CHAPTER III

RESULTS

1. Growth of A. halophytica and S. platensis

The growth of A. halophytica and S. platensis did not exhibit a lag phase and had a specific growth rate of 0.34 and 0.27 day^{-1} respectively (figures 2 and 3).

2. Relationship between Optical Density at 650 nm and Dry Weight

The relationships between optical densities at 650 nm (OD_{650}) and dry weights of A. halophytica and S. platensis were found to be linear (figures 4 and 5). One unit of OD_{650} corresponded to 0.58 mg dry weight/ml for A. halophytica and 0.46 mg dry weight/ml for S. platensis.

3. Accumulation of Lead

3.1 Rate of Accumulation

Both algae were able to accumulate lead rapidly. The accumulation of lead by A. halophytica increased at a rapid rate in the first 10 minutes and became saturated at about 90 ug/mg dry weight within 1 hour (figure 6). For S. platensis, the accumulation of lead

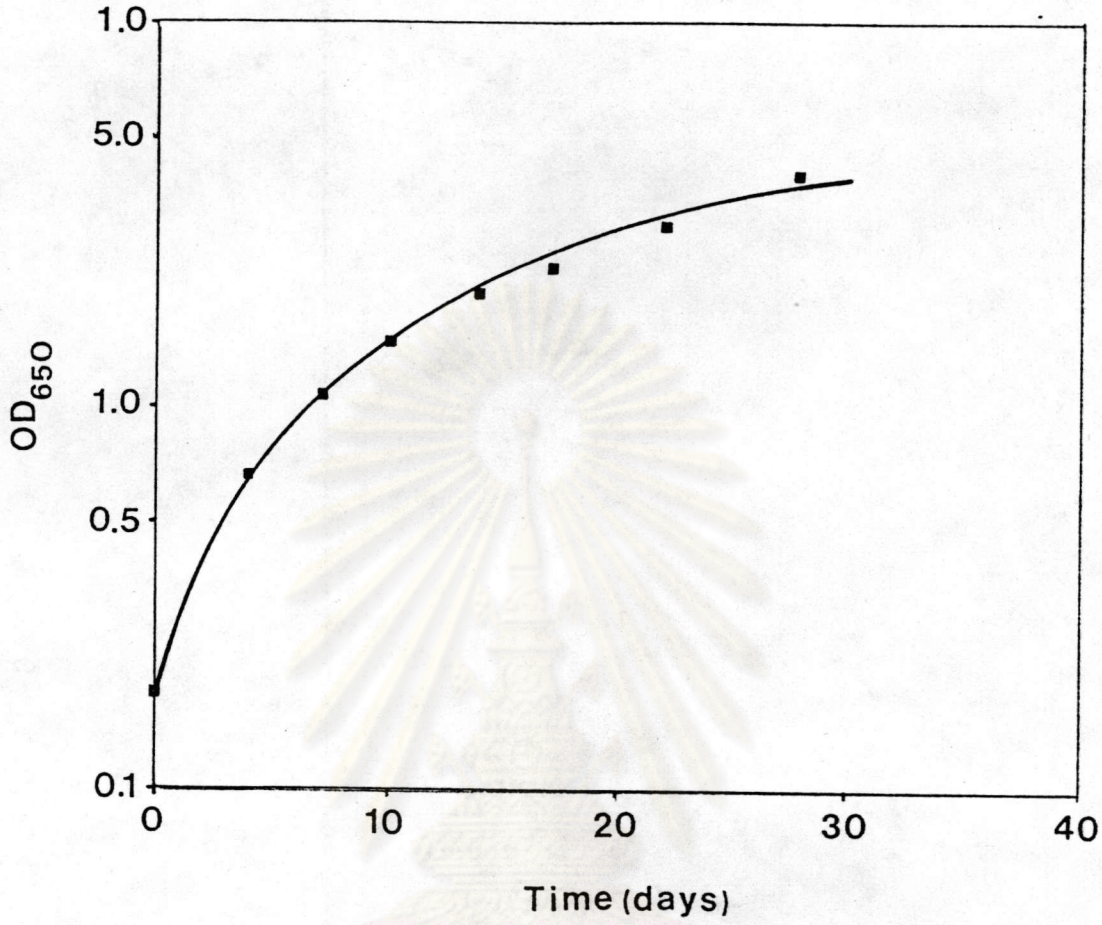

Figure 2 Growth of A. halophytica in Turk Island Salt Solution supplemented with BG 11 medium.

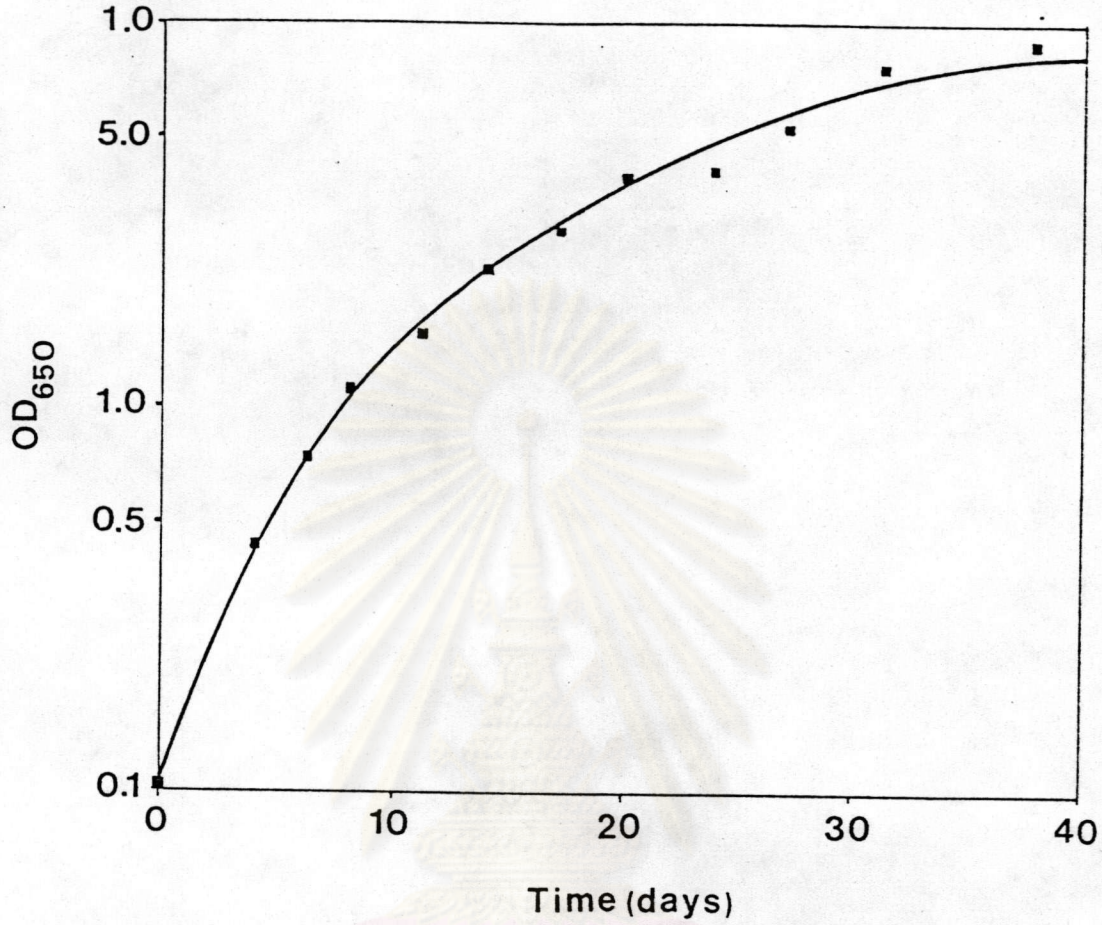

Figure 3 Growth of S. platensis in Zarrouk's medium.

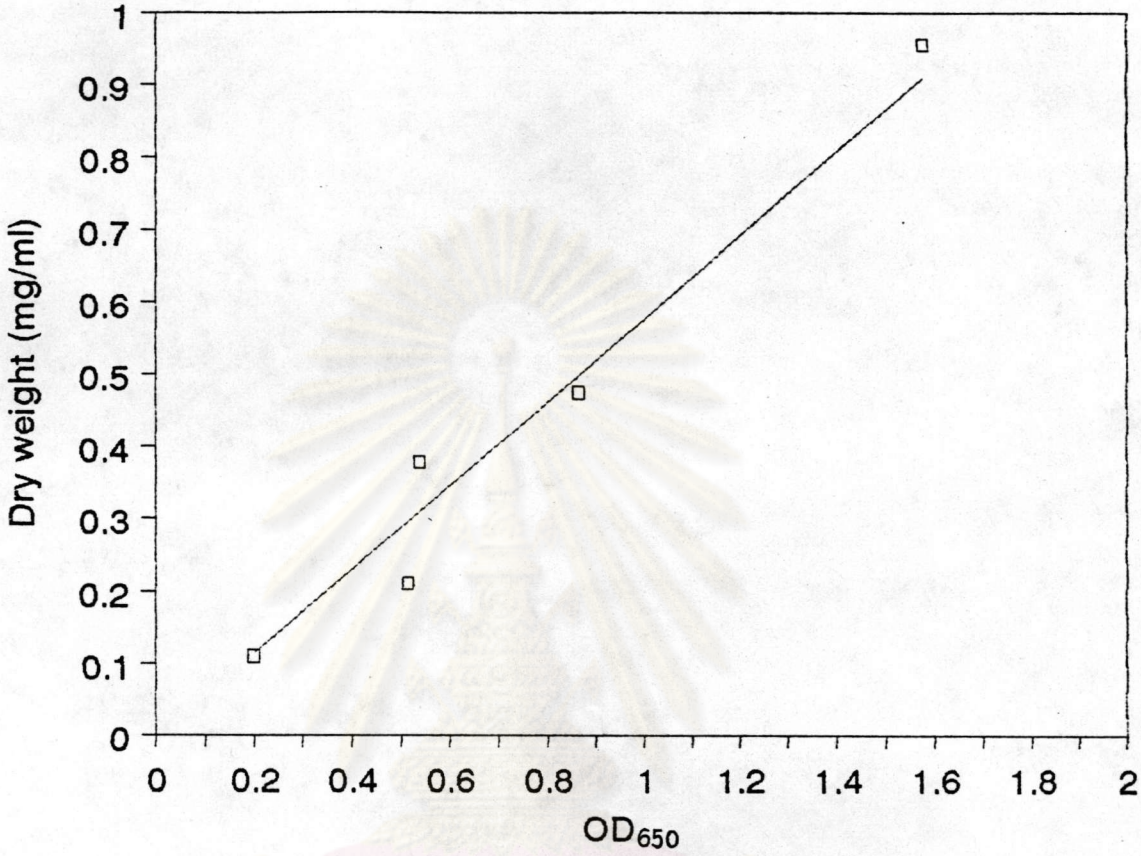

Figure 4 Relationship between OD_{650} and dry weight of A. halophytica

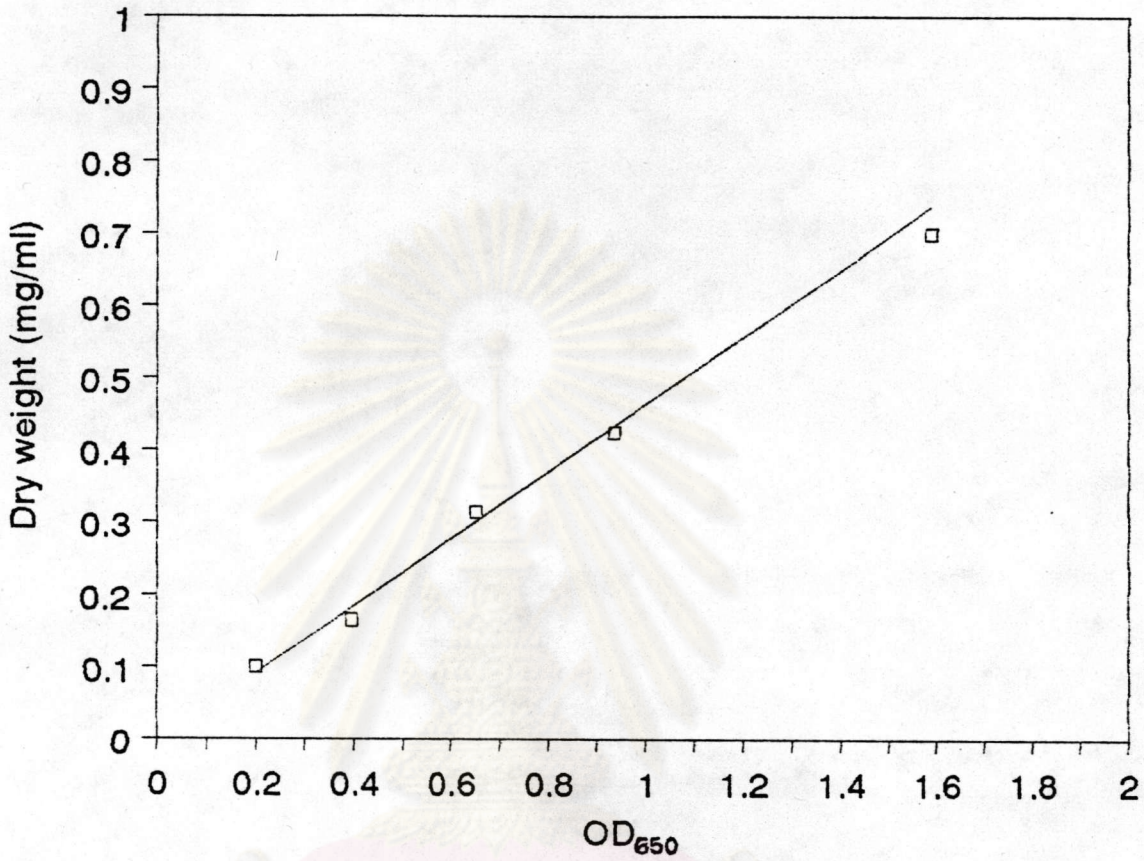

Figure 5 Relationship between OD_{650} and dry weight of S. platensis

was a biphasic phase. It rapidly increased in the first 10 minutes after which time the increase was at a slower rate of 15.6 ug/hr. mg dry weight (figure 7). Treatments of the lead accumulating algal cells with 10 mM EDTA resulted in the removal of about 90% for A. halophytica and 30-50% for S. platensis.

3.2 Effect of Metabolic Inhibitor

The accumulation of lead by A. halophytica was not affected by all three metabolic inhibitors tested, namely DNP, NaN_3 and DCCD (figure 8). However, for S. platensis the accumulation of lead was inhibited by 0.1 mM DNP and 1.0 mM NaN_3 (figure 9).

3.3 Effect of pH

The rate of lead accumulation by A. halophytica was highest at pH 6.5 and the rate declined when the pH was lower or higher than 6.5 (figure 10). The rate of lead accumulation by S. platensis was unchanged when the pH was in the range of pH 4.0 to 6.5. However, the rate increased sharply when the pH was higher than 6.5 (figure 11).

3.4 Effect of Cation

All the cations tested except zinc did not have any effect on the accumulation of lead by A. halophytica (figure 12). Zinc was found to double the rate of accumulation. For S. platensis, the effect of cations on the accumulation of lead varied (figure 13). Cobolt and manganese ions were without effect whereas other

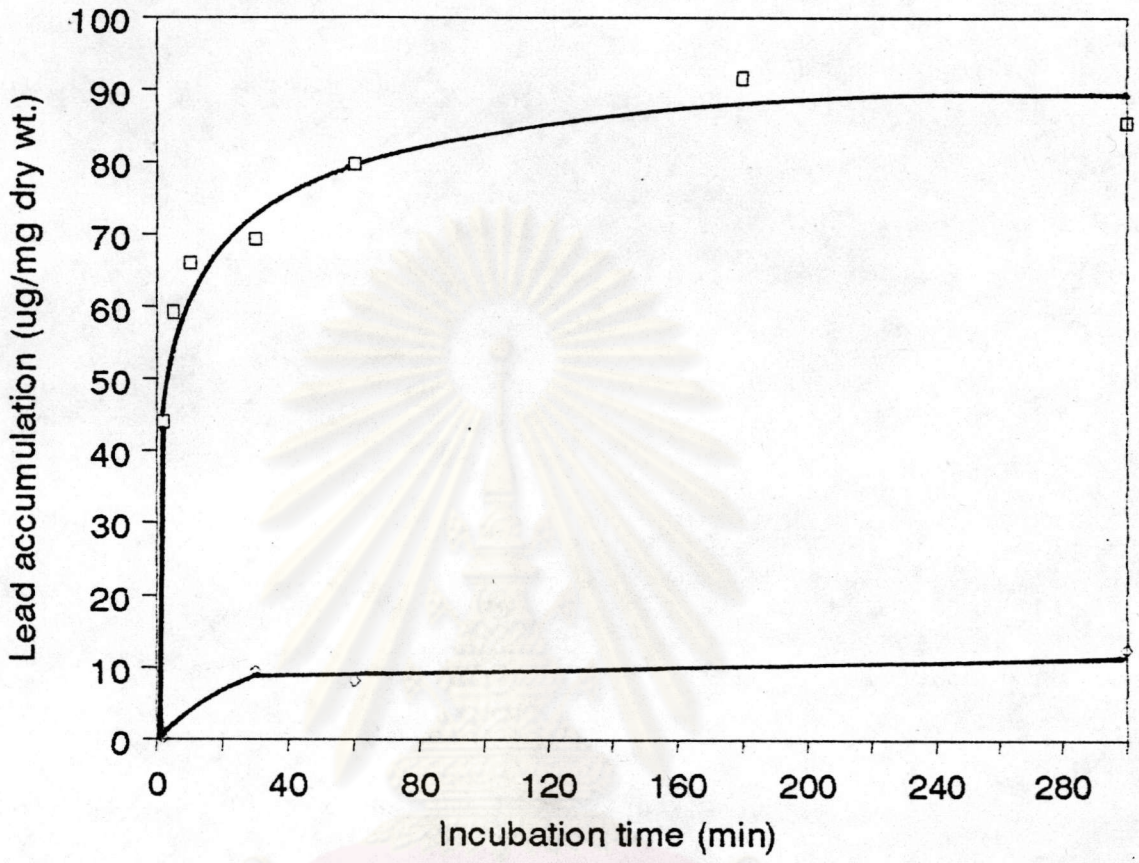

Figure 6 Accumulation of lead by A. halophytica. Cells were incubated with 500 ppm lead in 10 mM PIPES buffer pH 6.5 containing 0.6 M sorbitol. After incubation the cells were washed with PIPES-sorbitol buffer (□) or with 10 mM EDTA (◇).

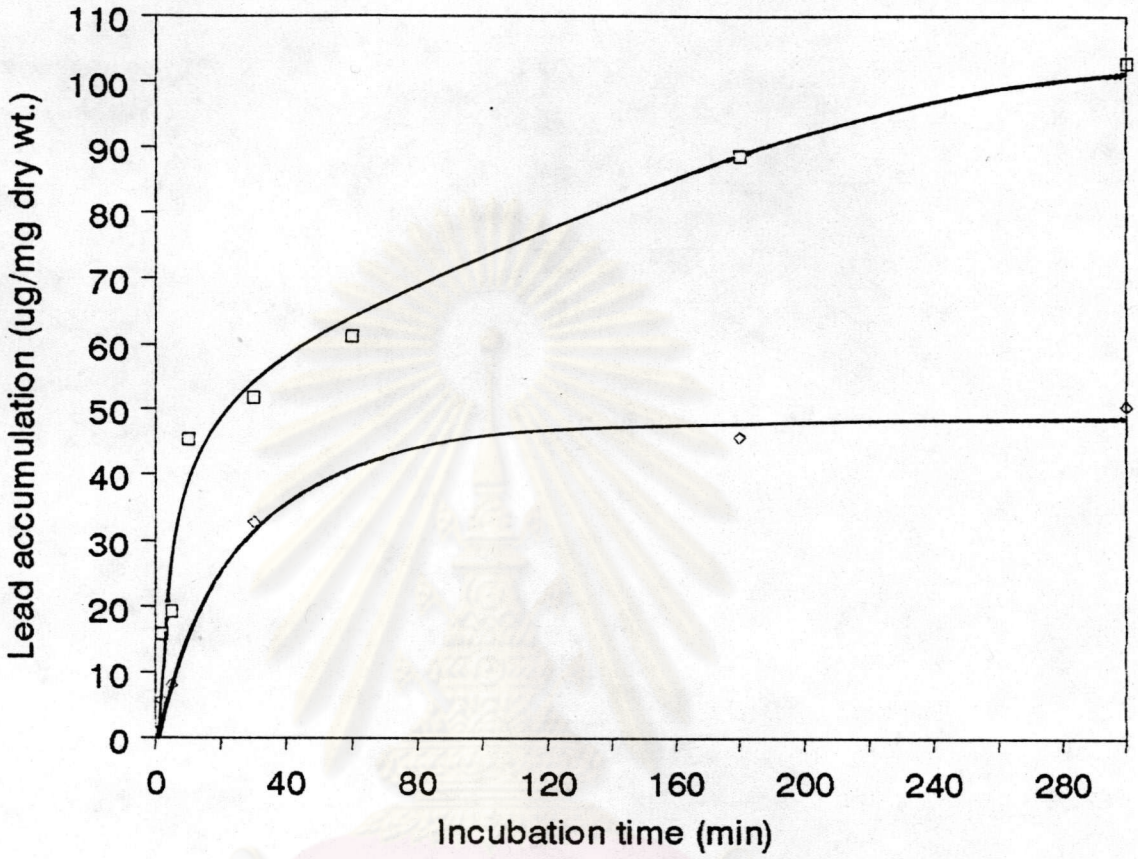

Figure 7 Accumulation of lead by S. platensis. Cells were incubated with 500 ppm lead in 10 mM acetate buffer pH 5.5. Afterincubation the cells were washed with acetate buffer (□) or with 10 mM EDTA (◇).

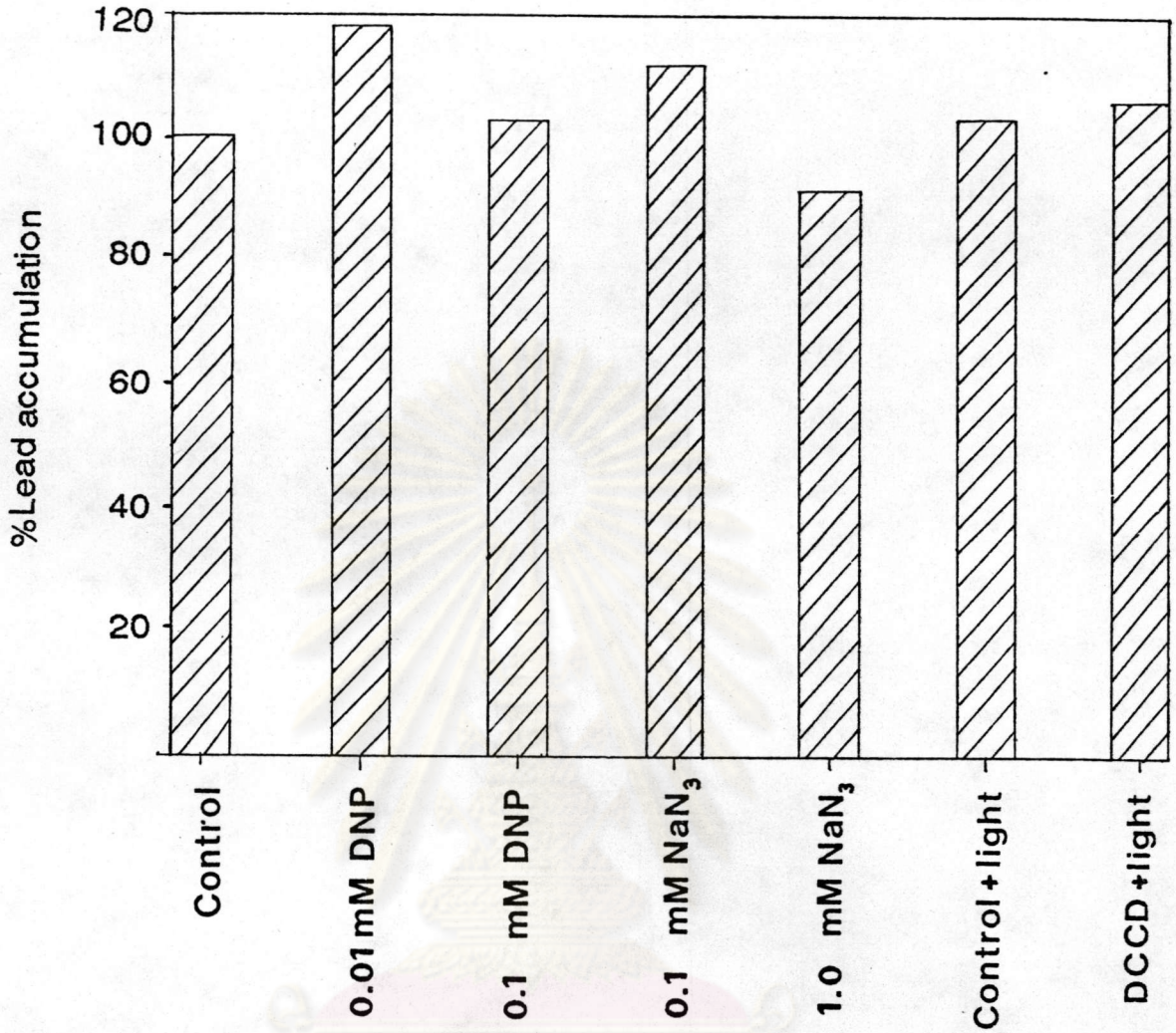

Figure 8 Effect of metabolic inhibitor on the accumulation of lead by A. halophytica. Cells were treated with various metabolic inhibitors at indicated concentration for 40 min in 10 mM PIPES buffer pH 6.5 containing 0.6 M sorbitol. Cells were then tested for the ability to accumulate lead as described in Materials and Methods.

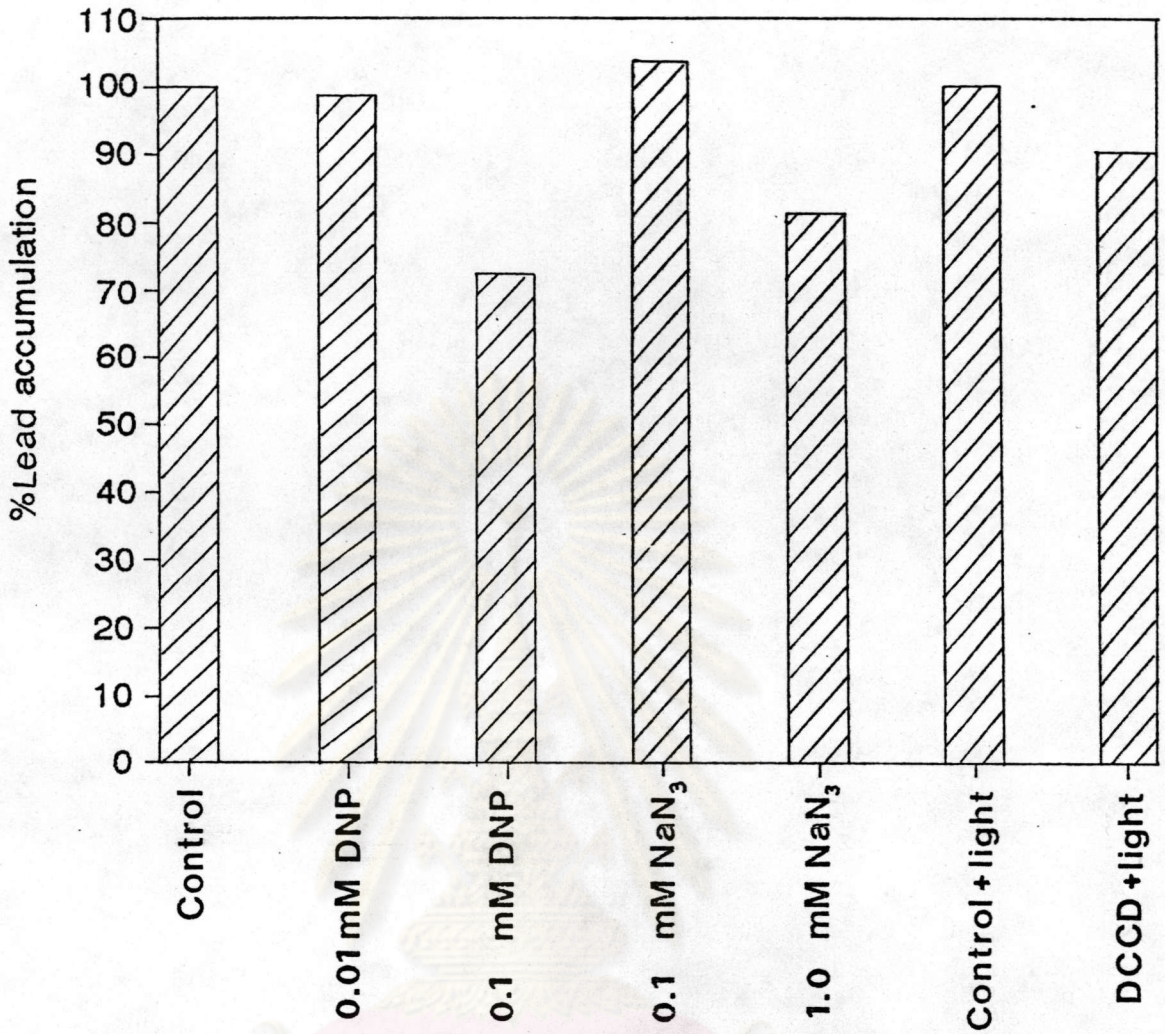

Figure 9 Effect of metabolic inhibitor on the accumulation of lead by S. platensis. Cells were treated with various metabolic inhibitors at indicated concentration for 1 hour in 10 mM acetae buffer pH 5.5. Cells were then tested for the ability to accumulate lead as described in Materials and Methods.

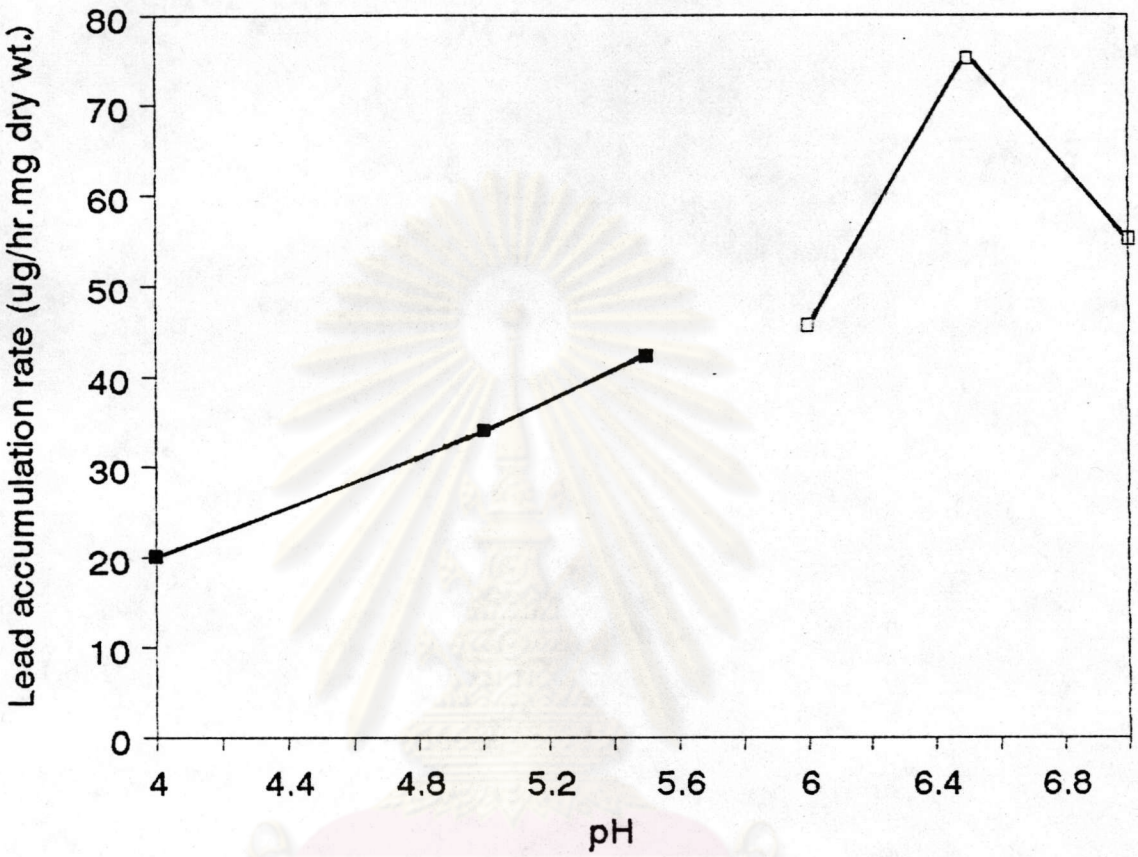

Figure 10 Effect of pH on the accumulation of lead by A. halophytica. Cells were incubated with 500 ppm lead in 10 mM acetate buffer containing 0.6 M sorbitol (■) and in 10 mM PIPES buffer containing 0.6 M sorbitol (□) for 40 min.

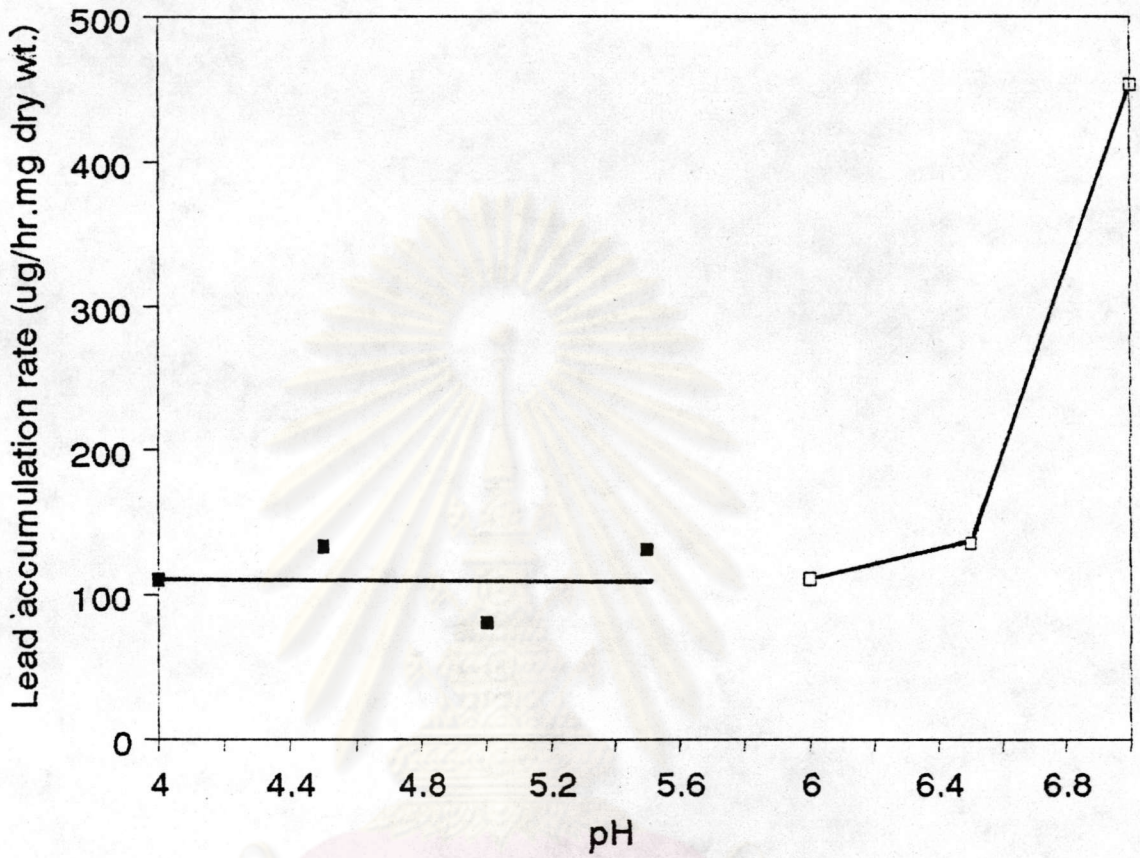

Figure 11 Effect of pH on the accumulation of lead by S. platensis. Cells were incubated with 500 ppm lead in 10 mM acetate buffer (■) and in 10 mM PIPES buffer containing 0.6 M sorbitol (□) for 1 hour.

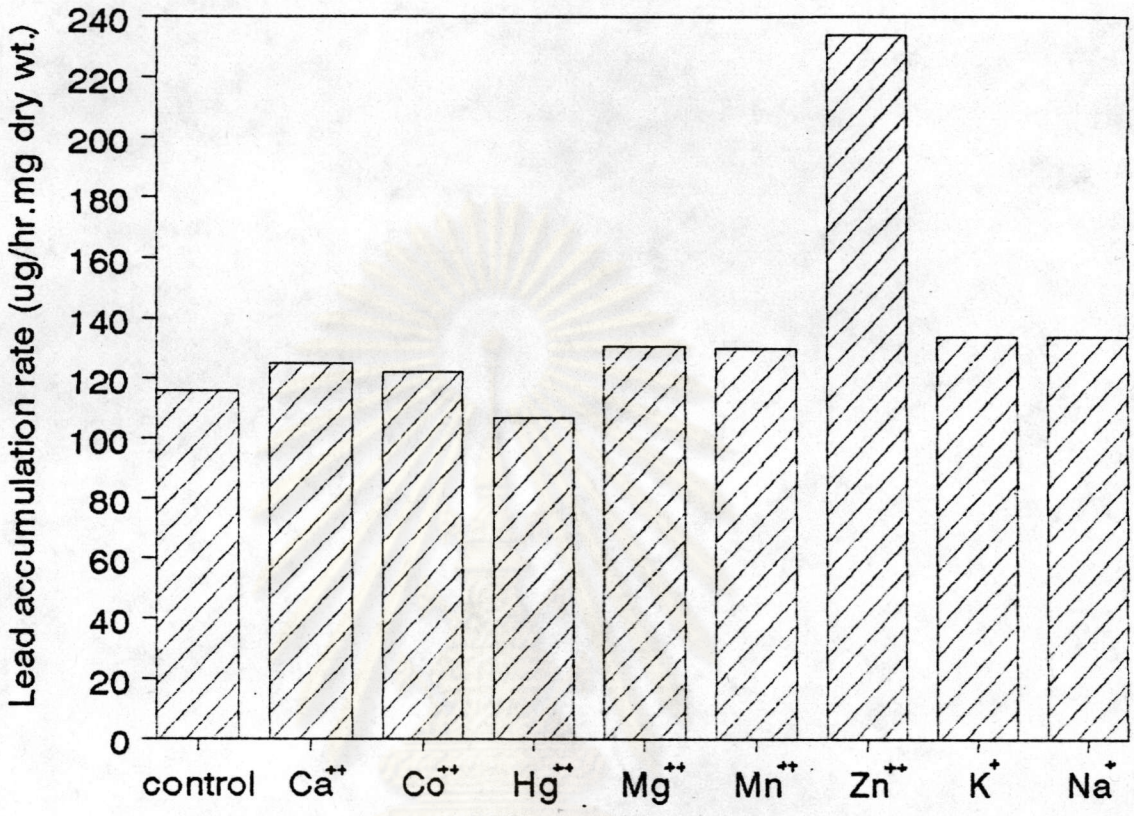

Figure 12 Effect of cations on the accumulation of lead by A. halophytica. Cells were incubated with 500 ppm lead plus an equimolar concentration of each cation in 10 mM PIPES buffer pH 6.5 containing 0.6 M sorbitol for 40 min.

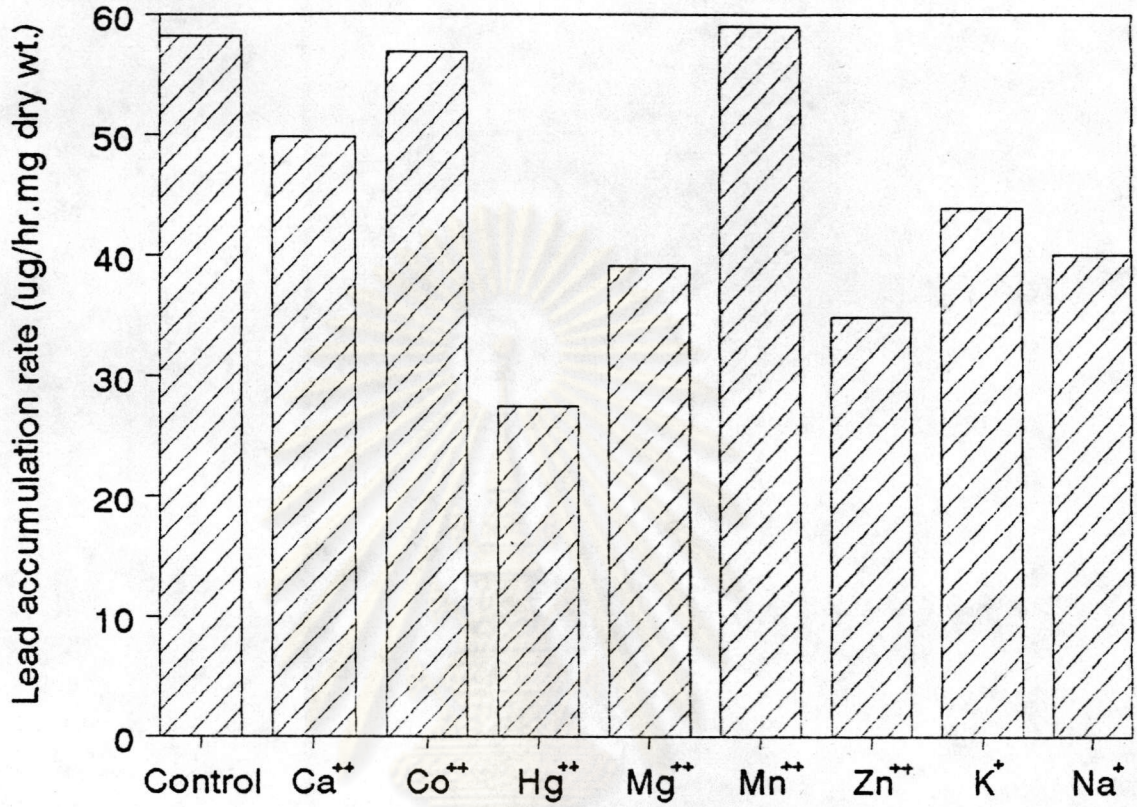

Figure 13 Effect of cations on the accumulation of lead by S. platensis. Cells were incubated with 500 ppm lead plus an equimolar concentration of each cation in 10 mM acetate buffer pH 5.5 for 1 hour.

tested ions elicited inhibitory effect. Mercuric ion was the strongest inhibitor, lowering the normal accumulation rate by one half.

3.5 Effect of Lead Concentration

The rate of lead accumulation by A. halophytica and S. platensis depended on lead concentration in solution when concentration of lead were lower than 200 and 300 ppm. respectively (figures 14 and 15).

3.6 Effect of Density of Cells

The total lead accumulation by both algae increased with increasing density of cells (figures 16 and 18) but the efficiency of accumulation was relatively unchanged when the density of cells was up to 4 mg dry weight/ml for A. halophytica (figure 17). For S. platensis, the efficiency of lead accumulation was reduced when the density of cells increased up to approximately 1.6 mg dry weight/ml and it appeared to be constant afterwards (figure 19).

3.7 Aging Effect

The ability of lead accumulation by A. halophytica was reduced when the age of cells increased up to 14 days. After 14 days there appeared to be slight decrease in the ability of the cells to accumulate lead (figure 20). The accumulation of lead by S. platensis increased up to 8 days and stayed relatively unchanged afterwards (figure 21).

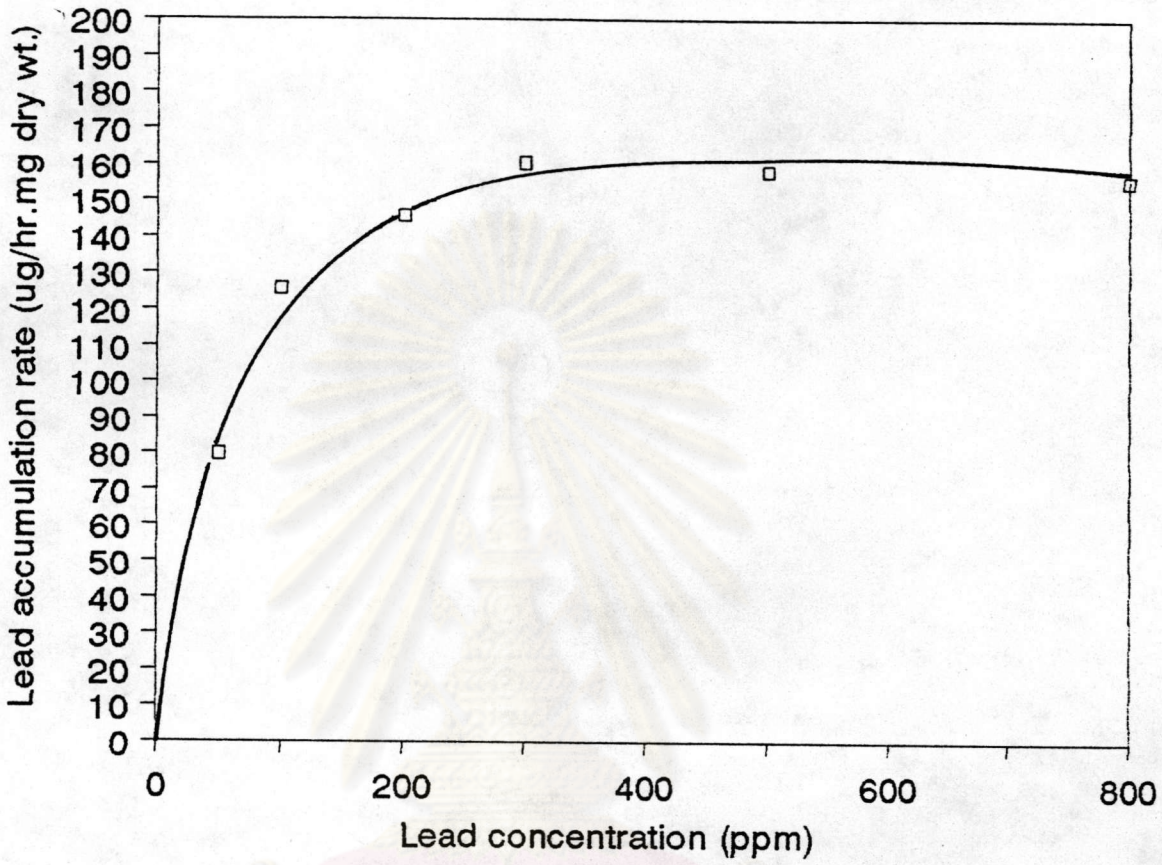

Figure 14 Effect of external lead concentration on the accumulation of lead by A. halophytica. Cells were incubated with various concentrations of lead for 40 min in 10 mM PIPES buffer pH 6.5 containing 0.6 M sorbitol.

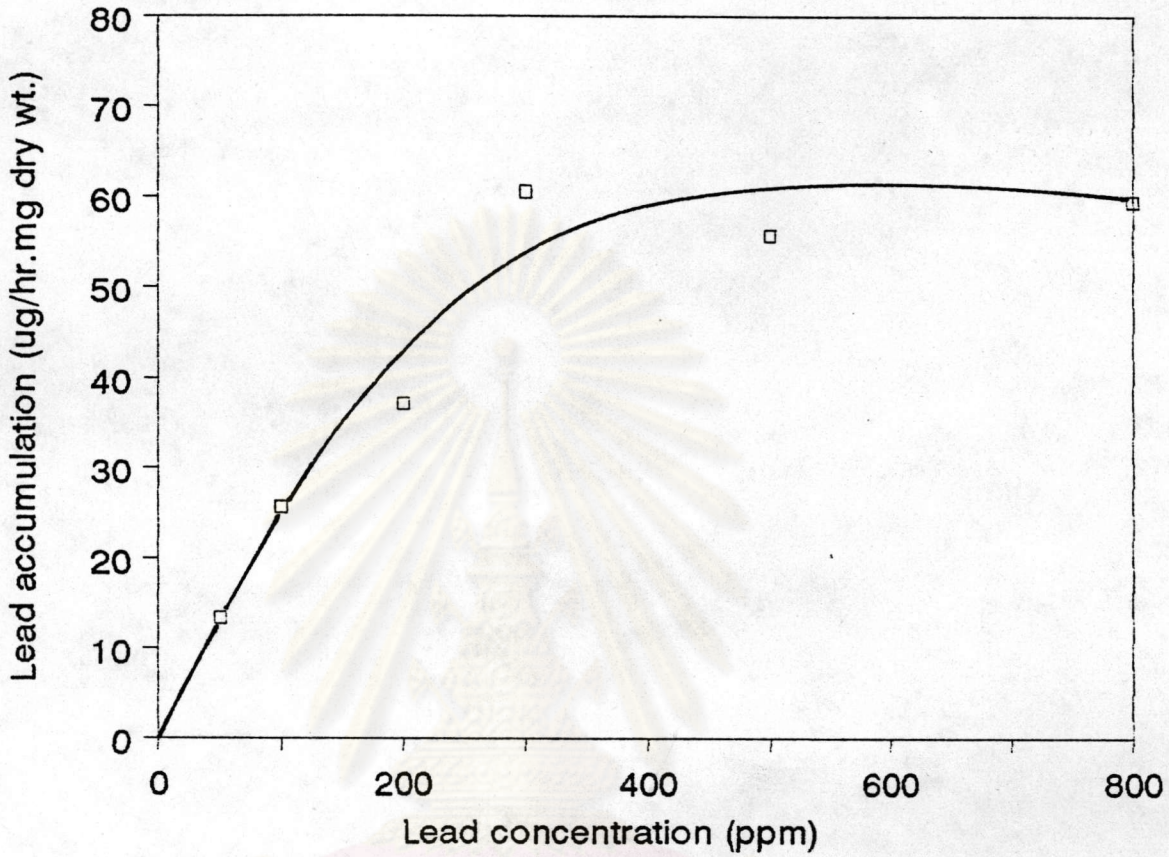

Figure 15 Effect of external lead concentration on the accumulation of lead by S. platensis. Cells were incubated with various concentrations of lead for 1 hour in 10 mM acetate buffer pH 5.5.

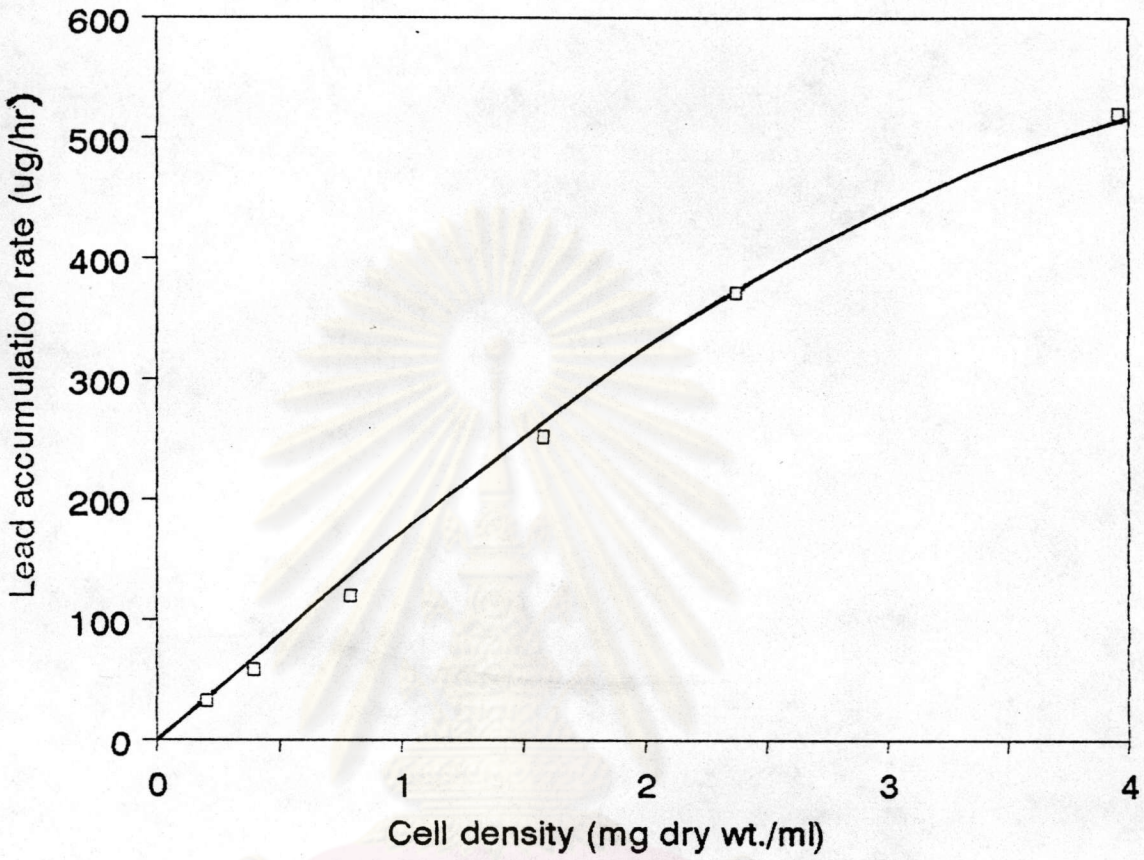

Figure 16 Effect of cell density on the accumulation of lead by A. halophytica. Various cell densities were incubated with 500 ppm lead in 10 mM PIPES buffer pH 6.5 containing 0.6 M sorbitol for 40 min.

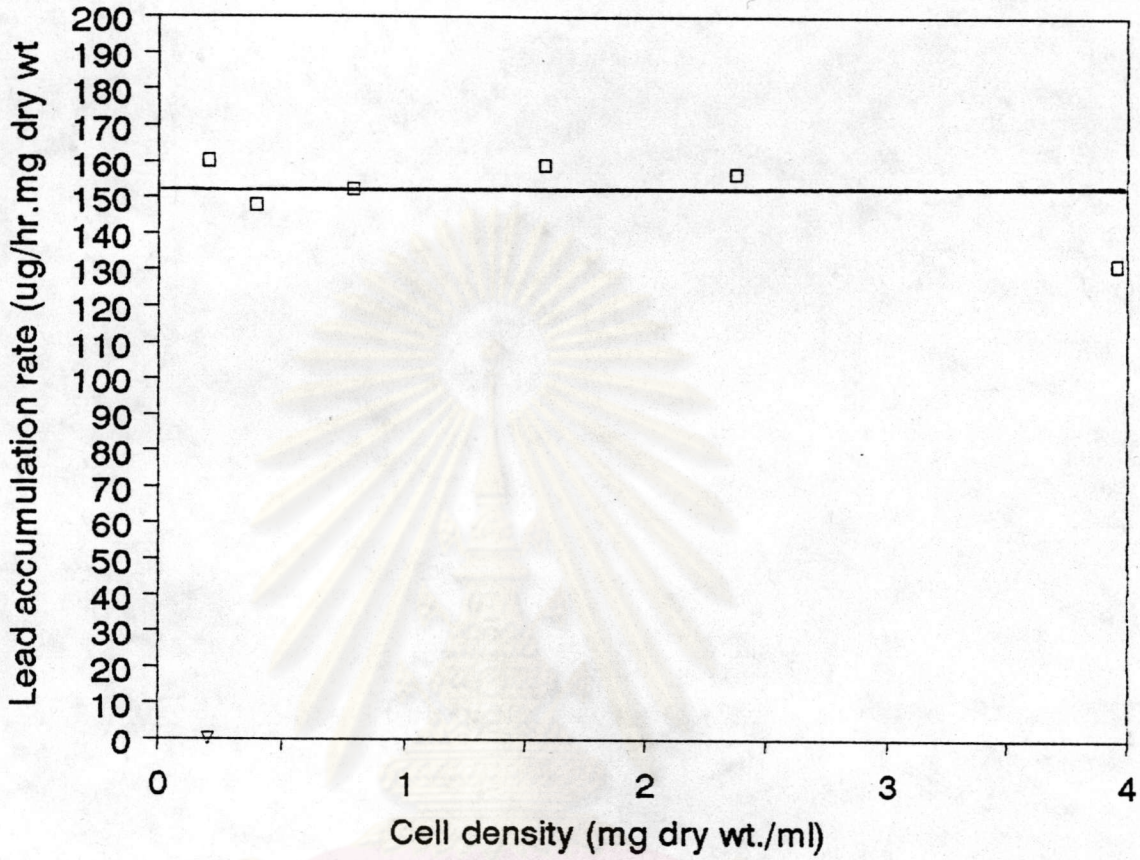

Figure 17 Capacity of A. halophytica in the accumulation of lead at increased levels of cell density. The experiment was the same as in figure 16.

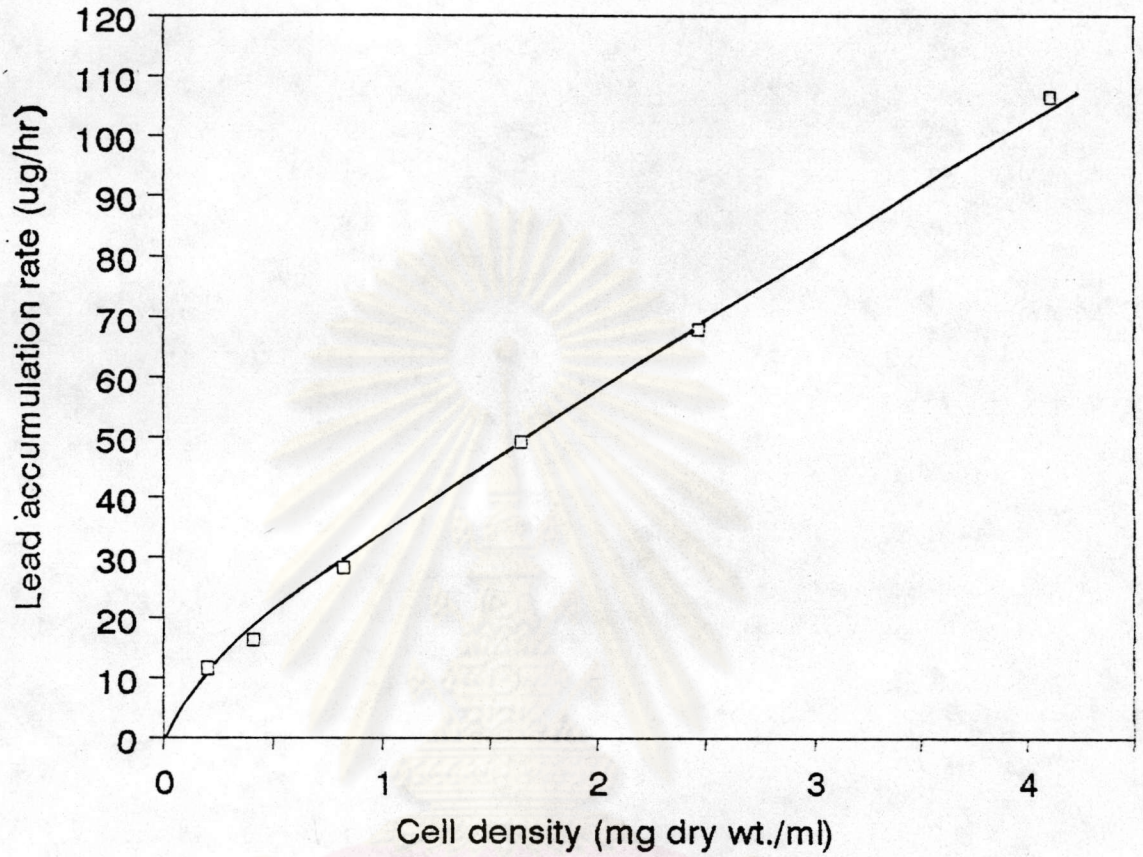

Figure 18 Effect of cell density on the accumulation of lead by S. platensis. Various cell densities were incubated with 500 ppm lead in 10 mM acetate buffer pH 5.5 for 1 hour.

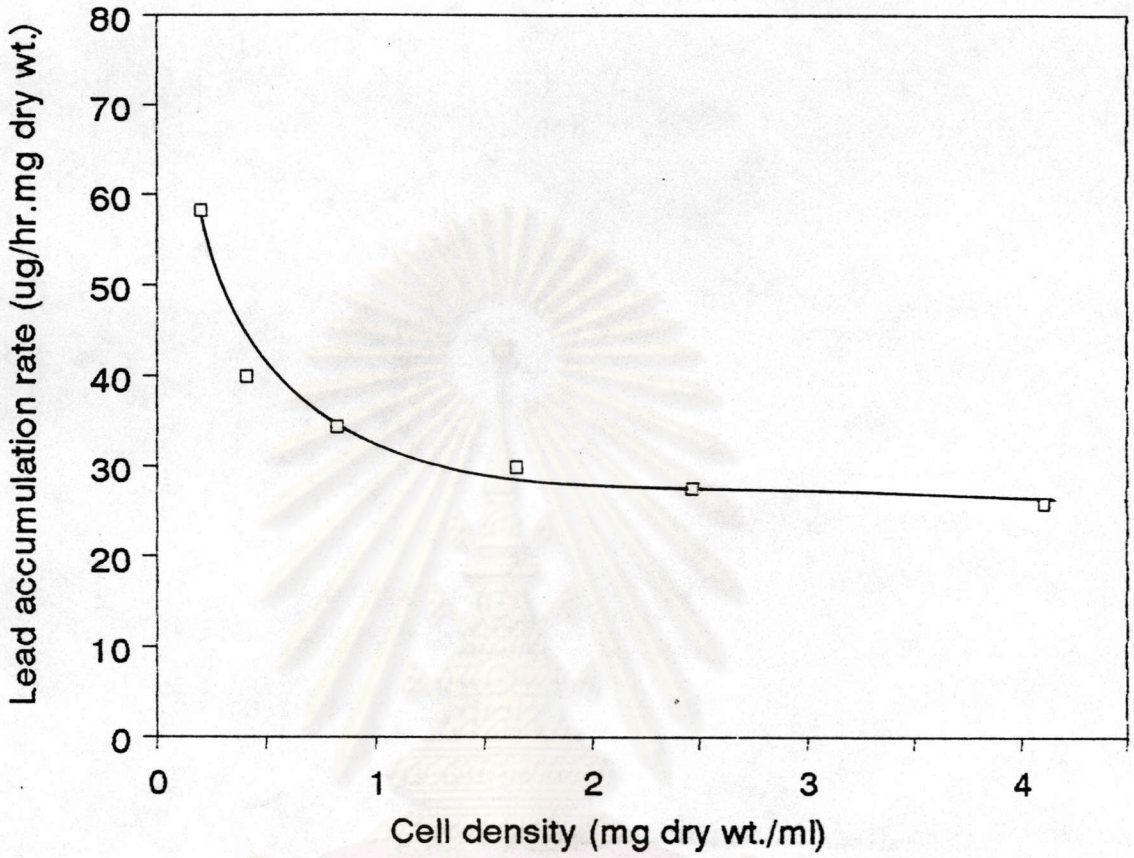

Figure 19 Capacity of S. platensis in the accumulation of lead at increased levels of cell density. The experiment was the same as in figure 18.

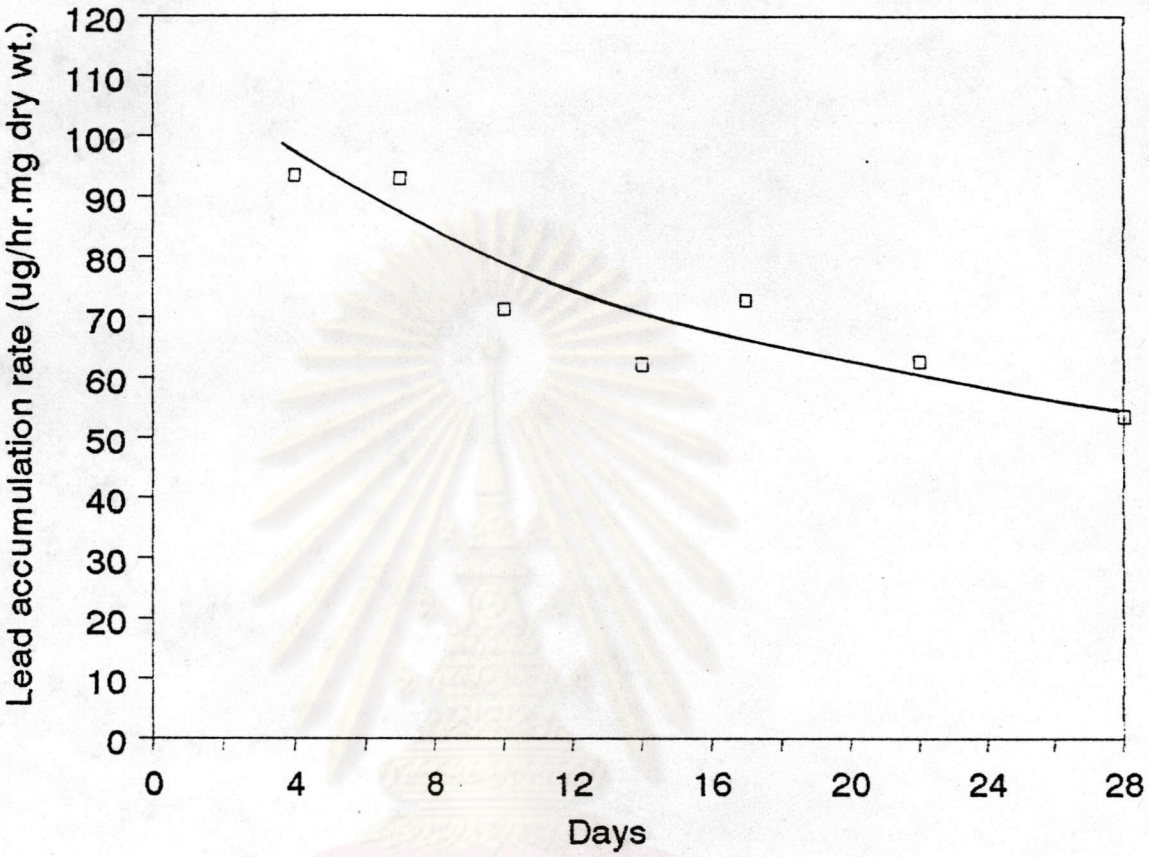

Figure 20 Effect of cell aging on the accumulation of lead by A. halophytica. Cells were grown in Turk Island Salt Solution supplemented with BG 11 medium. At indicated periods, cells were tested for the ability to accumulate lead as described in Materials and Methods.

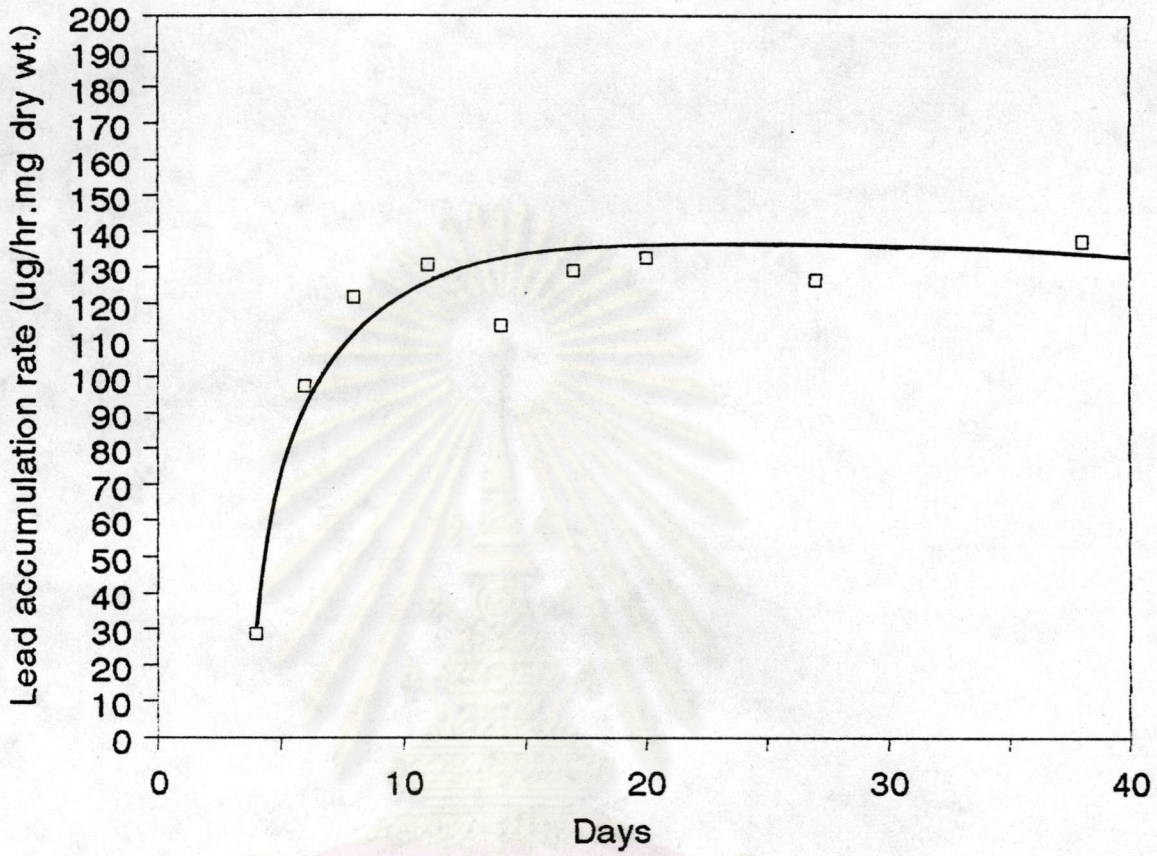

Figure 21 Effect of cell aging on the accumulation of lead by S. platensis. Cells were grown in Zarrouk's medium. At indicated periods, cells were tested for the ability to accumulate lead as described in Materials and Methods.

4. Accumulation of Zinc

4.1 Rate of Accumulation

Both algae were rapidly able to accumulate zinc. The zinc accumulation by A. halophytica was a biphasic phase, rapidly increased in the first ten minutes and thereafter the rate of increase was slower. This slower rate of increase was found to accumulate zinc at 7.8 ug/hr.mg dry weight (figure 22). The zinc accumulation by S. platensis increased linearly in the first hour with the rate of 15.6 ug/hr.mg dry weight and became saturated at about 19 ug/mg dry weight within 5 hours (figure 23). The zinc accumulation of both algae could be washed out completely with 10 mM EDTA.

4.2 Effect of Metabolic Inhibitor

The zinc accumulation by A. halophytica was not inhibited by metabolic inhibitors; DNP, NaN_3 and DCCD (figure 24). On the other hand, the zinc accumulation by S. platensis was inhibited by all 3 inhibitors at the concentrations tested (figure 25).

4.3 Effect of pH

The rates of zinc accumulation by both algae were stable in the pH range between 4.0 and 6.0 and sharply increased above pH 6.0 (figures 26 and 27).

4.4 Effect of Cation

The accumulation of zinc by A. halophytica was inhibited by Ca^{2+}, Co^{2+}, Hg^{2+}, Mg^{2+}, Mn^{2+} about 48, 12, 54, 24, 43 % respectively while Pb^{2+} completely

inhibited zinc accumulation. On the other hand, K^+ and Na^+ were not inhibitory (figure 28). For S. platensis, the extents of inhibition by divalent cations were different from those in A. halophytica except for Ca^{2+} which showed similar inhibition in both A. halophytica and S. platensis (figures 28 and 29). For monovalent cations, both K^+ and Na^+ could inhibit the zinc accumulation by about 50 and 60 % respectively (figure 29).

4.5 Effect of Zinc Concentration

The rate of zinc accumulation by A. halophytica and S. platensis depended on zinc concentration and reached the saturation when zinc concentrations were above 80 and 300 ppm respectively (figures 30 and 31).

4.6 Effect of Density of Cells

The total zinc accumulation by both algae increased with increasing cell density (figures 32 and 34). However, the rate of zinc accumulation per hr. mg dry weight decreased with increasing cell density and became stable after the cell density reached 0.5 mg dry weight/ml for A. halophytica (figure 33) and 0.6 mg dry weight/ml for S. platensis (figure 35).

4.7 Aging Effect

The age of A. halophytica up to 26 days did not appear to affect the ability of cells to accumulate zinc (figure 36). For S. platensis the ability of cells to accumulate zinc was independent of the cell age up to 20 days after which the accumulation of zinc slightly decreased (figure 37).

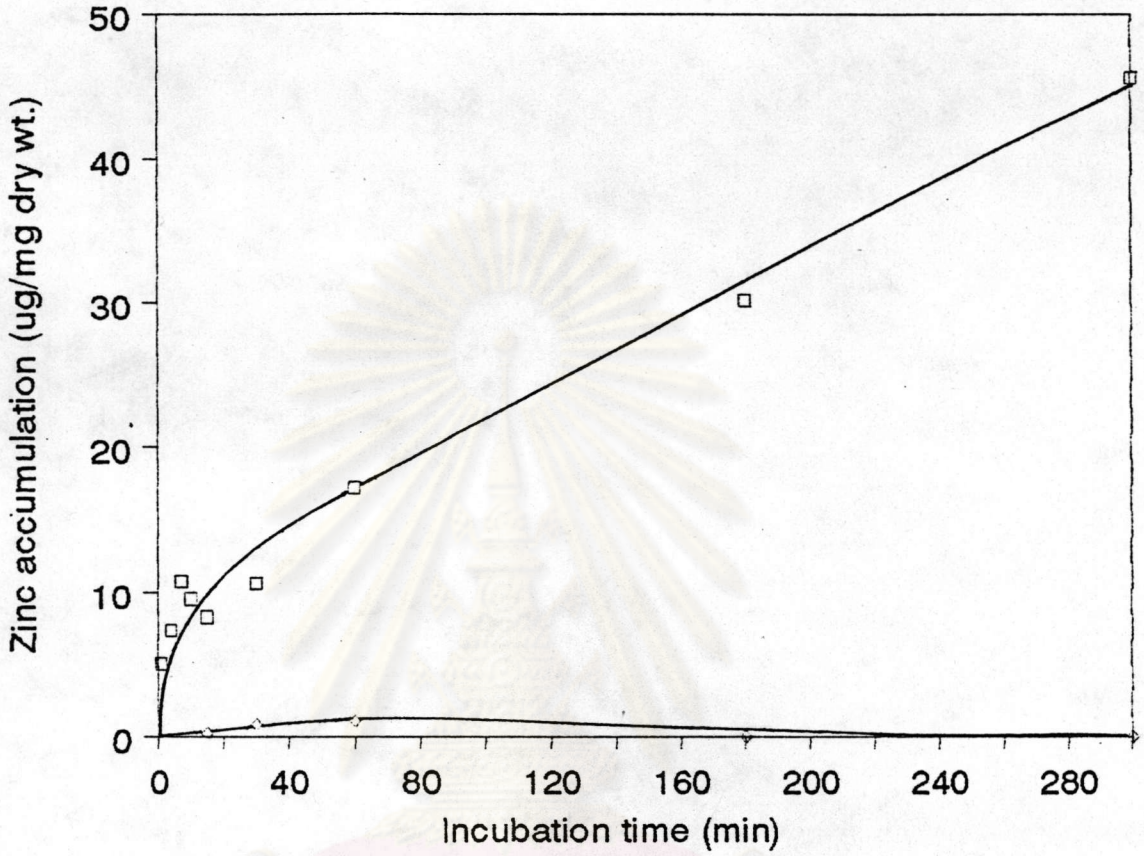

Figure 22 Accumulation of zinc by A. halophytica. Cells were incubated with 100 ppm zinc in 10 mM PIPES buffer pH 6.5 containing 0.6 M sorbitol. After incubation the cells were washed with PIPES-sorbitol buffer (□) or with 10 mM EDTA (◇).

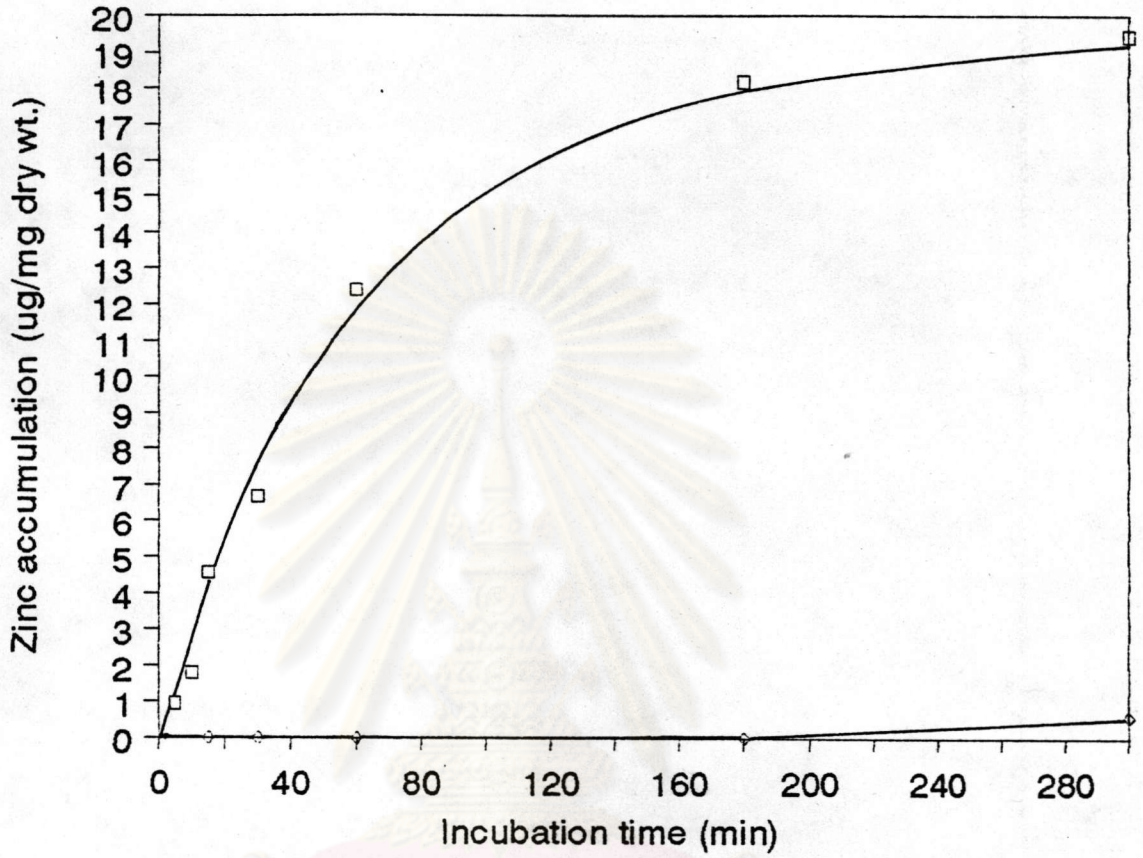

Figure 23 Accumulation of zinc by S. platensis. Cells were incubated with 500 ppm zinc in 10 mM PIPES buffer pH 6.5. After incubation the cells were washed with PIPES buffer (□) or with 10 mM EDTA (◇).

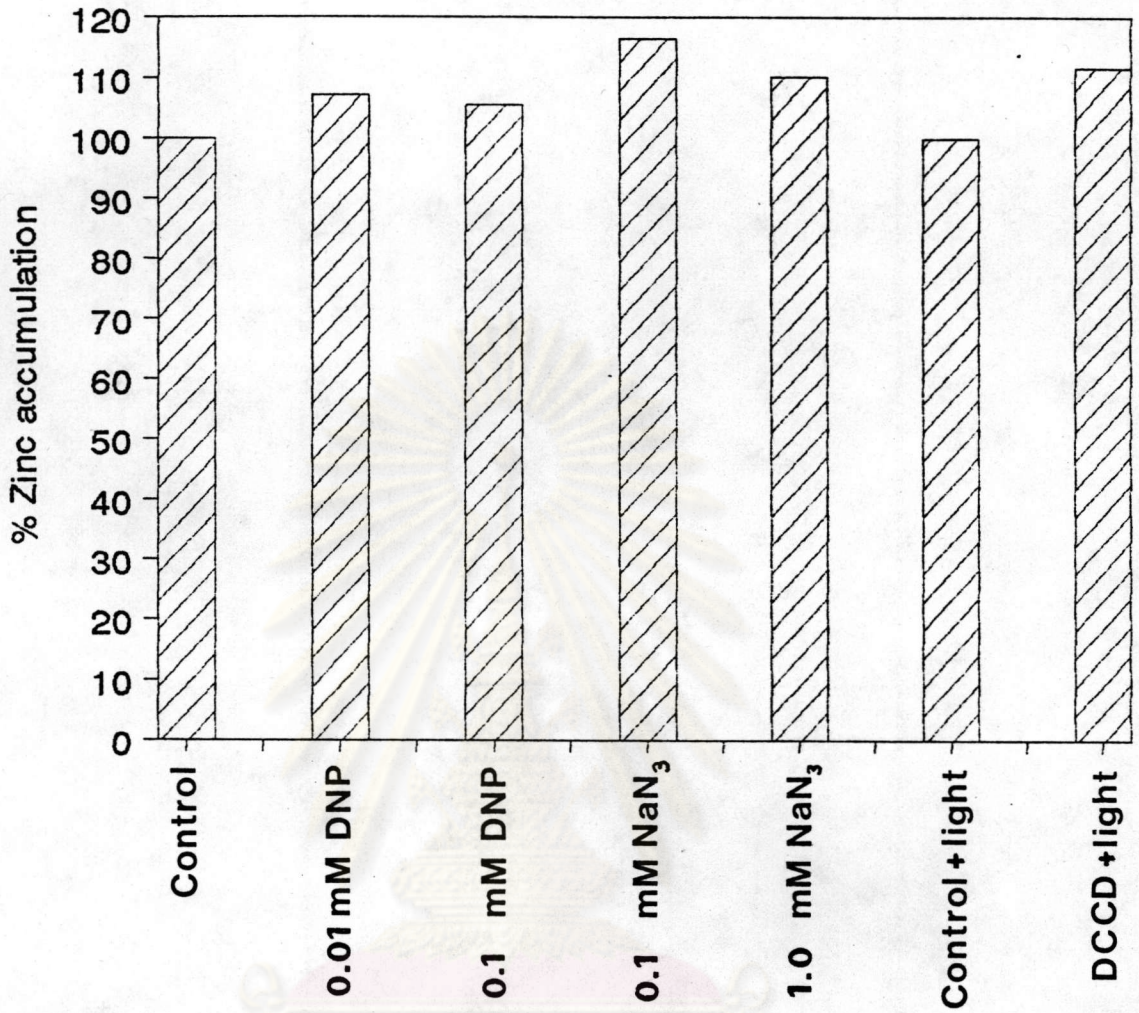

Figure 24 Effect of metabolic inhibitor on the accumulation of zinc by A. halophytica. Cells were treated with various metabolic inhibitors at indicated concentration for 1 hour in 10 mM PIPES buffer pH 6.5 containing 0.6 M sorbitol. Cells were then tested for the ability to accumulate zinc as described in Materials and Methods.

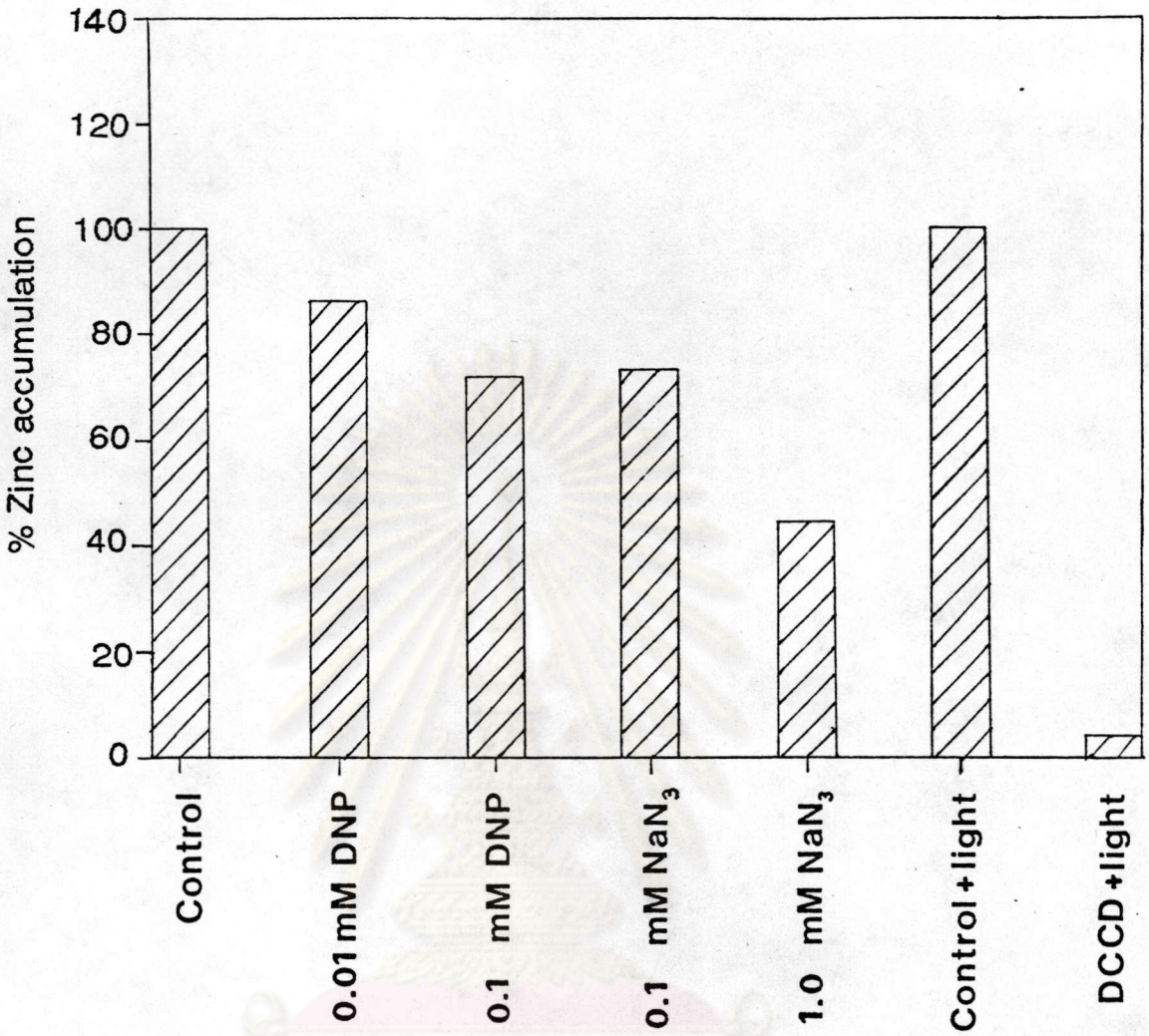

Figure 25 Effect of metabolic inhibitor on the accumulation of zinc by S. platensis. Cells were treated with various metabolic inhibitors at indicated concentration for 30 min in 10 mM PIPES buffer pH 6.5. Cells were then tested for the ability to accumulate zinc as described in Materials and Methods.

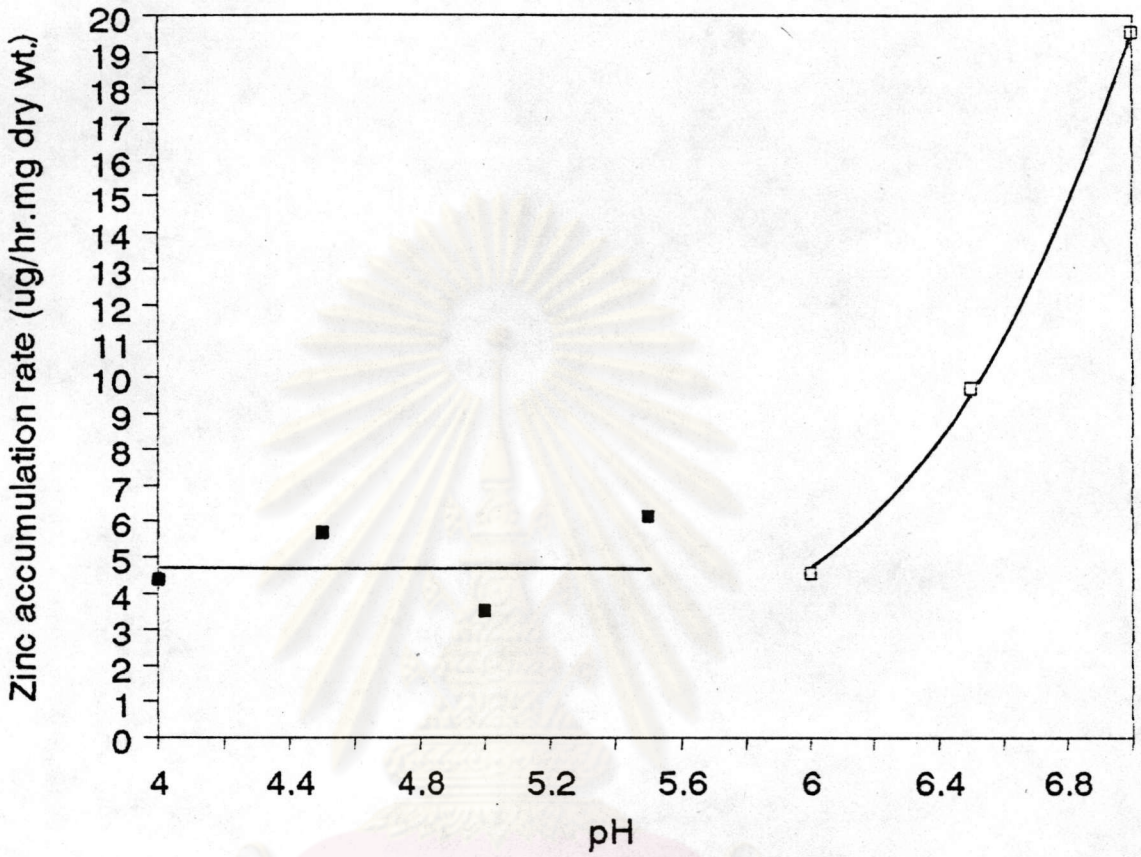

Figure 26 Effect of pH on the accumulation of zinc by A. halophytica. Cells were incubated with 100 ppm lead in 10 mM acetate buffer containing 0.6 M sorbitol (■) and in 10 mM PIPES buffer containing 0.6 M sorbitol (□) for 1 hour.

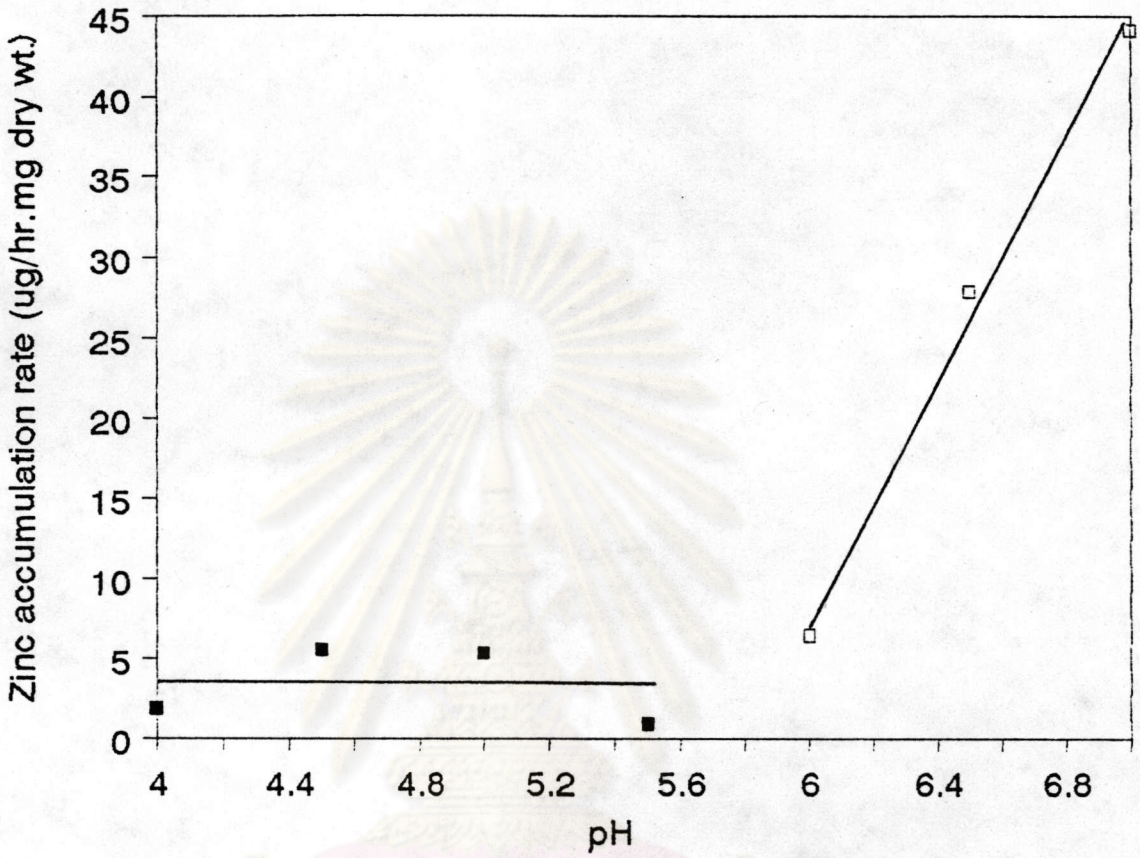

Figure 27 Effect of pH on the accumulation of zinc by S. platensis. Cells were incubated with 500 ppm lead in 10 mM acetate buffer (■) and in 10 mM PIPES buffer (□) for 30min.

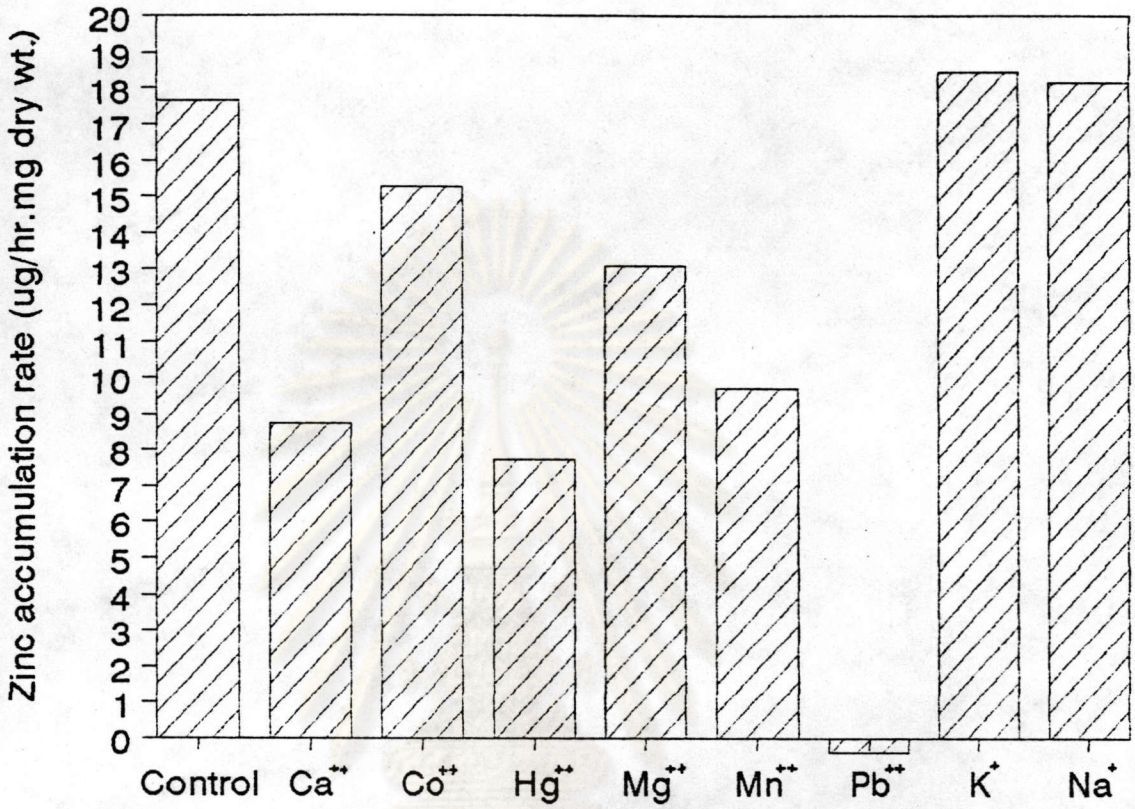

Figure 28 Effect of cations on the accumulation of zinc by A. halophytica. Cells were incubated with 100 ppm zinc plus an equimolar concentration of each cation in 10 mM PIPES buffer pH 6.5 containing 0.6 M sorbitol for 1 hour.

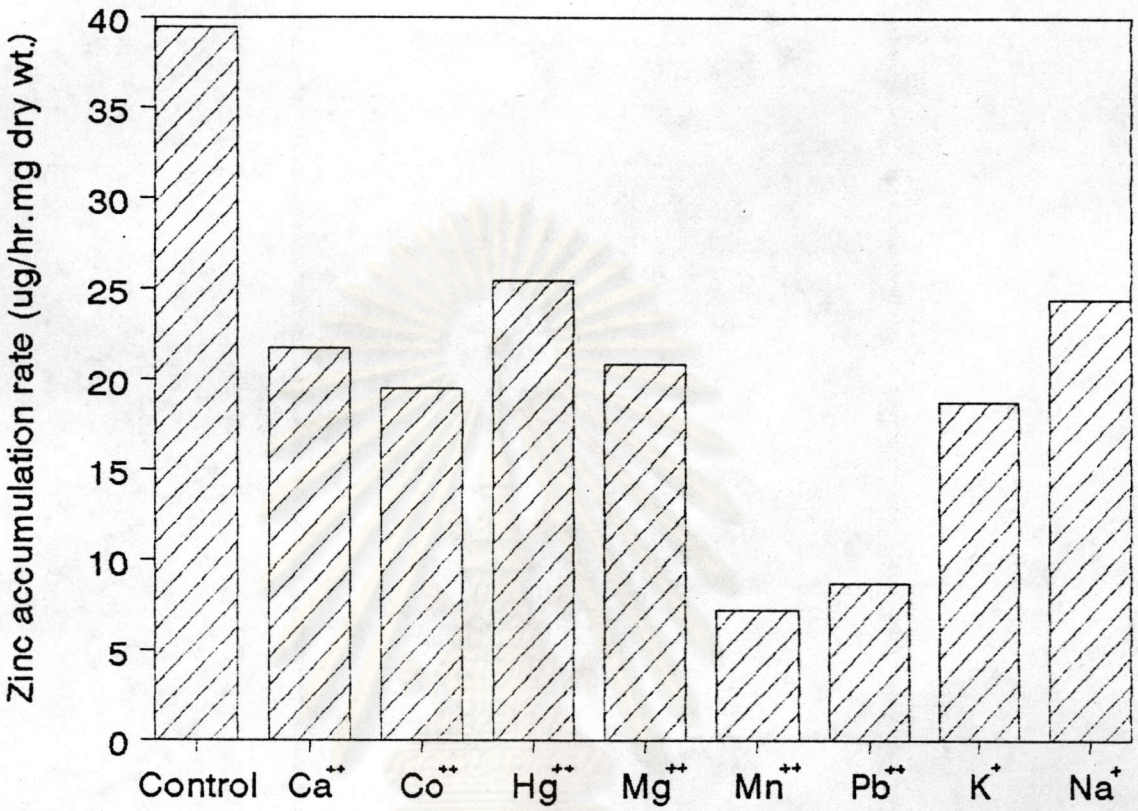

Figure 29 Effect of cations on the accumulation of zinc by S. platensis. Cells were incubated with 500 ppm zinc plus an equimolar concentration of each cation in 10 mM PIPES buffer pH 6.5 for 30 min.

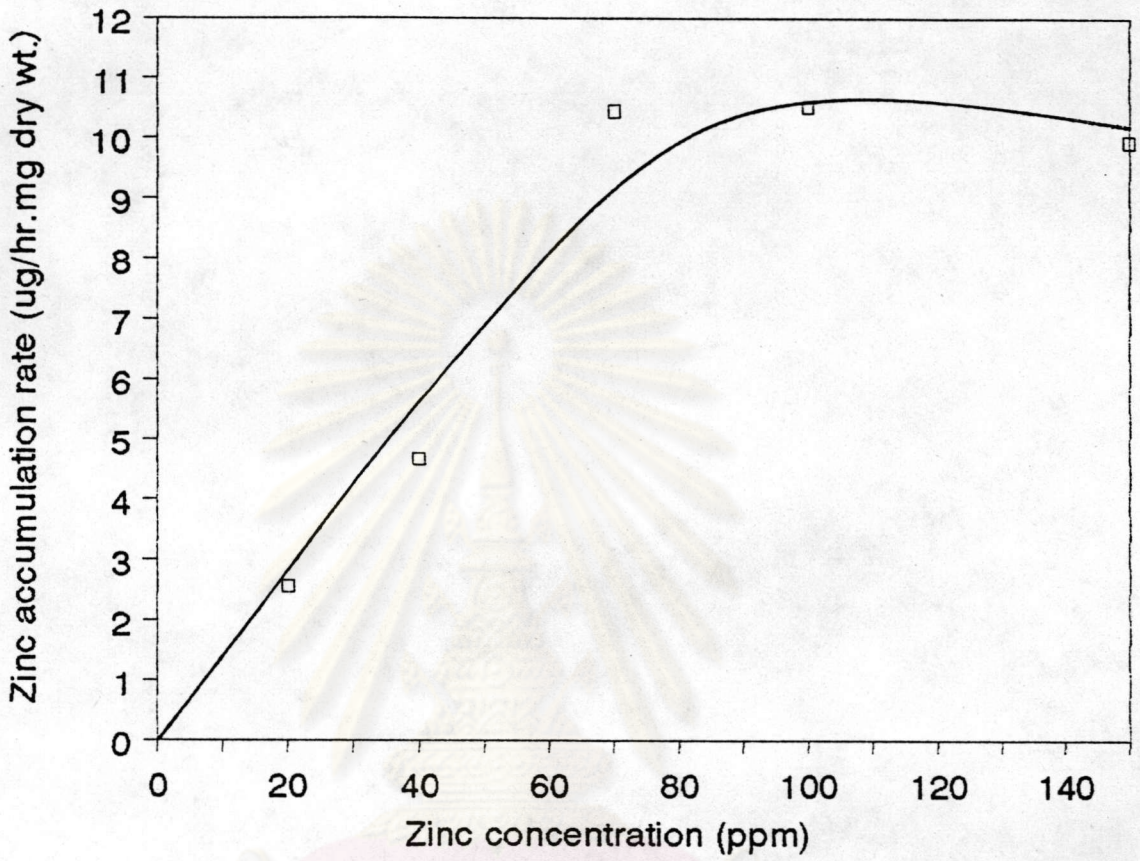

Figure 30 Effect of external zinc concentration on the accumulation of zinc by A. halophytica. Cells were incubated with various concentrations of zinc for 1 hour in 10 mM PIPES buffer pH 6.5 containing 0.6 M sorbitol.

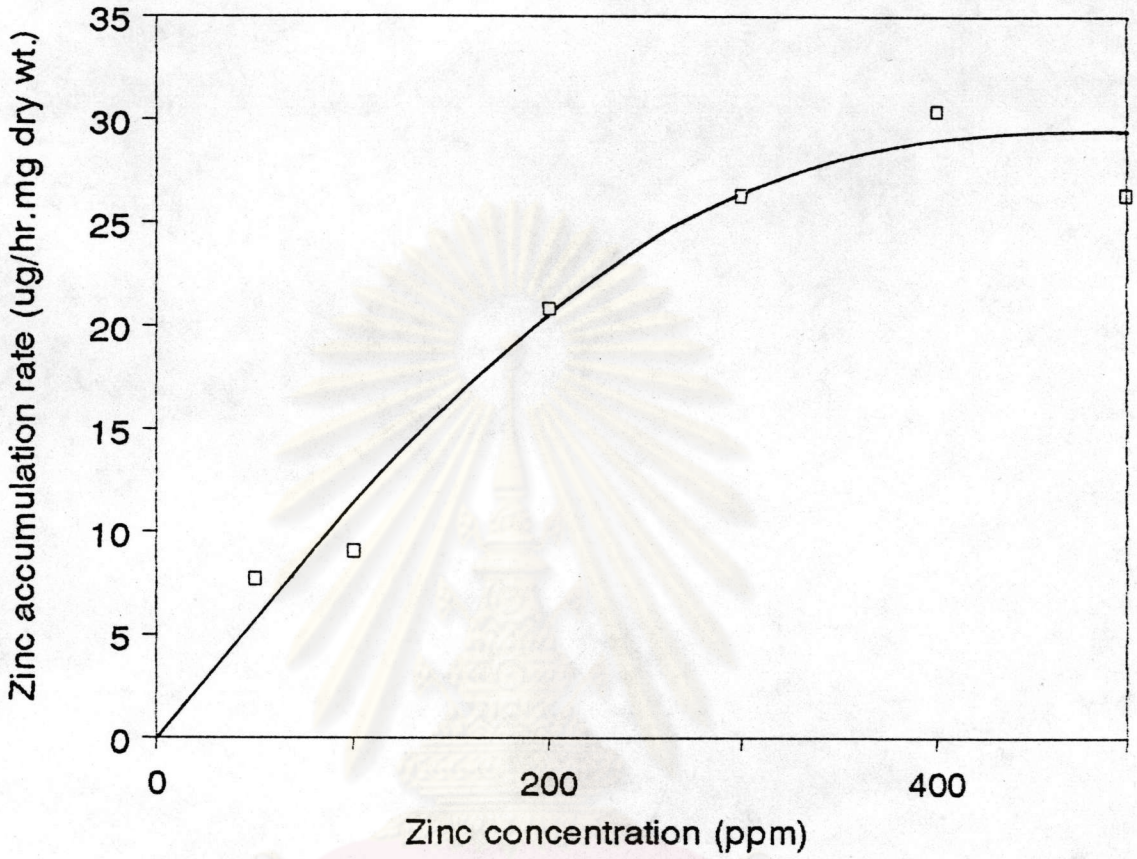

Figure 31 Effect of external zinc concentration on the accumulation of zinc by S. platensis. Cells were incubated with various concentrations of zinc for 30 min in 10 mM PIPES buffer pH 6.5.

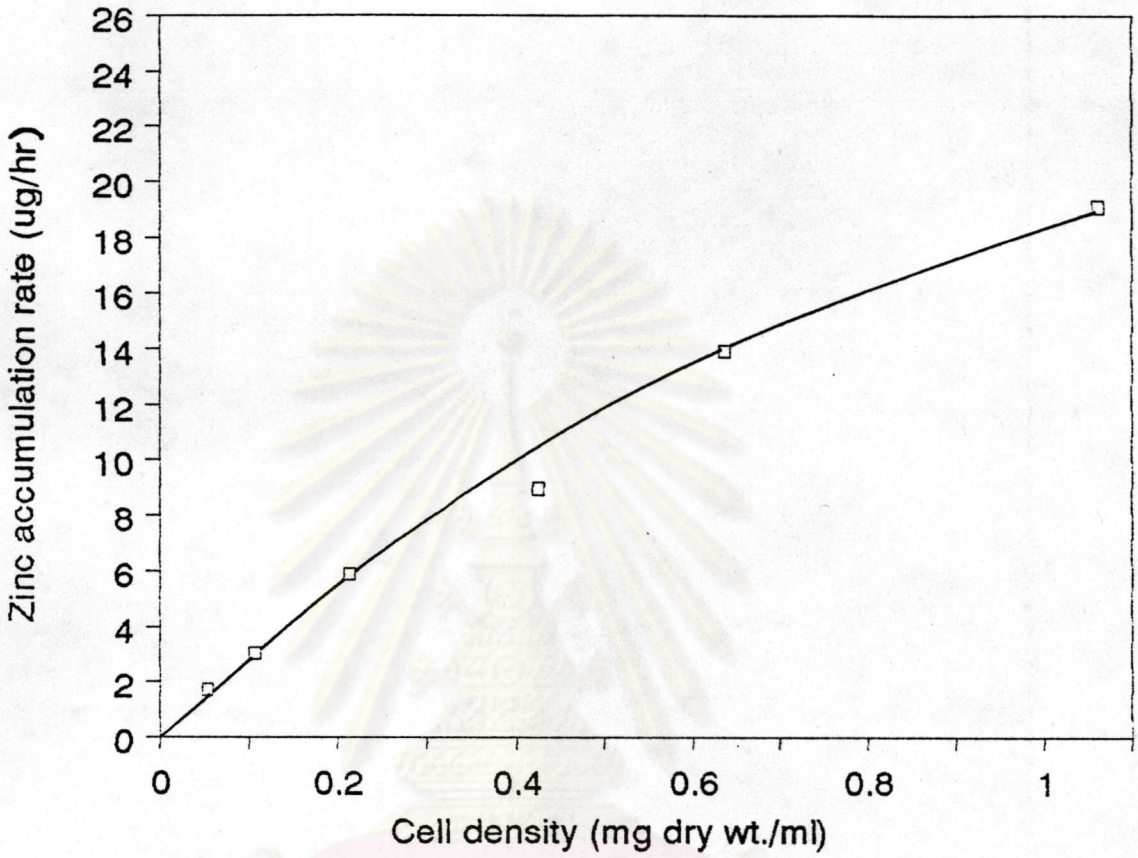

Figure 32 Effect of cell density on the accumulation of zinc by A. halophytica. Various cell densities were incubated with 100 ppm zinc in 10 mM PIPES buffer pH 6.5 containing 0.6 M sorbitol for 1 hour.

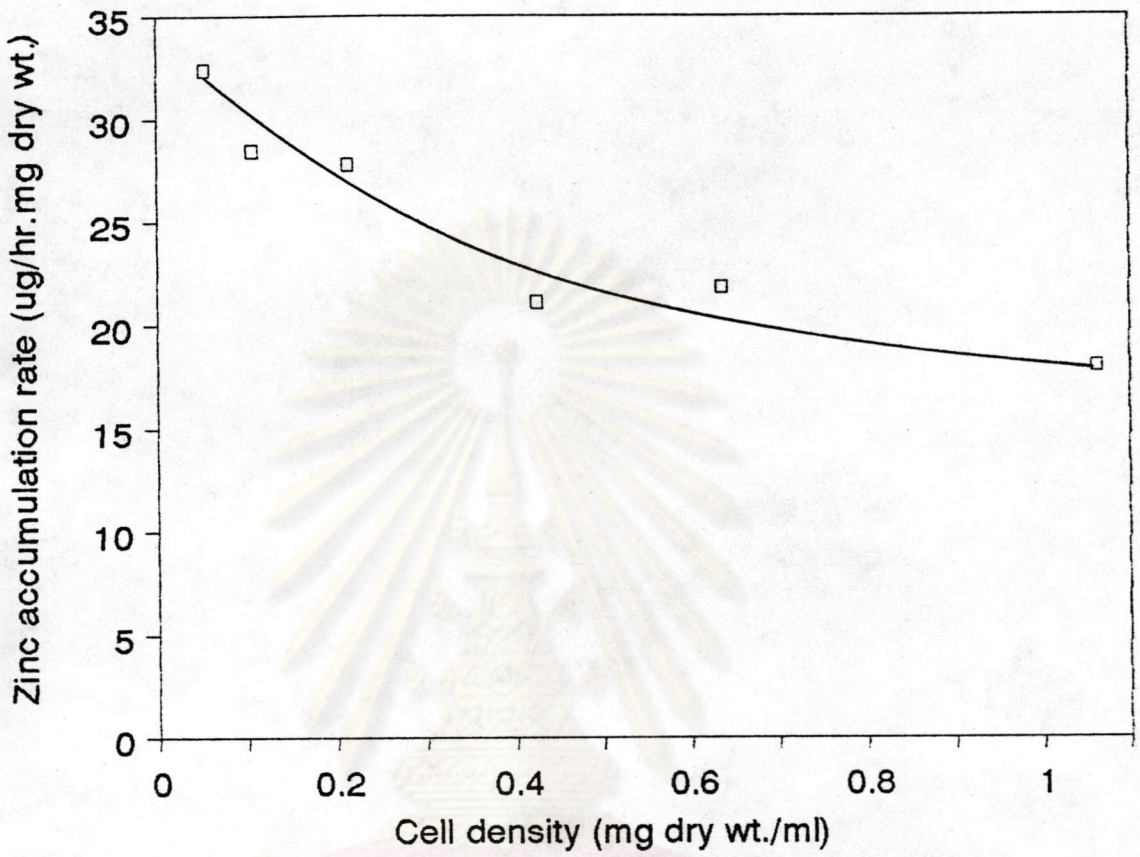

Figure 33 Capacity of A. halophytica in the accumulation of zinc at increased levels of cell density. The experiment was the same as in figure 32.

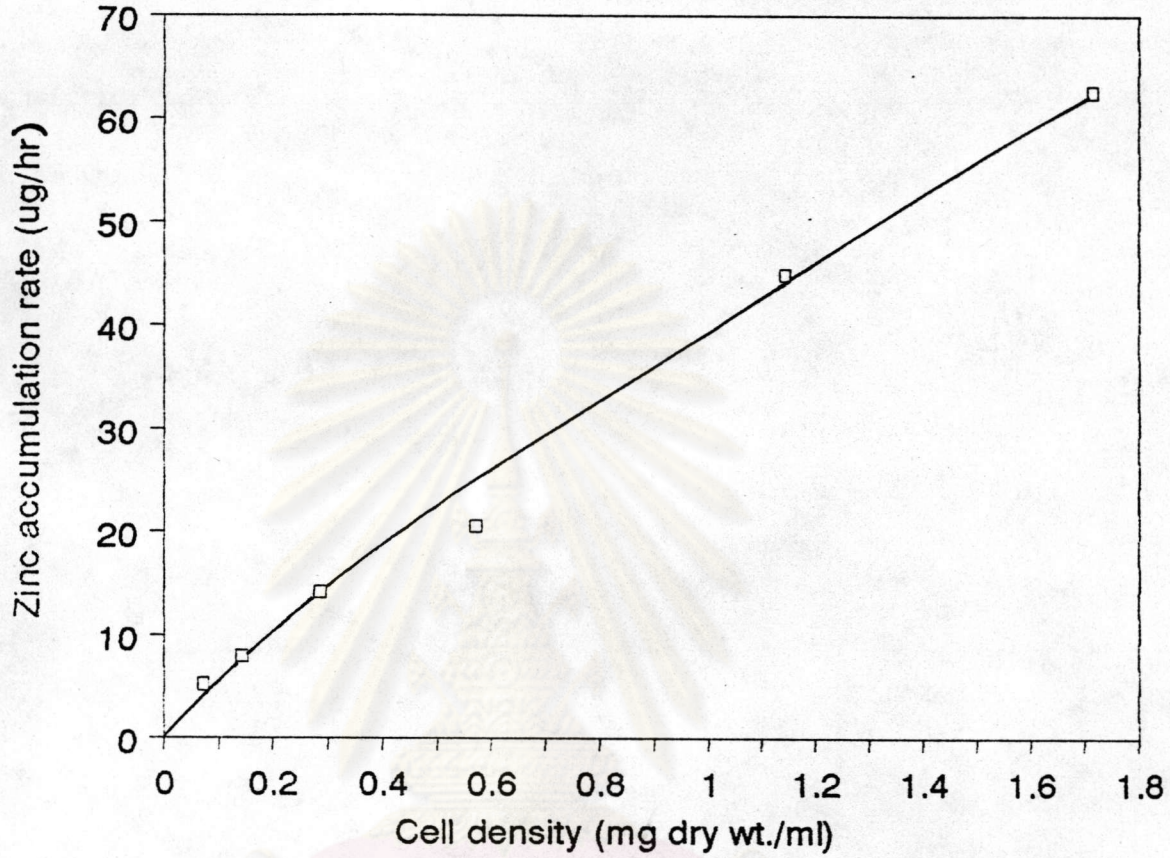

Figure 34 Effect of cell density on the accumulation of zinc by S. platensis. Various cell densities were incubated with 500 ppm zinc in 10 mM PIPES buffer pH 6.5 for 30 min.

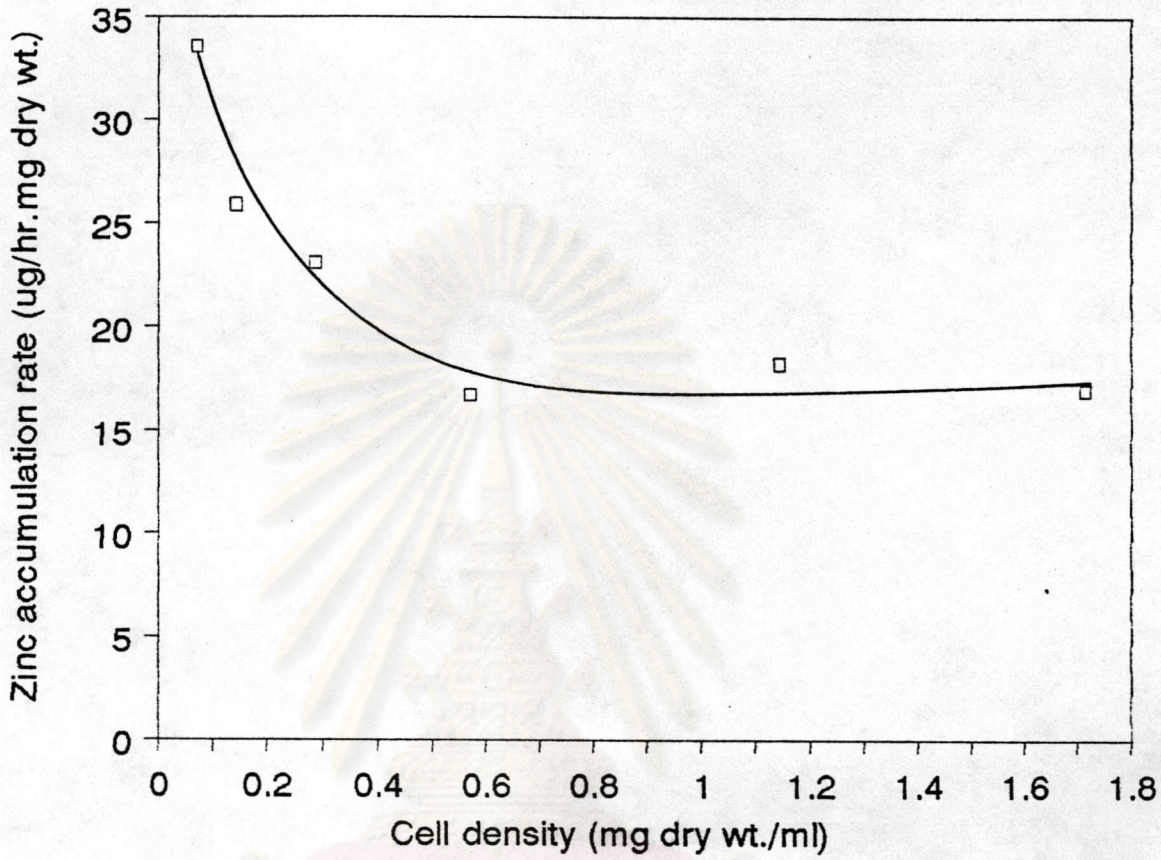

Figure 35 Capacity of S. platensis in the accumulation of zinc at increased levels of cell density. The experiment was the same as in figure 34.

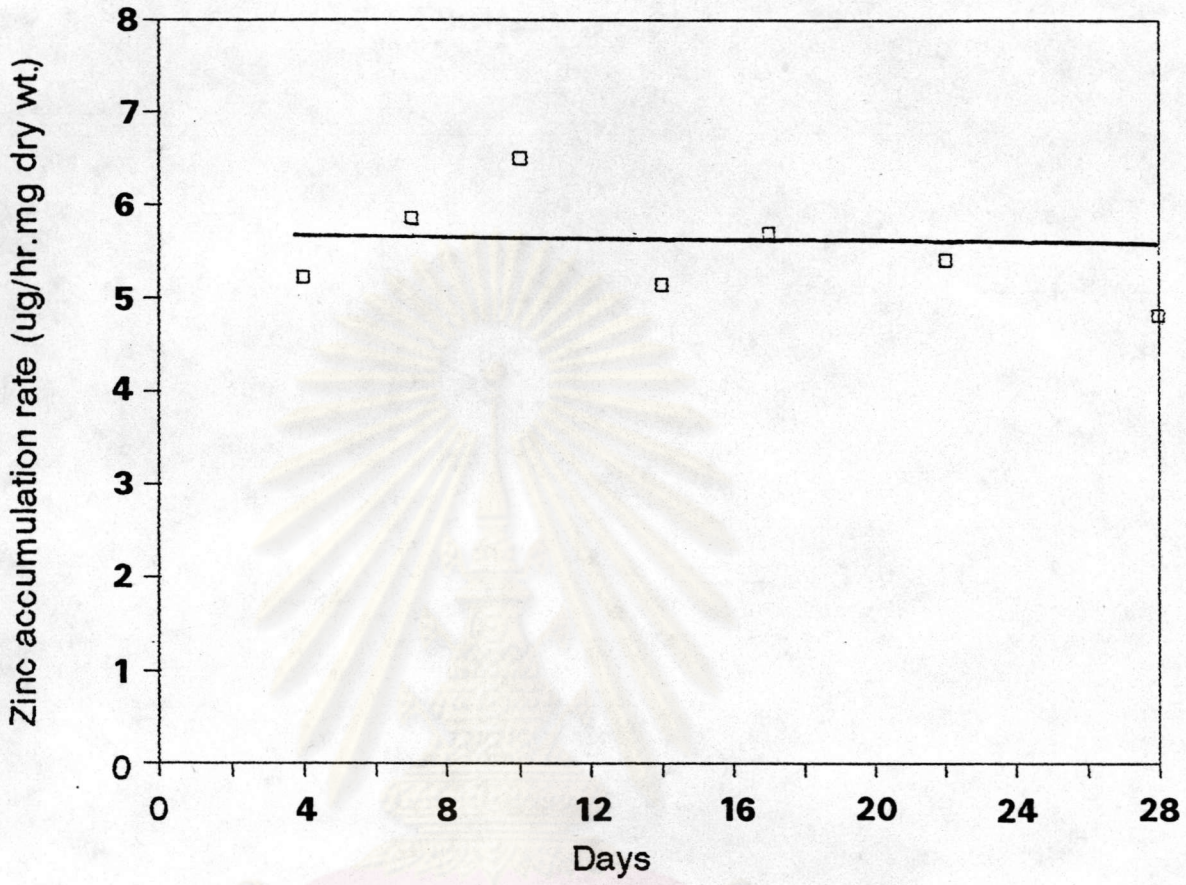

Figure 36 Effect of cell aging on the accumulation of zinc by A. halophytica. Cells were grown in Turk Island Salt Solution supplemented with BG 11 medium. At indicated periods, cells were tested for the ability to accumulate zinc as described in Materials and Methods.

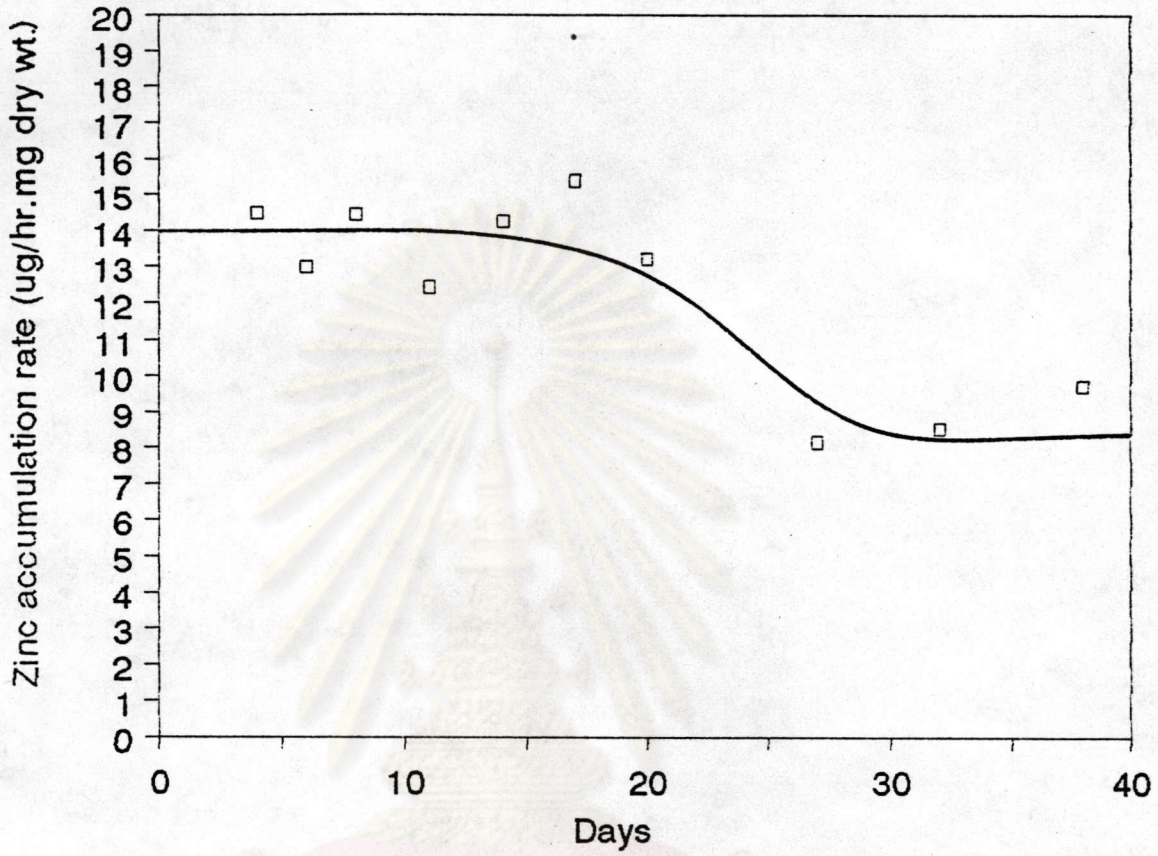

Figure 37 Effect of cell aging on the accumulation of zinc by S. platensis. Cells were grown in Zarrouk's medium. At indicated periods, cells were tested for the ability to accumulate zinc as described in Materials and Methods.

5. Determination of Cell Mortality

The cells of A. halophytica were treated with lead and zinc for 40 min. and 1 hour respectively. The cells were then checked for the mortality. The cells were still living approximately 71 % and 74 % after treating with lead and zinc respectively as compared to the control which showed 87 % living cells. For S. platensis, most cells died after treating with lead for 1 hour but the majority of cells were still living after treating with zinc for 30 min..

6. Removal of Lead and Zinc from Waste Water

6.1 Removal of Lead from Waste Water

The waste water obtained from the Battery Organization had a pH value of 2.23 and contained 1.549 ppm of lead. After the waste water was treated with Aphanothece halophytica for 1 hour or S. platensis for 3 hour , the concentration of lead in the supernatant after removing the cells was found to sligthly decrease. A. halophytica and S. platensis exhibited 21.7 and 34.6% efficiency for lead removal respectively.

6.2 Removal of Zinc from Waste Water

The waste water samples obtained from Samart Engineering Co. Ltd. were collected from 2 ponds, ie., the first one with pH 1.2 and 58.8 ppm zinc. and the second one with pH 12.5 and 8 ppm zinc. Table 1 showed that both A. halophytica and S. platensis exhibited a higher

efficiency of zinc removal at high pH than at low pH. Furthermore, A. halophytica was much more efficient than S. platensis in the zinc removal at basic pH. The second waste water sample obtained from the Waste Water Eradication Service Center, had a pH of 1.48 with 1881 ppm zinc. The efficiency of zinc removal by both algae was low at acidic pH with S. platensis being slightly more efficient than A. halophytica. The sample from Samart Engineering Co. Ltd. with pH 12.5 was adjusted by the addition of HCl so that the pH was reduced to 9, 6.5, 4 and 2. The efficiency of both algae at different pH values was shown in figure 38. At all pH values tested, A. halophytica was a more efficient organism to remove zinc from the waste water. There was no increase in the efficiency of zinc removal for S. platensis at the acidic pH range whereas increased efficiency was observed for A. halophytica. At pH 12.5, the efficiency of zinc removal was best for both algae with A. halophytica having almost 3 times higher efficiency than S. platensis.

The effect of increasing cell density on the efficiency of zinc removal is shown in figure 39. It appeared that there was a linear relationship between the increase in A. halophytica concentration and the increase in the efficiency of zinc removal.

Aphanothece halophytica was not available for

Source of Waste Water	pH	Zinc Concentration Before Treatment (ppm)	After Treatment with Blue-green Algae			
			Zinc Concentration (ppm)		% efficiency	
			A. halophytica	S. platensis	A. halophytica	S. platensis
Samart Engineering Co. Ltd. 1st pond	1.2	58.8	59.2	57.9	0	1.5
2nd pond	12.5	8.0	1.8	4.8	71.5	40.7
Waste Water Eradication Service	1.48	1881.0	1723.8	1626.5	8.4	13.5

Table 1 Percent efficiency of zinc ion removal from waste water by A. halophytica (1.16 mg dry wt./ml) incubated with the waste water for 5 hours and S. platensis (1.37 mg dry wt./ml) incubated with the waste water for 2 hours.

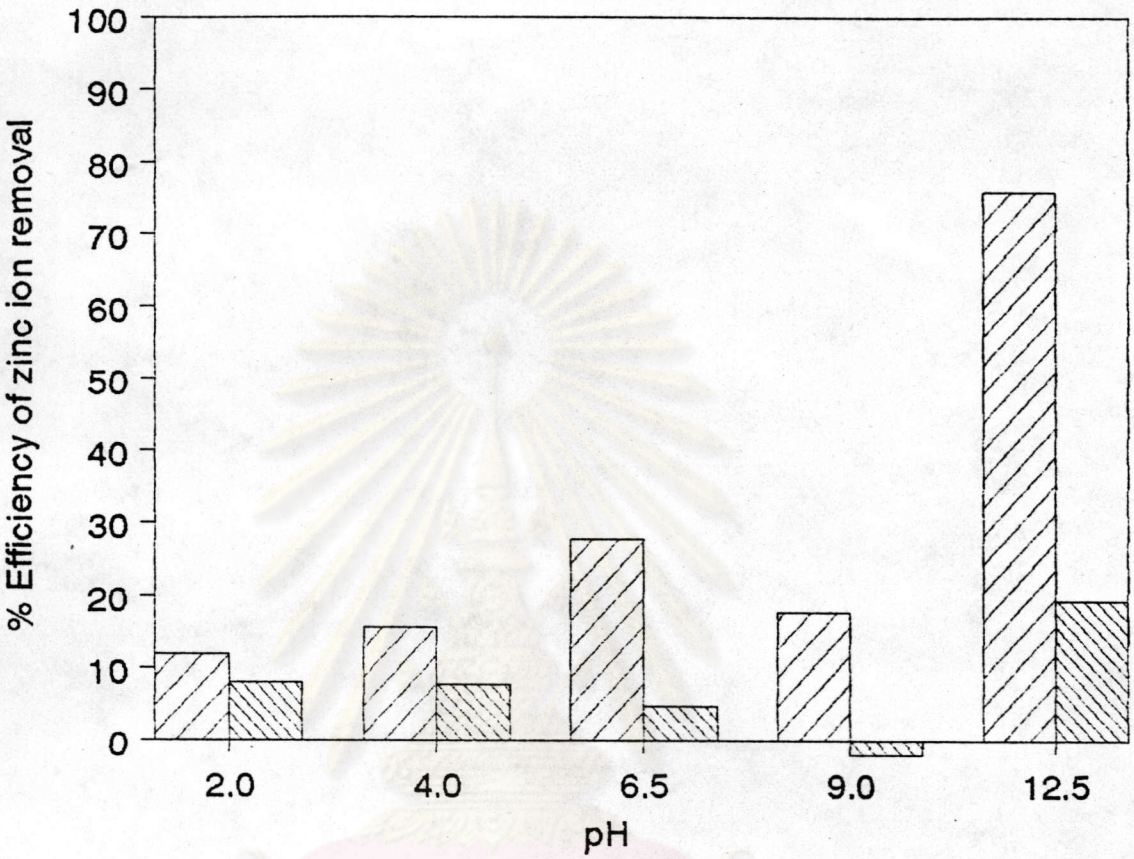

Figure 38 Percent efficiency of zinc removal at various pH from Samart Engineering Co. Ltd. The pH of the original waste water (pH 12.5) was adjusted by HCl. Cells of A. halophytica (▨) at 0.69 mg dry wt/ml were incubated with the waste water for 3 hours whereas cells of S. platensis (▧) at 1.28 mg dry wt/ml were incubated with the waste water for 2 hours.

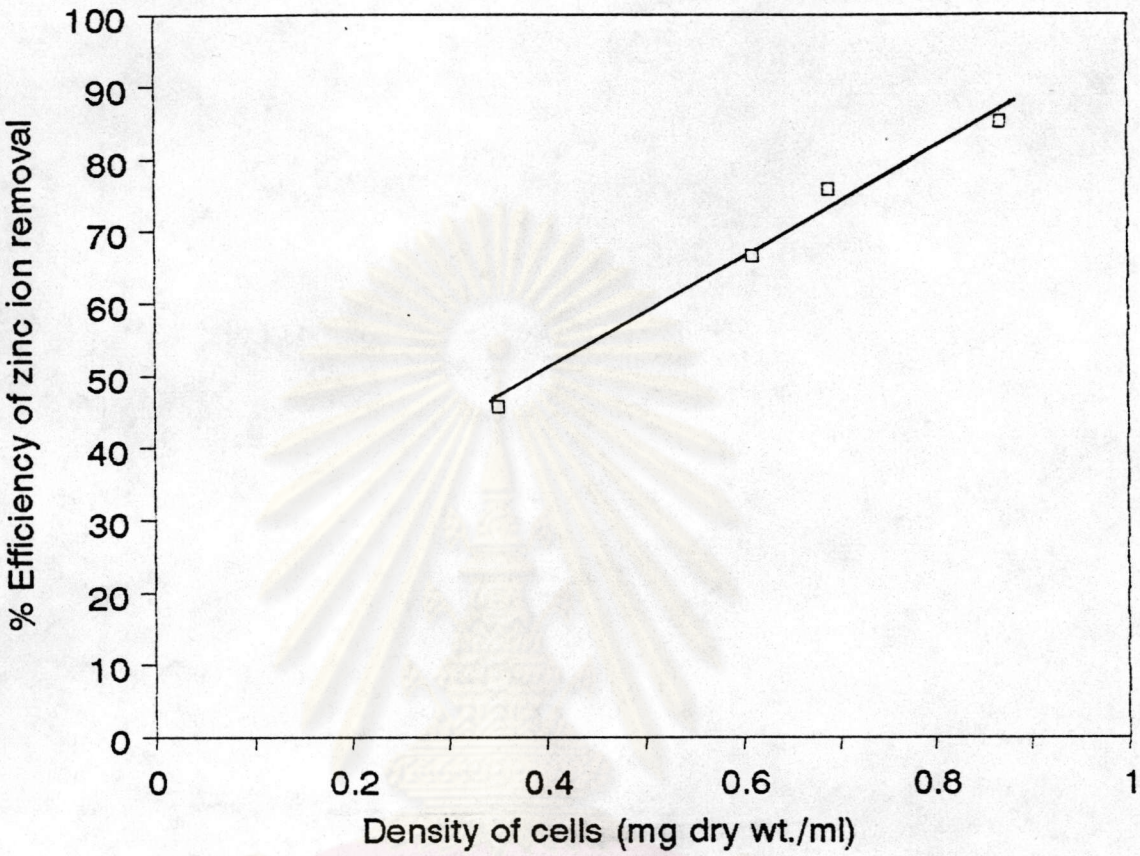

Figure 39 Percent efficiency of zinc removal from Samart Engineering Co. Ltd. (pH 12.5) by A. halophytica at various cell densities. Cells were incubated with the waste water for 3 hours.

reutilization experiments. It completely lost the ability of zinc accumulation after washing by EDTA. This was reasonable since EDTA washing might remove zinc and other metal cations essential for forming ionic and coordinate bonds with lipopolysaccharide and lipoprotein. As a consequence, the lipopolysaccharide and lipoprotein which have negative charges, might be released from cell walls (Asbell and Eagon 1966, 664-671; Leive 1965, 290-296; Gray and Wilkinson 1965, 385-399).

7. Sedimentation of Cells in Waste Water

7.1 Sedimentation of A. halophytica

Figure 40a showed that sedimentation of incubated A. halophytica in lead-containing waste water occurred faster than the control. This phenomenon might be due to the coagulation process resulting from the neutralization of metal ions contained in waste water and from the high ionic strength of waste water causing the decreased distance of repulsive forces toward the particle surfaces. Eventually, the net-charge curve drops entirely into the attractive-force region, and the particles are attracted to each other by the van der Waals forces (Schroeder 1977, 137-142). Sedimentation of A. halophytica in lead-containing waste water in dark condition was a little faster than in light condition. It might be due to the floating of the cells in the light condition. These cells accumulated oxygen in their vacuoels which is

generated from photosynthesis. However, in zinc-containing waste water the difference in light and dark conditions was not observed (figure 40b). Some of A. halophytica might lyse and die, and the pellet fast sedimented. Other small particles and living cells still remained in waste water and sedimented later.

7.2 Sedimentation of S. platensis

In lead-containing waste water, S. platensis showed a brownian motion of cells during incubation and consequently these cells coagulated and formed floc rapidly whereas in the control the cells slowly floated to the surface of the medium. At a longer time of incubation, the floc grew to a larger size and sedimented to the bottom of cylinder (figure 41a). These phenomena were similar in light and dark conditions. In zinc-containing waste water, similar phenomenon occurred but the floc of cells floated to the surface of the solution (figure 41b). This different phenomenon might be due to the difference in the pH of the waste water samples ie. pH 2,23 for lead containing waste water and 12.5 for zinc containing waste water.

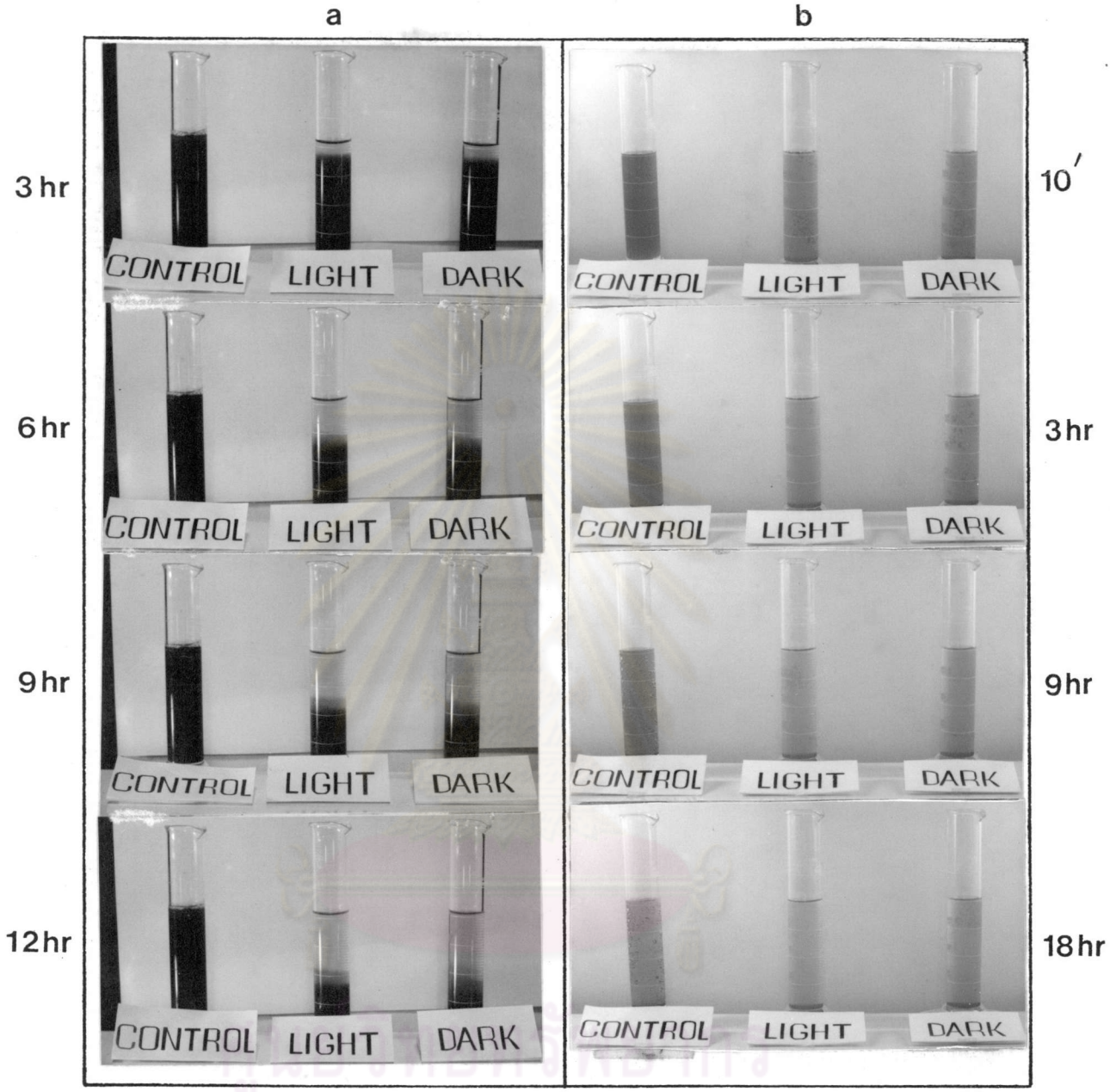

Figure 40 Sedimentation of A. halophytica at various standing times in light and dark conditions after incubating the cells with lead -containing waste water from Battery Industrial Co. Ltd. for 1 hour (a) and zinc -containing waste water from Samart Engineering Co. Ltd. for 3 hours (b).

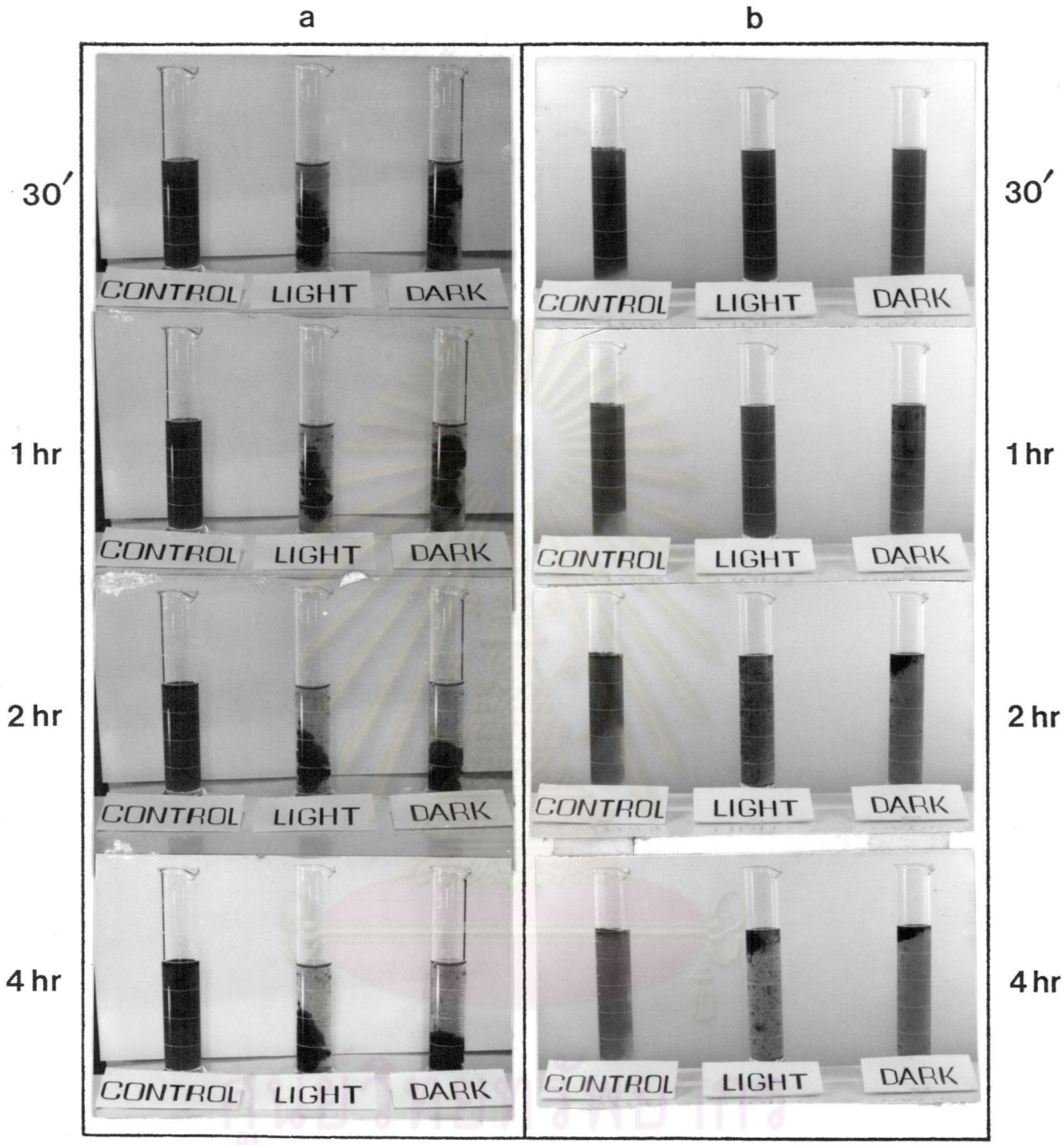

Figure 41 Sedimentation of S. platensis at various standing times in light and dark conditions after incubating the cells with lead -containing waste water from Battery Industrial Co. Ltd. for3 hours (a) and zinc -containing waste water from Samart Engineering Co. Ltd. for 2 hours (b).