J. mar. biol. Ass. India, 1973, **15** (1) : 302—305

Zn^{65} UPTAKE BY LABORATORY CULTURES OF *MICROCYSTIS LITTORALIS* AND *CHLORELLA VULGARIS**

K. VIJAYAKRISHNAN NAIR, M. C. BALANI AND S. G. SHRINGARPURE

Health Physics Division, Bhabha Atomic Research Centre, Bombay-85

ABSTRACT

Uptake of Zn^{65} was followed in *Microcystis littoralis*, a common littoral blue-green alga of Bombay and *Chlorella vulgaris*, a green alga for periods of fifteen days. *Microcystis* showed a maximum uptake value after five hours and there was an inverse relationship between the uptake and population growth patterns. Apparent equilibrium was reached in ten to fourteen days when the concentration factor was found to be 273. In *Chlorella* the uptake pattern followed closely that of the population growth and after about fourteen days the concentration of activity in the medium was considerably depleted. Zn^{65} activity in the control (filtered seawater into which activity has been added) remained fairly constant in both cases indicating that the loss of activity due to adsorption on the sides of the container was negligible.

INTRODUCTION

ZINC is now known to be a universal constituent of living matter. Of recent, the role of zinc in the food chain, both aquatic as well as terrestial has become particularly important with the introduction of Zn^{65} which is an induced radionuclide. It is reported (Chipman *et. al.*, 1958) that oysters accumulate Zn^{65} to the maximum compared to any other organism. Nair *et.al.* (1958) have found Zn^{65} to be the most predominant radionuclide in the brackishwater molluscs from Kerala, particularly in the back water clam *Villorita cochinensis*.

In the present paper, results are reported of experiments on the uptake of Zn^{65} by *Microcystis littoralis*, a common littoral blue-green alga and *chlorella vulgaris*, a green alga. *Microcystis* was chosen as a representative of the first link in the marine intertidal food chain of Bombay as larval decapods were found feeding on it in the laboratory (Sankoli, 1964).

Our thanks are due to Dr. A.K. Ganguly, Head, Health Physics Division for kindly going through the manuscript and offering helpful suggestions.

EXPERIMENTS

Microcystis littoralis and *Chlorella vulgaris* were isolated into pure cultures and used in these experiments. Both the cultures were grown in Millipore (size, 0.45 μ) filtered seawater enriched with nutrient solutions (Loosanoff and Davis, 1963). The experimental set up consisted of an active culture, the control culture and the control medium. The active culture consisted of seawater into which the appropriate number of cells and the desired amount of activity had been added. The control culture did not have Zn^{65} and was used to follow the population growth. The control medium consisted of millipore-filtered seawater into which Zn^{65} had

* Presented at the 'Symposium on Indian Ocean and Adjacent Seas — Their Origin, Science and Resources' held by the Marine Biological Association of India at Cochin from January 12 to 18, 1971.

been added. 50 ml samples of active culture were withdrawn at twentyfour hour intervals. Sampling at shorter intervals for the first twentyfour hours were carried out in the case of *Microcystis littoralis*. The algal cells were filtered on to a millipore membrane (0.45 μ), dissolved in dilute KOH and made upto 10 ml volume prior to measurement of radioactivity using a NaI (Tl) well counter and 512 channel analyser. Activity determinations were also carried out on 10 ml of the filtrate and an equal volume of the control medium. The number of cells per liter was determined from about 2 ml of the culture after vigorously shaking it. The average of four cells counts made with an improvised haemocytometer was taken as the population size.

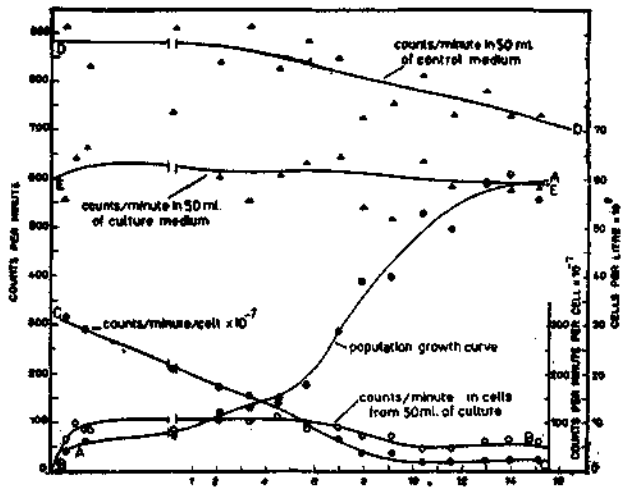

Fig. 1. Zinc^{65} uptake by laboratory cultures of *Microcystis littoralis*.

RESULTS AND DISCUSSION

The results of experiments using *Microcystis* and *Chlorella* are plotted and given in Figs. 1 and 2 respectively. In both the figures, curve 'A' represent the population growth. Curves 'B', 'D', and 'E' give respectively the results of activity determinations in the algal cells, control medium and culture medium. Curve 'C' represents the calculated value of counts per cell per minute. In *Microcystis* it is seen that the maximum uptake of activity occurred after five hours and thereafter for five days, there was no increase in the uptake of activity, although the population size was slowly increasing. From the fifth day to the 10th day the population size rapidly increased whereas the uptake curve showed a downward trend. From the 10th to the fourteenth day there was very little increase in the number of cells or the uptake of activity and during this phase the concentration factor $\left\{\frac{\text{concentration of activity in 1g of the algal cells}}{\text{concentration of activity in 1ml of the medium}}\right\}$ was found to be 273. The greatest amount of activity per cell was also at the end of five hours, thereafter there was a steady decrease till the 10th day. From the 10th to the 14th day activity per cell showed no appreciable change.

In *Chlorella*, the uptake of Zn^{65} followed closely that of the population growth, although there was a sudden spurt in population growth from the first to the fourth day which is not reflected in the uptake pattern. From the 6th to the

15th day the uptake of activity was very rapid and during this period the activity in the medium was considerably depleted. The activity per cell showed the maximum value at the end of fifteen days. The $Zinc^{65}$ activity in the control medium remained fairly constant in both the experiments indicating that the loss of activity due to adsorption on the sides of the container was negligible.

The pattern of Zn^{65} uptake by the two species of algae presents contrasting pictures. In the case of *Microcystis* although Zn^{65} was initially picked up by the cells, five days after there was a loss of activity from the cells. There is seen no appreciable depletion of activity in the medium although the population strength had increased twelve fold towards the end of the experiment. After the initial uptake,

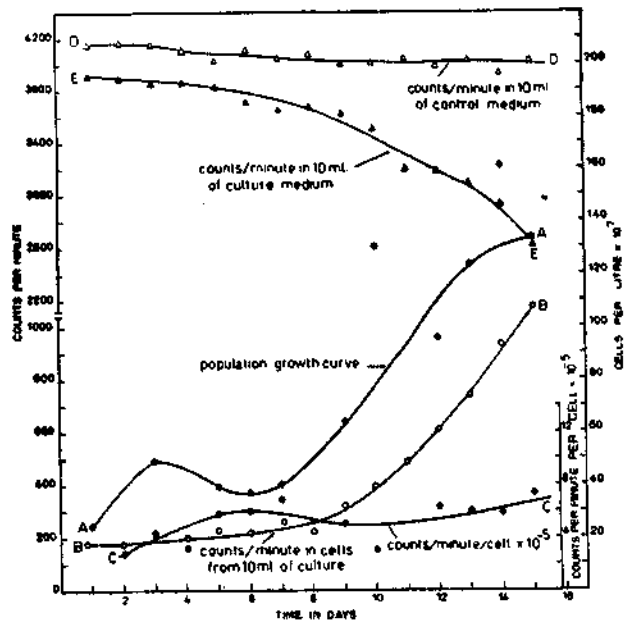

Fig. 2. $Zinc^{65}$ uptake by laboratory cultures of *Chlorella vulgaris*.

the cells do not seem to take up any $Zinc^{65}$ from the medium till the end of the experiment. This could possibly be due to the lack of affinity for zinc for this species of algae. In the case of *Chlorella* the algae seems to show a definite affinity for Zn^{65} as is evident from the increase in the uptake of activity with population growth. This is also seen from the activity per cell which is the highest towards the end of the experiment as contrasted with that of *Microcystis* in which case it was the lowest. The metabolic role of Zinc as a micronutrient in many species of algae is known. Walker (1954) has reported symptoms of zinc deficiency in *Chlorella pyrenoidosa* when grown in a medium containing less than 1 μg Zinc per litre. Borooughs *et.al.* (1957) has shown that at a concentration of 100 μg/l a culture of *Nitzschia* cells removed all the available Zinc in less than four days. Zinc is also known to be toxic at higher concentrations. Chipman *et.al.* (1958) studying the toxicity in *Nitzschia* culture has found 250 μg/l to be the lowest concentration which reduced the division rate of cells.

REFERENCES

BOROUCHS, H., W.A. CHIPMAN AND T. R. RICE 1957. Laboratory experiments on the uptake accumulation and loss of radionuclides by marine organisms. In: *The Effects of Atomic Radiation on Oceanography and Fisheries.* Publication No. 551, NAS, NRC, Washington D.C.

CHIPMAN, W.A., T.R. RICE AND T.J. PRICE 1958. Uptake and accumulation of Radioactive Zinc by Marine Plankton, fish and shell fish. *Fishery Bulletin,* 135, Vol. **58.** United States Dept. of the Interior, fish and wildlife service.

LOOSANOFF, V.L., AND H.C. DAVIS 1963. Rearing of Bivalve molluscs. *Advances in Marine Biology.* Academic press. London, **1** : 58, 59.

NAIR, K.V.K., (THE LATE) Y. M. BHATT AND G. R. DOSHI 1969. Fallout radioactivity in three brackish water molluscs from Kerala. *Curr. Sci.,* **38** (14): 332-333.

SANKOLLI, K.N. 1964. Personal communication.

WALKER, J.B. 1954. Inorganic micronutrient requirement of *chlorella.* II. Quantitative requirement for Iron, Manganese and Zinc. *Arch. Biochem. Biophys.,* **53** : 1-8.