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# UPTAKE AND LOSS OF RADIOACTIVE ZINC-65 BY TWO SPECIES OF MARINE CHLOROPHYCEAN FLAGELLATES

K. V. K. NAIR AND C. D. MULAY

*Health Physics Division, Bhabha Atomic Research Centre, Trombay, Bombay* - 400 085

ABSTRACT

Uptake and loss of $^{65}Zn$ was followed in laboratory cultures of two species of marine chlorophycean flagellates, *Dunaliella primolecta* and *Chlamydomonas* sp. In both the species the uptake of activity in the cells closely followed the population growth pattern. *Chlamydomonas* sp. removed almost all the added $^{65}Zn$ within a period of about 20 days, whereas 40% of the initial activity remained in the medium even after 28 days in the case of *Dunaliella primolecta*.

Radioactive cells lost only less than 2% of the activity when washed four times in nonactive medium. *Chlamydomonas* sp. cells resuspended in nonactive medium lost about 28% of the activity to the medium in 14 days, whereas *Dunaliella* sp. lost very little activity to the medium during the same period. With the addition of EDTA to the medium, *Chlamydomonas* sp. lost 50% of the activity within five hours and all the activity within 14 days. *Dunaliella* sp. lost 6.6% within five hours and it took 14 days to lose 50% of the activity.

At apparent equilibrium, *Chlamydomonas* sp. showed a concentration factor of 14,400 whereas *Dunaliella primolecta* showed a concentration factor of only 8,000. If the assimilated fraction of $^{65}Zn$ is only taken for the calculation of concentration factor the figures are not significantly different for both the species.

## INTRODUCTION

$^{65}$ZINC was the most predominant radionuclide in fishes after the nuclear bomb tests in the Pacific Ocean (Donaldson, 1960). The occurrence of $^{65}Zn$ has also been reported in zooplankton (Osterberg, 1964) and bivalves (Nair *et al.*, 1969). In laboratory experiments phytoplankton have shown an ability to accumulate this radionuclide to a very great extent (Chipman *et al.*, 1958). In laboratory experiments it has also been demonstrated that, for organisms in the higher trophic levels food chain is the more efficient pathway for the uptake of radioactive zinc (Baptist and Lewis, 1969).

The purpose of the present study was (1) to find the accumulation pattern of $^{65}Zn$ by two species of marine chlorophycean flagellates, (2) to follow the loss of $^{65}Zn$ from the algae under different experimental conditions and (3) to make an attempt to distinguish between the assimilated and unassimilated fractions of $^{65}Zn$ in the algal cells.

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## MATERIAL AND METHODS

Uptake of radiozinc was followed in two species of marine flagellates:(1) *Chlamydomonas* sp., a unialgal culture isolated from Bombay waters and being maintained in the Naval Chemical and Metallurgical Laboratories, Bombay and (2) *Dunaliella primolecta*, a unialgal culture originally obtained from the Plymouth Marine Biological Laboratory and maintained in the Taraporewala Marine Biological Station, Bombay. Both the cultures were grown at a temperature of 24±2°C under constant illumination with daylight fluorescent lamps. The culture medium used was natural seawater

enriched with nutrients according to the composition used by Loosanoff and Davies (1963).

The radionuclide $^{65}Zn$ was obtained from the Isotopes Division, Bhabha Atomic Research Centre and was in the form of zinc chloride in acid solution. 1 ml of this stock activity was made upto 100 ml with filtered seawater. This was allowed to stand for fifteen days and then filtered through a millipore membrane (0.45 $\mu$) and this filtrate was used as the working solution of stock activity for all the experiments. This working solution of $^{65}Zn$ in seawater had a carrier zinc concentration of 1.8 $\mu$g/ml and a specific activity of 1.8 $\mu$Ci/ml.

The experimental setup consisted of an active culture and a control medium. Millipore filtered seawater in which algal cells and $^{65}Zn$ activity were added formed the active culture. Similarly millipore filtered seawater in which $^{65}Zn$ alone was added formed the control medium. The latter was used to follow the loss, if any, of $^{65}Zn$ adsorption on the sides of the glass vessel. The cultures were grown for a period of 30 days and samplings were done every day for the first ten days and at less frequent intervals subsequently. 20 ml samples were millipore filtered (0.45 $\mu$) to separate the cells from the medium. The algal cells, filtrate and the control medium were counted separately.

Radioactivity measurements were carried out using a NaI (Tl) scintillation spectrometer. The number of cells per litre was determined from the average of two cell counts made with an improved Neubaur haemocytometer.

## Results

### *Uptake of $^{65}Zn$ by the cells*

In both the species the uptake of activity by the cells closely followed the population growth pattern. *Chlamydomonas* sp. removed almost all the added $^{65}Zn$ within a period of about 20 days whereas 40% of the initial activity remained in the medium even after 28 days in the case of the species *Dunaliella primolecta* (Fig. 1). In both the species the uptake of activity was very little during the first five days and there was a spurt in population growth and uptake of activity thereafter. In *Dunaliella* the uptake of activity as well as population growth reached near static conditions on the 26th day.

In *Chlamydomonas* the activity in the cells remained constant after the 22nd day whereas the cell number showed a decline. At this time it was noticed that the cells were fast settling on to the bottom. Significantly there was an increase of activity in the medium from the 22nd to the 29th day.

The control medium showed a reduction in activity on the second day and there was no significant change in the activity level thereafter.

Taking the activity levels in the medium and in the algae as those at apparent equilibrium conditions, the concentration factor, concentration of $^{65}Zn$ in 1 g algae/concentration of $^{65}Zn$ in 1 ml medium was calculated. The wet weight of the algae were determined by the method described by Rice (1956), except that the cells were centrifuged from the medium. The concentration factor obtained by this method was 14,400 for *Chlamydomonas* and 8,000 for *Dunaliella*.

Activity per cell was found to be maximum after four hours and showed considerable reduction thereafter (Fig. 2). The reduction in activity per cell could probably be due to a reduction in size of the cell with every division. Activity per cell was higher for *Dunaliella* when compared to *Chlamydomonas*.

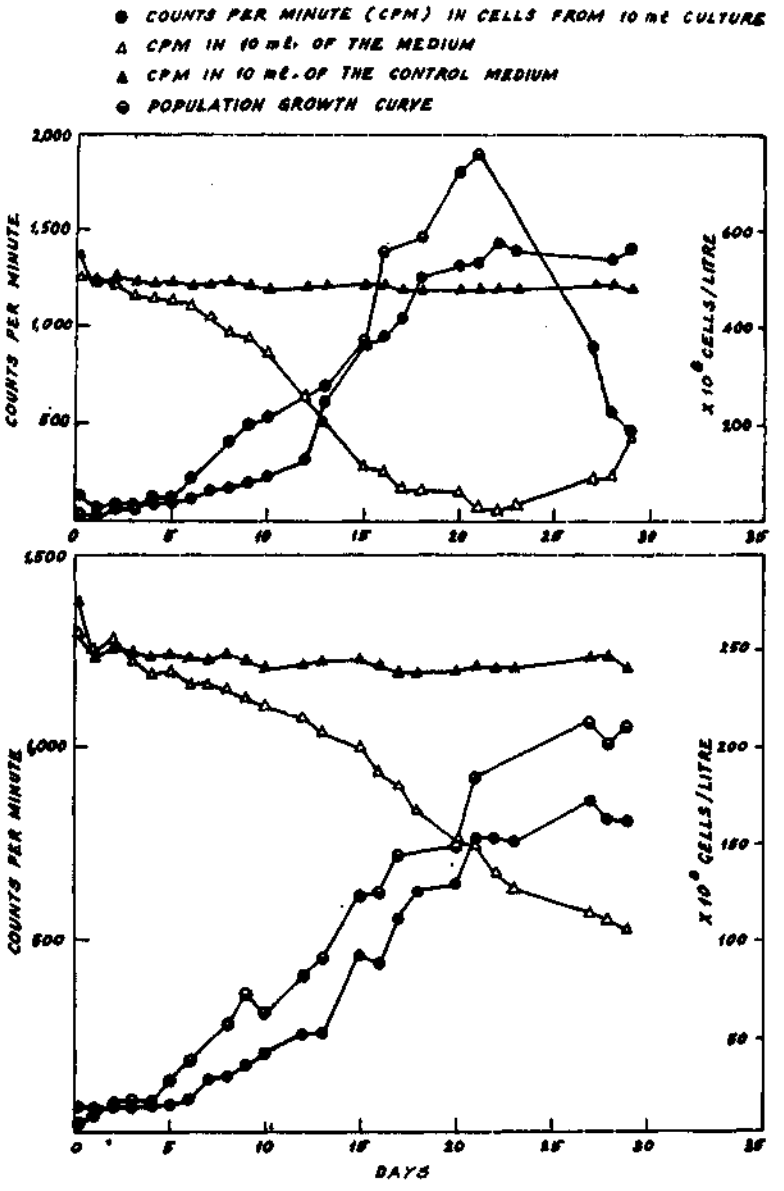


Fig. 1. Uptake of $^{65}Zn$ by *Chlamydomonas* sp. (top) and *Dunaliella primolecta* (bottom).

*Loss of $^{65}Zn$ from the cells*

Radioactive algal cells were centrifuged off from the radioactive medium and divided into three equal portions. One portion was resuspended in seawater and centrifuged at 3500 rpm for 30 minutes. Centrifugation after the addition of fresh seawater was repeated four times and the total loss of $^{65}Zn$ was found to be negligible (Table 1).

Part of the radioactive cells were resuspended in non-active seawater and the activity appearing in the medium as well as that remaining in the cells were periodically determined (Table 2).

TABLE 1. *Loss of $^{65}Zn$ from cells to medium in repeated washings*

| Washings | Percentage loss of $^{65}Zn$ to the medium | |
|---|---|---|
| | *Chlamydomonas* sp. | *Dunaliella primolecta* |
| 1 | 0.87 | 0.82 |
| 2 | 0.17 | 0.40 |
| 3 | not determined | 0.25 |
| 4 | 0.07 | 0.13 |

Cells of both the species lost about 5% each of the initial activity to the medium after 5 hours. *Chlamydomonas* sp. cells continued to lose activity to the medium and after 14 days 28% of the activity was in the medium. During the entire period of the experiment, *Dunaliella* cells lost negligible amount of activity to the medium.

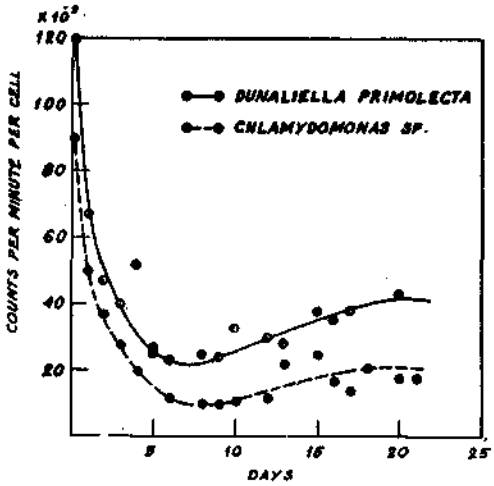


Fig. 2. Variation of $^{65}Zn$ activity per cell in *Dunaliella primolecta* and *Chlamydomonas* sp.

Seawater generally contains several natural chelating substances. As an attempt to follow the action of these chelating substances radioactive cells were also resuspended in seawater containing the sodium salt of ethylene diamine tetraacetic acid (EDTA) at a concentration of 300 mg/l. In *Chlamydomonas* sp. 5 hours after the addition of EDTA, 50% of the total activity appeared in the medium and all the activity was lost from the cells by the 14th day (Fig. 3). In *Dunaliella* only 6.6% of the activity appeared in the medium after 5 hours and it took 14 days to lose 50% of the activity to the medium.

TABLE 2. *Loss of $^{65}Zn$ from radioactive cells on resuspension in non-active seawater*

| Time (Days) | Percentage $^{65}Zn$ in the medium | |
|---|---|---|
| | *Chlamydomonas* sp. | *Dunaliella primolecta* |
| 0.2 | 5.2 | 5.3 |
| 2 | 9.5 | 3.9 |
| 4 | 11.2 | 9.0 |
| 9 | 16.8 | 4.0 |
| 14 | 27.6 | 0.0 |

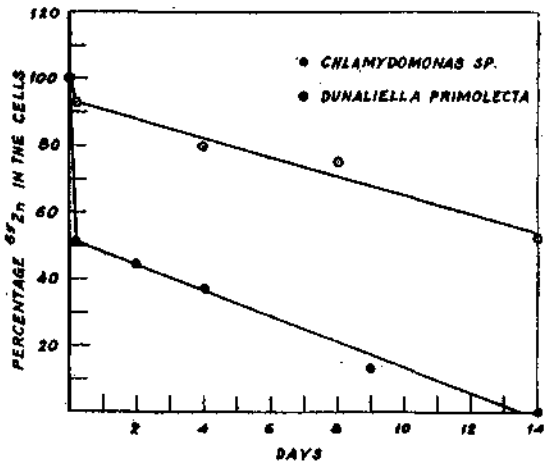


Fig. 3. Loss of $^{65}Zn$ from algal cells resuspended in seawater in the presence of EDTA.

## DISCUSSION

Thus the activity uptake pattern was broadly similar in both the species. At apparent equilibrium, *Chlamydomonas* sp. showed a concentration factor of 14,400 whereas *Dunaliella primolecta* showed a concentration factor of only 8,000. In *Chlamydomonas* sp. activity

reappeared in the medium with the ageing of the population. Thomas and Dumas (1970) observed that the protein content of the cells declines from a concentration of 250 ng/million cells to start with to a concentration of 30 ng/million cells in a 11-day old population. Proteins are known to provide the necessary binding sites for metal ions within the cells. At the beginning of the experiment probably the concentration gradient between the zinc in the medium and zinc within the cells, free ionic as well as that bound to the proteins, favours passage of zinc from the medium into the cells. With the ageing of the cell population the protein content decreases and the protein-bound zinc gets released into the cell sap. The reappearance of $^{65}$Zn in the medium at this time could be due to the concentration gradient now favouring transport of zinc from within the cells across the cell membrane into the medium.

The activity per unit cell was higher in the case of *Dunaliella primolecta* compared to *Chlamydomonas* sp. This could probably be due to the bigger size of the *Dunaliella* cells, thus providing a larger surface for absorption and adsorption compared with *Chlamydomonas* sp. cells.

The negligible loss of activity on resuspension in seawater medium shows that $^{65}$Zn is not easily exchangeable from the cells. When grown in fresh seawater medium *Chlamydomonas* cells lost 28% of the activity during a period of 14 days, whereas *Dunaliella* lost no activity from the cells during the same period. The differences in the pattern of loss of activity to the medium in these two closely related species of algae is significant. Davies (1970), while studying the role of iron in determining the growth rate of *Dunaliella tertiolecta* suggested that the initial activity appearing in the medium on addition of EDTA could be due to the metal that is sticking to the surface of the cells. The slow rate of loss of activity displayed by the algal cells thereafter might be due to the metal leached out from the assimilated pool within the cells. Thus when the linear part of the loss curve (Fig. 3) in the present instance was extrapolated to zero time one could distinguish two fractions of $^{65}$Zn, one assimilated and the other unassimilated. The assimilated fraction was 51% and 93% of the total $^{65}$Zn in the cells of *Chlamydomonas* sp. and *Dunaliella primolecta* respectively. Taking the assimilated fraction of $^{65}$Zn for the calculation of concentration factor, the figures work out to be 7,440 for *Dunaliella primolecta* and 7,344 for *Chlamydomonas* sp. Thus the quantities of assimilated $^{65}$Zn in both the species are very nearly equal whereas the concentration factors based on total concentration of $^{65}$Zn at apparent equilibrium (14,400 and 8,000 respectively for *Chlamydomonas* sp. and *Dunaliella primolecta)* differ significantly.

The relatively high accumulation factors observed for assimilated $^{65}$Zn in both the species of algae are significant both from the point of view of transfer of radioactivity through food chains Baptist and Lewis (1969) and from the point of view of heavy metal pollution of coastal waters, since elevated levels of zinc in phytoplankton cells are known to inhibit carbon fixation (Davis and Sleep, 1979).

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