Journal of Environment and Aquatic Resources. 1(1): 87-98 (2009)
doi: 10.48031/msunjear.2009.01.07

In vitro lead accumulation in *Gracilaria coronopifolia* and *Gracilaria eucheumoides*

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ABSTRACT

The effects of lead at different concentrations (0.02, 0.10, 1.0 and 5.0 ppm) on growth rate, total chlorophyll content, and dissolved oxygen production of *Gracilaria coronopifolia* and *Gracilaria eucheumoides* were investigated under laboratory conditions. The amount of lead accumulated in the plant tissue and in the agar extracted from the two *Gracilaria* species was determined by using the Spectra AAS atomic absorption spectrometer. Results showed that the specific growth rate in biomass of the two *Gracilaria* species did not differ significantly ($P<0.05$) among treatments. Although the chlorophyll content in *G. coronopifolia* decreased and *G. eucheumoides* increased at the end of the culture period, both species did not differ significantly among treatments. Dissolved oxygen production in all treatments decreased on the 15th day of culture of both species. The amount of lead detected in the agar extracted from *G. coronopifolia* and *G. eucheumoides* was 2.1 to 31.4 times higher than the lead content in the tissues of both species. The amount of lead in the water medium significantly decreased after 15 days of culture. An important characteristic of *G. coronopifolia* and *G. eucheumoides* is their ability to absorb lead from water and survive in lead-contaminated waters with concentrations as high as 5ppm. These characteristics make both *Gracilaria* species good candidates for bioremediation as a means of improving water quality in nearshore ecosystems.

Keywords: seaweed, chlorophyll, bioremediation, heavy metals

INTRODUCTION

Lead (Pb) is one of the oldest metals known to man. Since medieval times it has been used in piping and building materials, soldering, paints, ammunition, and casting. Recently, lead has been used mainly in storage batteries, gasoline (as tetraethyl lead), cable covering, solders and chemicals (Demayo *et al.,* 1984). Lead occurs in the environment in a wide range of physical and chemical forms that greatly influence its behavior and effect on the ecosystem. Lead mostly exists in inorganic form in the environment, however, lead is also present in organic form such as tetraethyl lead from gas emissions of motor vehicles.

Studies on the transfer of Pb among marine organisms suggest that Pb is not biomagnified from lower to higher trophic levels in the marine ecosystem. Lead can be accumulated directly from seawater and the sediment by marine organisms that utilize gill tissue as the major nutrient uptake route (Sadiq, 1992). The survival of a certain species is often determined by its ability to adapt to environmental changes (Kuebler *et al.*, 1991). For example, it is widely accepted that adaptation allows seaweeds to optimize photosynthetic ability and respiration in response to changes in environmental conditions (Davison, 1987).

Some species of seaweeds and their derivatives have the ability to remove a range of heavy metals from water and can be used in wastewater treatment system (Aderhold *et al.*, 1996). Current algal research gives emphasis on *Gracilaria* because of its utility and wide distribution. *Gracilaria coronopifolia* and *G. eucheumoides* are good sources of agar and provide a good substitute to *Gelidium* (Que, 1992). On the other hand, these aquatic plants often occur in areas threatened by water pollution that may hinder their productivity. Factory discharges, mine tailings, oil spills and domestic effluents contribute greatly to deteriorating water quality. A study that investigates the ability of the different species of *Gracilaria* to tolerate and accumulate heavy metals such as lead can help solve certain environmental problems by the use of algal biomass for wastewater treatment. This study investigated the extent of lead accumulation by the plant tissue and agar extract, and the effects of lead on the growth and productivity of two species of *Gracilaria*.

METHODS

Sample collection

Gracilaria coronopifolia and *G. eucheumoides* thalli were collected during low tide from the reef flats of Jampason, Initao, Misamis Oriental (Fig. 1). Approximately three kg of each species were placed in plastic bags filled with seawater and then transported immediately to the MSU-IFRD Wet Laboratory.

Experimental Set-up

The collected samples were thoroughly cleaned using filtered seawater and the clean thalli were acclimated for one week before the start of the experiment conducted in a Completely Randomized Design (CRD) for each *Gracilaria* species. Five different concentrations of lead, namely, 0.02 (Treatment 2), 0.1 (Treatment 3), 1 (Treatment 4) and 5ppm (Treatment 5) were prepared using a known concentration of lead nitrate and deionized water. The experimental control (Treatment 1) contained only filtered seawater.

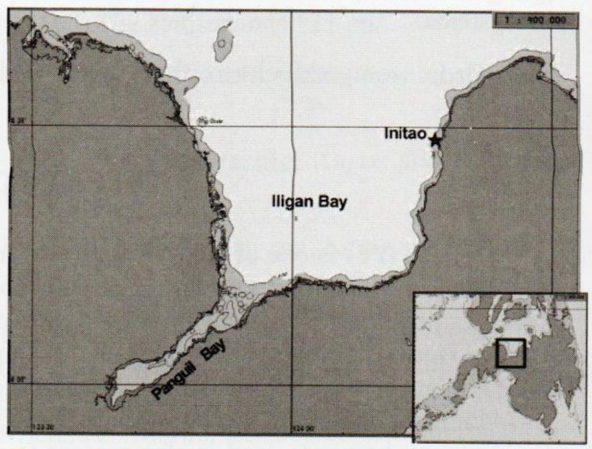


Figure 1. Map showing collection site in Iligan Bay. Inset is the Mindanao Island.

One hundred-gram samples of the two species of *Gracilaria* were placed in plastic jars each containing eight liters of the experimental lead concentration. Each plastic jar was provided with aeration fitted with a swab of cotton to filter impurities. After seven days, 1.3 g of thalli were collected from each jar for dissolved oxygen (1.0 g) and chlorophyll (0.3 g) measurements. This procedure was repeated on the 15th day of culture. All plants were harvested at the end of the culture period and weighed. The water and plant samples were processed for the analysis of lead accumulation and agar extraction.

Laboratory Analysis

Specific growth rate was determined by subtracting the initial weight (blotted) from the final weight of the plants over time. Chlorophyll *a* was determined using the UV-1201 Visible spectrophotometer (Shimadzu) applying the alcohol extraction procedure in the Standard Methods for the Examination of Water and Wastewater (APHA, 1995). The determination of dissolved oxygen produced by plants was done immediately after two hour incubation following the Winkler method (Strickland and Parsons, 1977).

Extraction of agar from each of the treatments of the two *Gracilaria* species used the modified alkaline extraction method of Hurtado and Umezaki (1988). Determination of lead in the water, tissue, and agar extract was done following standard methods of analyzing biological (AOAC International 1995) and water samples (APHA, 1995).

RESULTS

Effect of lead on growth

The specific growth rate in biomass of *G. coronopifolia* ranged from 0.04 to 0.92% d^{-1} after 15 days of culture, while *G. eucheumoides* had a negative specific growth rate in biomass in all treatments (Fig.2). There was, however, no significant difference in specific growth rate in biomass among treatments in both species (ANOVA $p>0.05$).

Total chlorophyll content

The total chlorophyll content of *G. coronopifolia* ranged from 5.8 to 7.0 $mg.g^{-1}$ DW and 4.4 to 5.6 $mg.g^{-1}$ DW after 7 (D_7) and 15 (D_{15}) days of culture, respectively (Fig. 3). Analysis of variance (ANOVA) showed that the chlorophyll content on D_7 and D_{15} culture was not significantly different among treatments. A comparison between the chlorophyll content of the five treatments of D_7 and D_{15}, however, showed total chl had declined on D_{15} (t-test $p < 0.05$).

The total chlorophyll content of *G. eucheumoides* ranged from 2.1 to 3.5 $mg.g^{-1}$ DW on D_7 of culture and from 2.4 to 3.7 $mg.g^{-1}$ DW on D_{15} (Fig. 3). As in *G. coronopifolia*, ANOVA showed that the chlorophyll content obtained on D_7 and D_{15} culture did not differ significantly among treatments. In contrast to *G. coronopifolia*, the total chlorophyll content of *G. eucheumoides* observed on D_7 did not differ significantly with the chlorophyll content on D_{15} (t-test $p > 0.05$). It was observed, however, that in the later part of the culture period there was discoloration or bleaching in *G. coronopifolia* grown in the leaded culture media, while a white substance formed on the surface tissue of *G. eucheumoides*.

Oxygen production

The dissolved oxygen level in the *G. coronopifolia* culture medium ranged from 1.3 to 1.5 mg $O_2.g^{-1}$ $DW.h^{-1}$ on D_7 and 0.2 to 0.3 mg $O_2.$ g^{-1} $DW.h^{-1}$ on D_{15} of culture (Fig. 4). Results show that oxygen production by both *Gracilaria* species declined after 15 days of exposure to Pb. Analysis of variance on the DO levels of *G. coronopifolia* on D_7, however, was not significantly different among treatments ($p>0.05$). No significant differences among treatments were also observed on D_{15} ($p>0.05$). On the other hand, DO level on D_7 was significantly higher than that on D_{15} (t-test $p<0.05$).

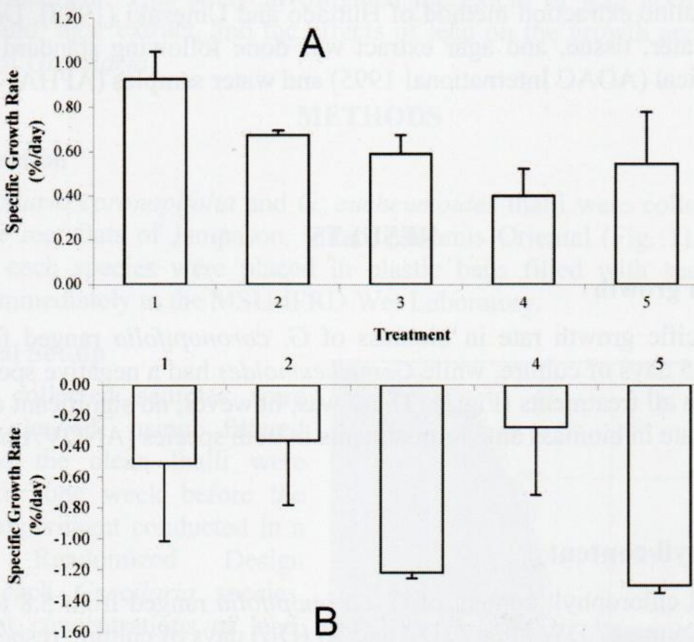
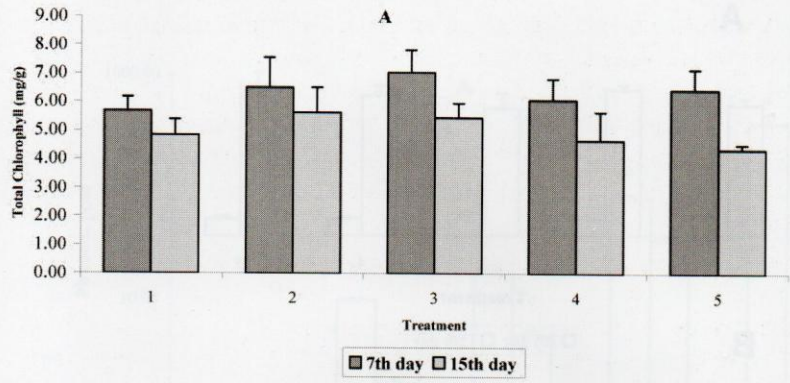


Figure 2. Specific growth rate in biomass of (A) *Gracilaria coronopifolia* and (B) *Gracilaria eucheumoides* (Error bars are standard errors with n =3).



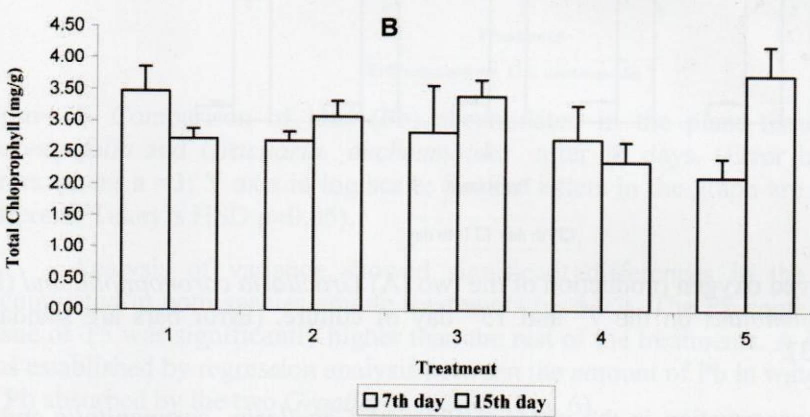


Figure 3. Total chlorophyll content of (A) *Gracilaria coronopifolia* and (B) *Gracilaria eucheumoides* on the 7 th and 15 th day of culture. (Error bars are standard errors with n =3).

Lead in water and plant tissue

The different lead concentrations in T2-T5 have average Pb concentrations of 0.02, 0.1, 1 and 5 ppm, respectively at the beginning of the experiment, but these decreased over 15 days. Table 1 shows the comparison of the initial and final lead concentrations of the culture medium. The lead content in water significantly decreased by 50-90% in *G. coronopifolia* and by 50-85% in *G. eucheumoides* at the end of the culture period (Table 1) while the amount of lead accumulated in the tissue of both species increased (Fig. 5).

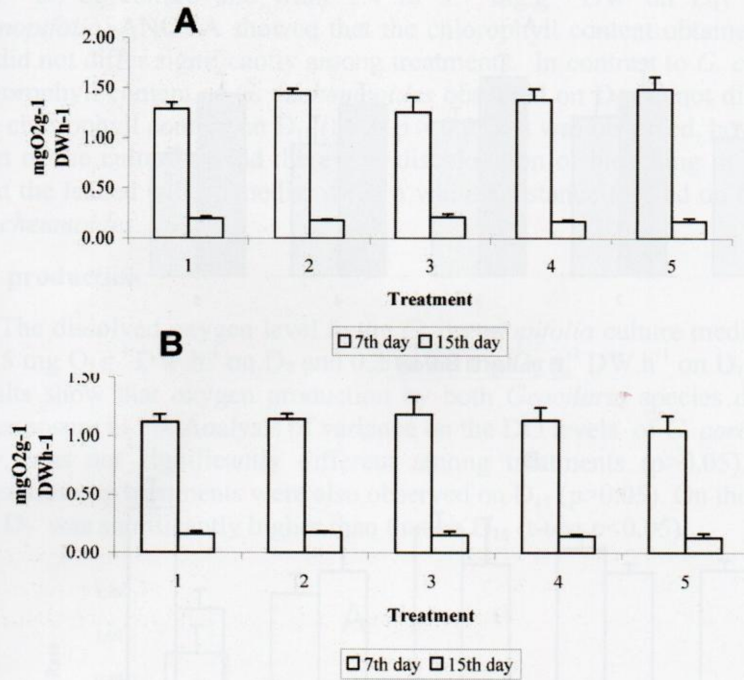


Figure 4. Dissolved oxygen production of the two (A) *Gracilaria coronopifolia and* (B) *Gracilaria eucheumoides* on the 7[th] and 15[th] day of culture. (Error bars are standard errors where n =3).

Table 1. Lead concentration in the water medium of *Gracilaria coronopifolia* and *G. eucheumoides* after 15 days of culture.

Treatment	Lead Concentration (ppm)		% reduction
	Initial	Final	
Gracilaria coronopifolia			
1(control)	na	nd	n/a
2	0.02	0.01	50
3	0.10	0.06	40
4	1.00	0.12	88
5	5.00	0.50	90
Gracilaria eucheumoides			
1(control)	na	nd	n/a
2	0.02	0.01	50
3	0.10	0.02	80
4	1.00	0.45	55
5	5.00	0.75	85

nd – not detected, na- no addition of Pb ; n/a – not applicable
% reduction = initial Pb conc – final Pb conc / initial x 100

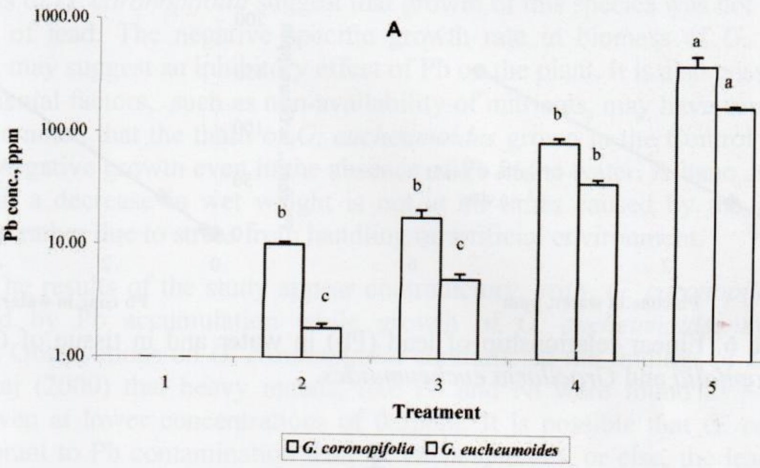


Figure 5. Comparison of lead (Pb) accumulated in the plant tissue of *Gracilaria coronopifolia* and *Gracilaria eucheumoides* after 15 days. (Error bars are standard errors where n =3; Y axis in log scale; Similar letters in the graph are not significantly different; Tukey's HSD p>0.05).

Analysis of variance showed significant differences in the amount of lead accumulated in both species among treatments ($p<0.05$). The Pb content in the seaweed tissue of T5 was significantly higher than the rest of the treatments. A linear relationship was established by regression analysis between the amount of Pb in water and the amount of Pb absorbed by the two *Gracilaria* species (Fig. 6).

Lead in the agar extract

Lead was detected in the agar extracted from the plant samples of *G. coronopifolia* and *G. eucheumoides* except in the control. The lead content in the agar extracted from the plant samples of *G. coronopifolia* ranged from 38.5 to 797.7 ppm with the highest amount obtained at 5 ppm (T5). The result showed that there was an increasing lead content in the agar relative to the amount of lead in the water (Fig. 7) as established by the linear relationship shown in Fig. 8. The lead content of agar extracted from *G. eucheumoides* was highest in 5ppm ranging from 26.1 to 368.8ppm, followed by 1.0, 0.1 and 0.02 ppm Pb concentrations. The lead content in the agar, however, was significantly higher in *G. coronopifolia* than in *G. eucheumoides* (Fig. 7). The profile of Pb content of agar extracts from both *Gracilaria* species shows that lead concentration in water was magnified more than a thousand times in the agar extracts.

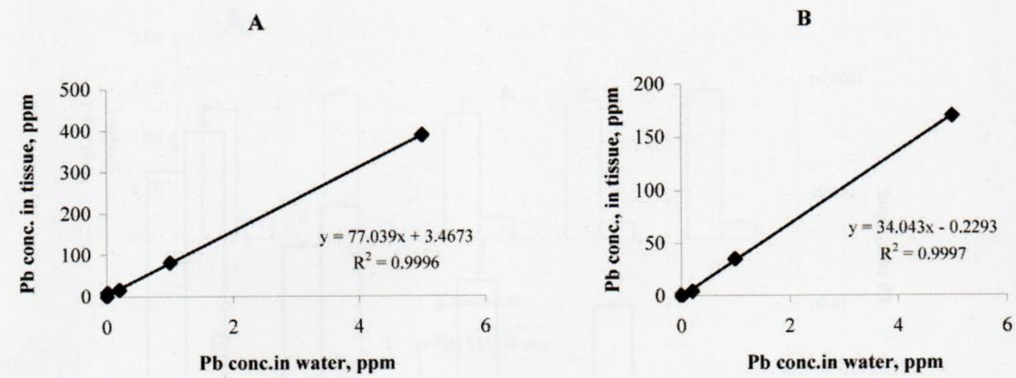


Figure 6. Linear relationship of lead (Pb) in water and in tissue of *Gracilaria coronopifolia* and *Gracilaria eucheumoides*.

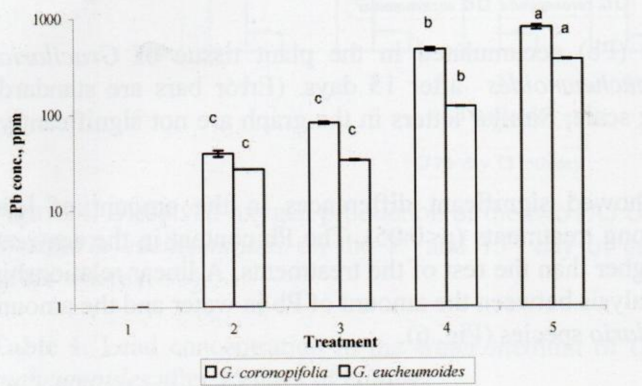


Figure 7. Lead content in the agar extracts of *Gracilaria coronopifolia* and *Gracilaria eucheumoides* (error bars are standard errors where n = Y axis in log scale; Similar letters in the graph arenot significantly different; Tukey's HSD p>0.05).

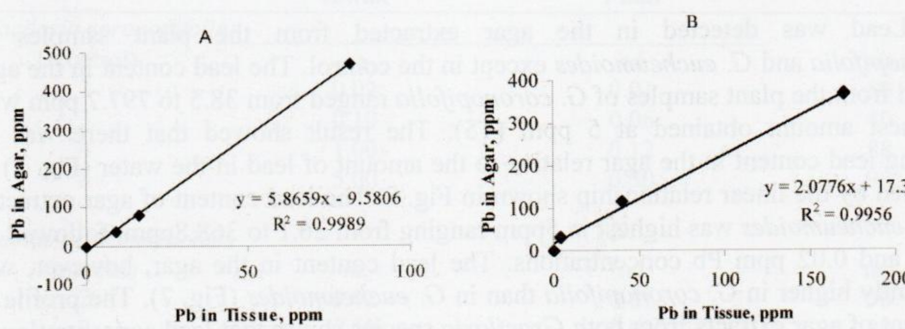


Figure 8. Linear relationship of lead (Pb) in tissue and agar of (A) Gracilaria *coronopifolia* and *Gracilaria eucheumoides*.

DISCUSSION

The non-significant differences in the specific growth rate in biomass among treatments of *G. coronopifolia* suggest that growth of this species was not affected by the presence of lead. The negative specific growth rate in biomass of *G. eucheumoides*, however, may suggest an inhibitory effect of Pb on the plant. It is also possible that other environmental factors, such as non-availability of nutrients, may have caused this effect. It must be noted that the thalli of *G. eucheumoides* grown in the Control (T1) treatment also had negative growth even in the absence of Pb in the water. Albano and Liao (1998) stated that a decrease in wet weight is not at all times caused by the introduction of metals but rather due to stress from handling or artificial environment.

The results of the study appear contradictory, with *G. coronopifolia* seemingly unaffected by Pb accumulation while growth of *G. eucheumoides* appeared to be inhibited. Observations on *G. coronopifolia* contradicted the findings of Saraswathy and Govindaraj (2000) that heavy metals, like Pb and Ni were found to be inhibitory to growth even at lower concentrations of 0.5ppm. It is possible that *G. coronopifolia* is more tolerant to Pb contamination than *G. eucheumoides*, or else, the lead levels of the culture medium (0.02 - 5ppm) may not be the range of Pb concentrations that can inhibit growth of this seaweed.

The significant decrease in chlorophyll content of *G. coronopifolia* was evident with bleaching observed in the higher levels of Pb concentration at the end of the culture period. This may imply that the health of *Gracilaria* species was altered, since any color change of the plant may manifest the effect of any environmental stress (Lee *et al.*, 1999).

The decrease in DO levels from D_7 to D_{15} is another indication that the ability of the two *Gracilaria* species to produce oxygen was affected by the presence of Pb in the culture medium. Reduced DO level may indicate that the plants were under stress possibly caused by exposure to Pb contamination. Skipnes *et al.*, (1975) reported that the accumulation of metals in algae involved a two-stage process, first a physical and chemical process of absorption on the exterior surface followed by intracellular uptake of the plant. The significant decrease of DO level at the end of the culture period may be due to Pb accumulation which had also affected the algal biomass, at least in *G. eucheumoides*. Kuebler *et al.* (1991) found that macroalgae in many habitats have various physiological mechanisms for responding to environmental changes, and the ability to tolerate environmental disturbances. The survival of a certain species is often determined by its ability to adapt to environmental changes.

The decrease in Pb concentration in the culture medium of both species indicates that the plants absorbed most of the Pb present in the water medium. These results conform to the findings of Aderhold *et al.* (1996) that some species of seaweeds have the ability to remove a range of heavy metals. These seaweeds do not regulate or limit the uptake nor discharge them, instead they can accumulate and tolerate very high concentrations of heavy metals (Phillips, 1977).

The linear relationship observed in the amount of Pb in water to the amount absorbed by the two *Gracilaria* species showed that the greater the concentration in the water the more lead is accumulated in the tissues. This result is similar to the findings of Favero *et al.* (1996) who found that heavy metal concentration in water has a direct relationship with metal concentrations in the tissues of the green leafy algae, *Ulva lactuca*. The direct relationship between Pb in the water and in the tissue of *Gracilaria* indicates that Pb concentrations of the water where the plants were taken can be estimated using the Pb in plant tissue. On the other hand, the application of this linear model is limited by the failure to establish a lethal threshold of Pb concentration for *Gracilaria* species.

Accordingly, seaweed is used as a bioindicator for heavy metal pollution because of its recorded ability to accumulate heavy metals in their cell walls (Mittal *et al.*, 2000). It is widely known that alginates bind with heavy metals such as lead, mercury, and other radioactive elements of strontium, barium and cesium (Gordin, 2000). This would imply that the Pb present in the water media was sequestered and accumulated in the plant tissue and concentrated in the agar extracts. This direct relationship between tissue and agar showed the probability of absorption. This indicates that either the plant tissue or agar can be used for detecting Pb content in the water. The ability of these plants to accumulate heavy metals is useful in predicting Pb content in the agar extracts although results show the danger of using agar for human consumption from Pb-contaminated seaweeds.

CONCLUSION AND RECOMMENDATIONS

The present study has shown some evidence that increasing lead concentrations can negatively affect growth and productivity of aquatic plants. The significant changes in DO level and total chlorophyll content analyzed on the D_7 and D_{15} of culture, however, cannot single out the presence of lead as the only cause. The reduction in algal biomass in the lead-free Control treatment was unexpected, so that it is possible that certain other conditions in the culture medium may have influenced the results. The fact that no plant samples died in all treatments during the 15 days of culture may indicate that *G. coronopifolia* and *G. eucheumoides* can survive in lead-contaminated waters with concentrations up to 5ppm.

An important characteristic of *G. coronopifolia* and *G. eucheumoides* is their ability to extract concentrations of lead from the water medium and absorb these in the plant tissue. The lead concentration in the water significantly decreased after the culture period and was found to have been bioaccumulated by the two *Gracilaria* species. The 5 ppm concentration used in this study is several times higher than the reported lead concentration of 0.2 ppm that has adverse effects on marine biota (Wong *et al.*, 1978). The amount of lead in plant tissue was not removed during the process of extracting agar. It was instead concentrated in the agar extracted from *G. coronopifolia* and *G. eucheumoides*.

This work has demonstrated promising results using *G. coronopifolia* and *G. eucheumoides* for removal of lead from the environment in the process known as phyto-remediation. These seaweeds are potential species for use in treatment of industrial wastewater particularly in poor economies where expensive treatment facilities are not available. On the other hand, caution must be taken in these novel efforts, as high levels of contamination in tissues and agar would render the plants not suitable for human consumption.

ACKNOWLEDGMENT

The authors are greatly indebted to the following: Professors Grace I. Prado and Milan Daitia or their comments and helpful discussion, MSU at Naawan and the MSU at Naawan Foundation for Science and Technology Development, Inc. for the funding support, and above all to our loved ones for the inspiration.

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