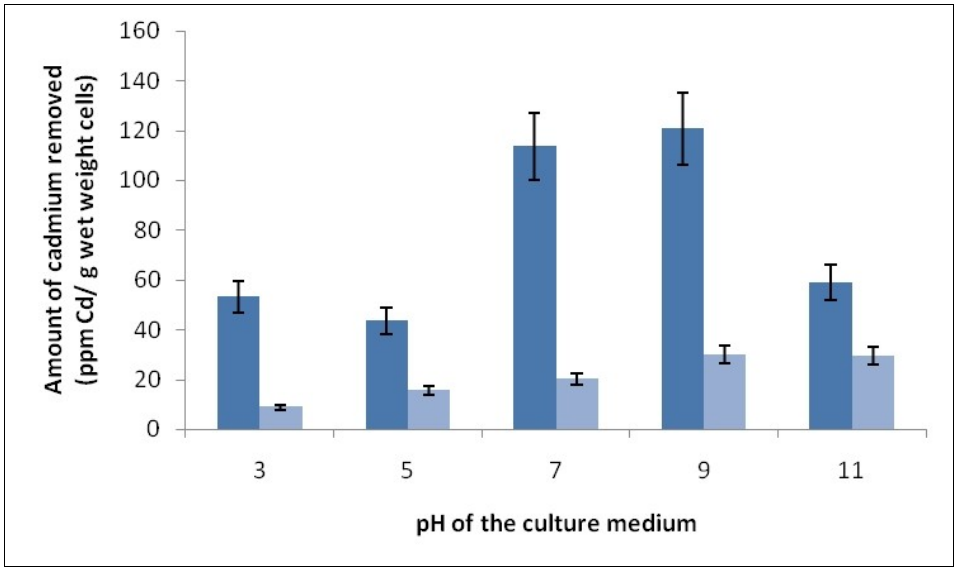

Figure 9. Cellular locations of cadmium ions removed b *H. welwitschii* as determined by pH of the culture medium. The removed cadmium ions were either located on the cell wall (■) or inside the cytoplasm (■). Error bars represent standard error of the means. The values represent mean ±SEM of three replicates.

two ions. It is also possible that Cu^{2+} did not compete with cadmium because, in trace amounts, it is required for cellular growth and maintenance of metabolic functions (Gilotra and Srivastava 1997).

Similar to the results obtained from the time course and effect of varying cadmium concentrations, significant amounts of cadmium ions were adsorbed on the cell surface of *H. welwitschii* cells compared to the amount of cadmium located intracellularly (Fig 7). The highest amount of cadmium assayed on the cell surface was 293.3 ppm/g wet weight cells in cultures exposed to 5 ppm of cadmium together with the same amount of copper. This amount is significantly higher compared to the adsorbed cadmium in cultures exposed to cadmium alone (176.2 ppm/g wet weight cells). The amount of cadmium detected on the cell surface in cultures exposed to 5 ppm cadmium together with the same amount of the divalent cation magnesium was not significantly different from the amount in cadmium alone (178.9 ppm/g wet weight cells). The least amount of cadmium was found on the cell surface of the blue-green algal cells in cultures exposed to cadmium together with the divalent cations cobalt and nickel (104.8 and 103 ppm/g wet weight cells, respectively).

The highest amount of cadmium found in the cytoplasm of the *H. welwitschii* cells was also noted in cultures exposed to 5 ppm cadmium together with the same amount of copper (76.2 ppm/g wet weight cells). On the other hand, a different observation was noted in the amount of intracellular cadmium taken up by the *H. welwitschii* cells when exposed to the same amount of cadmium and other divalent cations. In the case of cadmium adsorbed on the cell wall, the levels found in the cultures exposed to cadmium alone and in the cultures exposed to cadmium together with magnesium were not significantly different. The same was noted in cultures exposed to cadmium together with cobalt and nickel. In terms of the levels taken up intracellularly, the amounts of cadmium detected in cultures exposed to cadmium alone and together with cobalt were not significantly different (50.3 and 50.7 ppm/g wet weight cells, respectively). The least amounts of intracellular cadmium were found in cultures exposed to cadmium together with magnesium and nickel (30.6 and 31.5 ppm/g wet weight cells, respectively).

Effect of pH

A significantly lower amount of cadmium was removed at pH 3 and 5 (Fig. 8). The ability of the *H. welwitschii* cells to bind cadmium from the contaminated medium was greatly enhanced at pH 9. This amount however, is not significantly different from the amount removed by the cells at pH 7.5, which is the normal pH of its growth medium. The amount of cadmium removed at pH 11 was significantly lower than the amounts removed at pH 7.5 and 9, but significantly higher than the amount removed at pH 3 and 5. Again, in terms of cellular distribution of cadmium in the *H. welwitschii* cells, a significantly higher amount was detected in the cell wall than within the cytoplasm regardless of the pH of the culture medium (Fig 9).

The pH dependence of the removal of cadmium by *H. welwitschii* is not much different from the observation made by

Yilmaz and Ensari (2005) with the bacterium, *Bacillus circulans* strain EB1, where maximum cadmium removal by the cells was at pH 7.0. Most algal species show maximum concentration factor values between pH 7.0 and 9.0 and this alkaline condition of the medium may allow more binding sites for cadmium. The significantly lower amount of cadmium removed by *H. welwitschii* when exposed to pH 3.0 and 5.0 is similar to the reduced binding reported by Brady and Duncan (1994) for copper by *Saccharomyces cerevisiae*. This is probably because, at low pH values protons compete with the cations for binding sites, thus lowering the extent of biosorption (Ahuja et al. 1997).

The results of this study have shown that under controlled laboratory conditions, the binding of cadmium by *H. welwitschii* cells is directly proportional to the available concentration of cadmium in the medium up to a concentration of 10.0 ppm. It is also time-dependent in the initial phase of binding and is optimum at pH 9. The presence of Cu^{2+} greatly increased the amount of cadmium bound to the cells, while other divalent cations such as Co^{2+}, Mg^{2+}, and Ni^{2+} had the opposite effect. However, there are still uncertainties regarding the potential use of *H. welwitschii* in bioremediation since an efflux mechanism was noted in this study. More work must be done, which will look into utilizing the binding capability of the organism while at the same time eliminating the efflux mechanism, so that it can be harnessed as a bioremediation agent for cadmium.

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