An Electron Microscope Autoradiographic Investigation of the Accumulation of Zinc-65 by a Species of *Eutreptia*

Marine organisms have been found to concentrate many elements occurring in seawater in extreme dilution. Analyses of marine plants and animals have shown that zinc is accumulated[1–3] to higher levels (up to 1000 × that in their environments) than many elements more abundant in the environment and is readily transferred through marine food chains[4–6]. The uptake and accumulation of zinc by marine plants is considered to be the result of one of three possible processes: physical adsorption, ion exchange, or metabolically mediated absorption[5]. Zinc uptake has been observed to increase with increasing pH[7], increasing light intensity[7], increasing zinc concentration in the medium[8], increasing surface area to weight ratio[9], and may be related to the chemical composition of the cell wall and competition by other cations[10].

Eutreptia sp. is a single celled organism, very similar to *Euglena viridis*, which occurs in marine eutrophic waters, for example near large cities[11]. Near Port Pirie, South Australia, there is an abundance of these otherwise rare organisms living in high concentrations of zinc (~ 30 µg/ml) resulting from industrial effluent. Electron microscope autoradiography was employed to investigate the intracellular localization of zinc in the species of *Eutreptia* occurring in an environment containing levels of zinc which would be toxic to many other organisms[8]. Autoradiography localized the zinc intracellularly and was used to indicate the amount of zinc incorporated, on a comparative basis, between one organelle and another[12].

Materials and methods. Eutreptia sp. were isolated by hand from samples of the industrial effluent by micropipette and maintained in sterile seawater with $Zn^{65}Cl_2$ added (30 µg/ml total zinc concentration; 0.45 µCi Zn^{65}) for periods of 12 and 24 h. The suspension of *Eutreptia* sp. was filtered onto Millipore filters (0.45 µm pore size) and fixed with 4% glutaraldehyde followed by 2% OsO_4, dehydrated in an alcohol series and embedded in Spurr's medium – all on the Millipore filter[13].

The leakage of Zn^{65} from various fixation solutions during specimen preparation was compared: Na-cacodylate buffered glutaraldehyde; phosphate buffered glutaraldehyde; dithizone treatment (which precipitates Zn intracellularly[14]) before fixation with Na-cacodylate buffered glutaraldehyde. Na-cacodylate buffered fixative showed least loss of Zn^{65} and so specimens prepared using this fixative were used for autoradiography.

Pale gold sections were collected on carbon-coated copper grids, coated with a light carbon film, and coated with Ilford L4 emulsion using a variation of the loop method[15]: grids were arranged on round coverslips, attached by a thin strip of double-sided transfer tape and the coverslips attached to a glass rod and inverted through a dry emulsion film which had been made by dipping the open end of a straight-sided glass vial into the liquid emulsion diluted 1:4 with distilled water. Only those films which produced a gold interference colour (corresponding to a monolayer of silver halide crystals[15]) and free from flaws were used. The filmed coverslips were attached to glass microscope slides and stored in a light proof box with a small bag of silica gel at 4 °C for 50 days. The grids were developed in Kodak Microdol X developer for 5 min at 20 °C.

The resulting autoradiographs were examined in a Siemens Elmiskop I microscope and cells were scored for the number of developed grains over various organelles

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Cell No.	Total cell	Nucleus	Chloroplasts	Mitochondria	Remainder
1	17/ 801	4 (2.13)/124	5 (3·12)/181	2 (0·61)/38	6/458
2	12/ 704	—	5 (2·22)/152	2 (0·25)/20	5/532
3	33/ 962	—	11 (3·83)/117 [b]	3 (0·92)/35	19/800
4	15/1102	6 (2.49)/185 [a]	4 (2·42)/232	0 (0·39)/54	5/631
5	26/ 540	—	13 (5·09)/102 [c]	0 (0·56)/19	13/419
6	5/1019	—	3 (0·39)/96 [b]	1 (0·08)/36	1/887
7	9/ 262	6 (1.27)/44 [c]	2 (0·22)/16 [a]	0 (0·01)/ 3	1/199
8	25/ 667	—	12 (4·44)/114	0 (0·59)/25	13/528
9	21/ 382	1 (2.59)/38	1 (4·87)/82 [a]	0 (0·51)/ 7	19/255
10	20/1570	—	4 (0·71)/58 [b]	1 (0·18)/17	15/1495
11	10/ 909	3 (3.64)/393	3 (1·89)/134	0 (0·21)/20	4/362
12	7/ 955	5 (3.46)/463	1 (0·55)/127	0 (0·07)/26	1/339

Autoradiography scores: The top figure in each row is the number of developed grains over the organelle (with the expected number of grains in brackets). The last figure is the point count giving the area of the organelle in arbitrary units – this, divided by the total point count for the cell, is an estimate of relative area of that class of organelle in the cell. —, there was no nucleus present in the section; [a] significant at the 0·5–0·01 probability level; [b] significant at the 0·01–0·001 probability level; [c] significant at the < 0·001 probability level.

(nucleus, chloroplasts, mitochondria) and the relative areas of each organelle estimated from point counts[16].

Results. The data were analysed using χ^2 tests, where the expected number of grains over an organelle was

$$E(n_i) = A_i/A_i + A_j \cdot N$$

where A_i was the area of the first organelle, A_j was the area of the second organelle, and N was the total number of grains over the 2 organelles.

The hypothesis being tested was that the amount of label over the organelles was expected to be proportional to their relative areas. Because expectations in classes for individual cells were frequently less than 5, probabilities (from the information statistic) of the amount of label over the organelle compared with the remainder of the cell were calculated for each cell. Those organelles which had significant probabilities are indicated in the Table.

Examination of the data (see Table) indicated that there was little difference between the expected number of grains and the actual number of grains over the nucleus and mitochondria; the values for the mitochondria being significantly less than the expected. The organelles were compared with the remainder of the cell when values for nucleus chloroplasts and mitochondria had been extracted. The cell wall was too thin to be resolved autoradiographically and was included in the values for the 'remainder' of the cell. The number of grains over the chloroplasts was in most cases, greater than the expected number of grains. Apart from 2 nuclei, the only significant increase in concentration of Zn^{65} occurred in the chloroplasts.

Discussion. Zinc-65 autoradiographs of *Eutreptia* sp. indicated that zinc was accumulated by these single-celled algae and localized in the chloroplasts. The *Eutrepia* sp. used in this experiment were obtained from a high zinc environment and the nature of the accumulation of zinc in the chloroplasts cannot be established on the basis of these results alone. It is tempting to link the high levels of zinc-65 in the chloroplasts with the role of carbonic anhydrase, which contains 0.33% zinc[17], and which occurs predominantly in the chloroplasts.

Eutreptia sp. occurs at the bottom of the food chain, therefore its ability to accumulate and possiblyincorporate zinc at high levels is of major importance since zinc belongs to the group of heavy metals which are poisonous to most organisms when occurring in excessive quantities.

Résumé. On a montré par les techniques de microscopie électronique et de l'autoradiographie que le zinc-65 s'accumule dans les chloroplastes de l'*Eutreptia* sp.

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Boron Tolerance and Enhancement of Boron Toxicity by Chloride Ions in Alkali Sacaton during Germination of *Sporobolus airoides* Torr.

Alkali sacaton (*Sporobolus airoides* Torr.) is an important forage grass of southwestern United States. In an effort to introduce it in Pakistan, preliminary studies on its salt tolerance and cation interaction have already been reported[1]. Since soil salinity and boron toxicity are closely associated, the present work was undertaken to determine the boron tolerance and the interaction of boron with other ions of growth medium during germination of this grass.

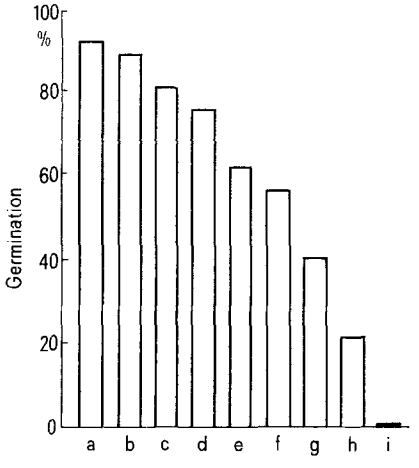

Fig. 1. Boron level in the culture solution. a, Control; b, boron-level 100 ppm; c, 200 ppm; d, 300 ppm, c; 400 ppm; f, 500 ppm; g, 600 ppm; h, 700 ppm; i, 800.

Germination tests were performed during summer season at room temperature in plastic dishes containing 200 ml nutrient solutions. Healthy seeds were selected and sterilized with 0.1% $HgCl_2$ for 1 min. These seeds were soaked for 18 h in deionized water prior to imposing treatments. 50 seeds were placed on nylon mesh and suspended over the nutrient solution in each plastic dish. The nutrient solution used was 1/8 dilution of Hoagland solution. Boron was added in the forms of boric acid in the desired concentrations. All the solutions were prepared with deionized water. Counting of the germinated seeds was made every second day from the start of the experiment to 12th day when the experiment was concluded. Germinated seeds were discarded after counting. Germination in 1/8 Hoagland solution served as control. Treatments were replicated 4 times.

1. *Boron tolerance.* Boron level in the culture solution ranged from 100 ppm to 800 ppm. Figure 1 shows that percentage of germination decreased as the level of boron in the solution increased. Reduction in germination became more pronounced beyond 500 ppm boron and the seeds virtually stopped germination at the highest concentration of boron used.

2. *Effect of various salts on boron toxicity.* In studying the interaction of various salt with boron, its concentration in the nutrient medium was maintained at 100 ppm. At this concentration of boron NaCl, KCl, $CaCl_2$ and $MgCl_2$ at 5 mEq/l were added separately to observe their effect on

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