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UPTAKE OF COBALT 60 BY *DUNALIELLA SALINA*

Riassunto — *Accumulo di Cobalto 60 in* Dunaliella salina. E' stato studiato l'accumulo del Cobalto 60 in soluzione da parte di *Dunaliella salina*, allevata in condizioni sperimentali controllate, nelle fasi di crescita comprese tra la fase logaritmica e quella stazionaria, usando tre diverse concentrazioni del radioisotopo. L'accumulo massimo nei vari periodi di sviluppo varia in accordo con le diverse dosi, ma il fattore di concentrazione risulta comunque inferiore alla concentrazione del radionuclide nelle colture sperimentali.

Abstract — The green alga *Dunaliella salina* was used to study the accumulation of radiocobalt 60 under controlled experimental conditions. Cobalt 60 was added to the growth medium of the alga at various concentrations. The maximum uptake by *Dunaliella*, from log to stationary growth phase, varied according to the doses, however the concentration factor resulted lower than the radionuclide concentration in the experimental cultures.

Key words — Dunaliella, algae, growth, Cobalt 60, uptake, accumulation.

The rapid worldwide increase in nuclear energy imposes the necessity for more accurate controls on environmental marine contamination caused by release of radionuclides as fission and corrosion products from reactor plants. Cobalt 60 (half-life = 5.24 y) is one of the most important neutron activation products of nuclear plants. As sea water is the final depository of all soluble and particulate materials released in inland water, the effects of radionuclides are evidenced in marine organisms. Since the phytoplankters are considered to be at the base of the trophic chain as primary producers, the green flagellate *Dunaliella salina* was used to study the accumulation of ^{60}Co. *D. salina* has the advantage of

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being easily cultured under controlled laboratory conditions and it can be viewed as a good representative of the primary producers. The results obtained can then give an idea of radionuclide absorption and accumulation by similar organisms in the marine environment.

Besides, one can study the radionuclide accumulation at other steps of the trophic chain by using *D. salina,* pretreated with radionuclides, as a food for other marine organisms.

Material and methods

A strain of the green alga *Dunaliella salina,* cultured for many years in the Zoology Institute of the University of Pisa was used. The alga was grown at 18° ± 1°C in continuous diffused light (4300 °K) of about 1500 lux in Erd-Schreiber culture medium. This culture medium consists of doubly pasteurized sea water to which 150 ml/l of autoclaved soil extract infusion was added. The infusion (stock solution) was prepared once for all, and stored frozen to ensure the same chemical composition for all the experiments. As it is difficult to maintain axenic cultures of the algae during the experimental procedure, expecially when grown in pasteurized sea water, each experiment was performed in duplicate: two sets of 1 l Erlenmeyer flasks were prepared each containing 500 ml of culture medium, to which ^{60}Co was added (see below). One of the sets was inoculated with algae culture while a same amount of *Dunaliella*-free old medium was added to the other. The *Dunaliella*-free old medium was obtained by filtering *Dunaliella* culture through a 0.8 μm membrane filter. Both sets were then allowed to develop in the same conditions. This was done on the assumption that the growth of contaminating bacteria in the cultures of the algae would correspond to the growth of bacteria in the algae-free culture medium. From the two sets the algae and the bacteria were separately harvested by filtration through 0.45 μm Millipore membrane and analyzed for their ^{60}Co content.

The ^{60}Co uptake by bacteria could then be measured and subtracted from the ^{60}Co uptake of coeval algae.

In all experiments, cultures of algae starting from an initial concentration of about 8.5×10^7 cells/ml reached a final concentration of about 10^9 cells/ml at the beginning of stationary phase.

Cell concentration was ascertained during the fifth-sixth day of incubation by cell counting followed by the turbidometric method (DAVIS et al., 1973; CHARLOT, 1974). Every day from the onset of incubation to the 8th day of culturing, algae and bacteria contained in 50 ml of culture medium were separately harvested. The amount of collected algae varied from 5 to 25 mg in dry weight; the bacteria varied from about zero to 1.8×10^7 cells/ml. This measure was obtained by turbidometric determination having standardized the method by cell counting of *Pseudomonas aeruginosa* grown in parallel with the unknown bacteria cells.

These amounts of cells were used in the radiometric analysis by gamma spectrometry with a NaI (Tl) well type scintillation detector coupled to a 400 channel height pulse analyzer. The analysis of stable cobalt in the Erd-Schreiber stock solution and in the untreated algae was carried out by atomic absorption spectrometry with grafite furnace (Perkin Elmer 403, HGA 76, background corrector Deuterium). The collected algae and bacteria traced with 60 Cobalt were first washed with sea water and then with 2 ml of distilled water poured down over the filter containing cells to remove salts and finally dried up to a constant weight at 90°C (MEISCH and BIELIG, 1975). The distilled and sea water used to wash the cells did not show any appreciable radioactivity when analyzed at the gamma counter.

Samples of the stock solution, algae and bacteria were separately mineralized with nitric acid plus hydrogen peroxide at 36% w/v (KRISHNAMURITY et al., 1976) evaporated almost to dryness on a sand-bath. Concentrated hydrochloric acid was added to eliminate nitric acid. Cobalt was extracted in APDC-MIBK solution at pH 2.5 (BROOKS et al., 1967; BURRELL, 1967; PAUS, 1973; KINRADE and VAN LOON, 1974; MACHIROUX and DUPONT, 1976) and determined by the method of additions of standard solutions. The sensitivity of the determination method corresponds to 0.04 ng of cobalt under the working condition with a limit of theoretical detection of about 5.10^{-12} g of cobalt (SLAVIN, 1969; PINTA, 1971; WELZ, 1976).

The radioactive cobalt tracer ($^{60}CoCl_2$ in HCl) was added to the culture medium together with algae and bacteria. The ^{60}Co, in the form of $^{60}CoCl_2$, was first dissolved in hydrochloric acid to which sea water was added to reach a Co^{++} concentration of 5 mg% (w/v) and a 0.1 N HCl solution (tracer stock solution). The values of stable cobalt found in the various systems of our study are reported

in table 1. Three concentrations of the tracer 2, 0.5, 0.2, μCi/l were used, corresponding to 1, 0.25, 0.1 ml of stock solution of the tracer. Tracer scoring was performed every day on algae, bacteria and on the cell-free culture obtained through filtration on a 0.45 μm membrane as mentioned above.

TABLE 1 - Concentrations of stable cobalt.

Concentration of stable Cobalt	μg/ml	Standard deviation	n = 6
Stock solution	0.0293	± 0.00438	
Culture medium	0.0044		
Stock solution of the tracer	0.01		
Algae	1.11 (*)	± 0.19	

(*) value in μg/g dry weight.

RESULTS

The objective of the study was to measure the uptake of ^{60}Co by the alga *Dunaliella salina* during its growth phase. To be reasonably certain that all the ^{60}Co added to the solution was actually available to the cells in the medium, we first determined whether the tracer concentration in the culture medium declines as function of time. No variation of the tracer concentration was found in 7 days period when 0.2 μCi/l of ^{60}Co was used. This appears to exclude tracer adsorption on the vial walls. In the present analysis it is assumed that no diffusion gradient of the tracer is at work in the medium and around any single alga cell. The opportunity for each cell to take up the ^{60}Co is thus dependent only on the tracer concentration. The log phase lasts about three days, and the stationary phase is reached at the sixth day. For this reason only one growth curve is reported which fits well with the growth pattern of control and experimental cultures (Fig. 1).

Three cultures of algae and of bacteria at different time were carried on to measure the ^{60}Co uptake for each dose of the tracer. Every day a batch of algae and 2 ml of cell-free medium were taken from the growing cultures and analysed as described in Material

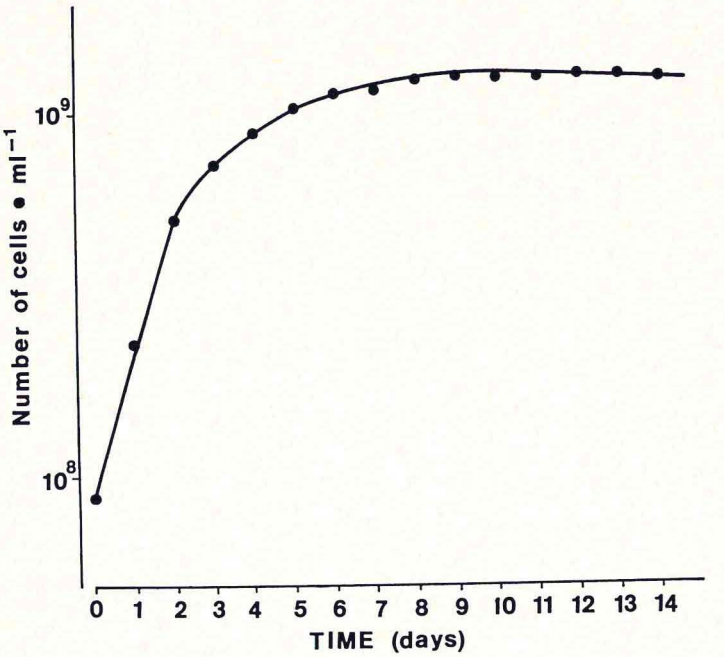

Fig. 1 - Growth of *Dunaliella salina*. Each point represents the mean of three tests in which the variation has been $\leqslant 10\%$ in number of cells ml^{-1}.

and Methods. At the same time bacterial cultures prepared as already explained (see Material and Methods) were similary analysed.

The uptake of ^{60}Co by bacteria varied according to the tracer and bacteria concentration in the cultures. The maximum uptake (12 dpm/mg of bacteria) was observed at the highest tracer concentration at the 8th day of culturing when bacteria had reached the highest growth level (1.8×10^9 cells/ml).

Whathever the value measured in bacteria, it was subtracted, as already mentioned, from the corresponding daily uptake by the algae. In this way the net uptake of ^{60}Co by the algae was determined, and the data, pooled together for each dose, are reported in Fig. 2. The uptake of samples taken immediately after tracer addition corresponds to the zero time values. In the figure the variation of ^{60}Co concentration in the medium during algae growth is also reported. The pH of algae cultures was found to gradually increase from 7.2 at inoculation time to 7.8 at the end of the experiments.

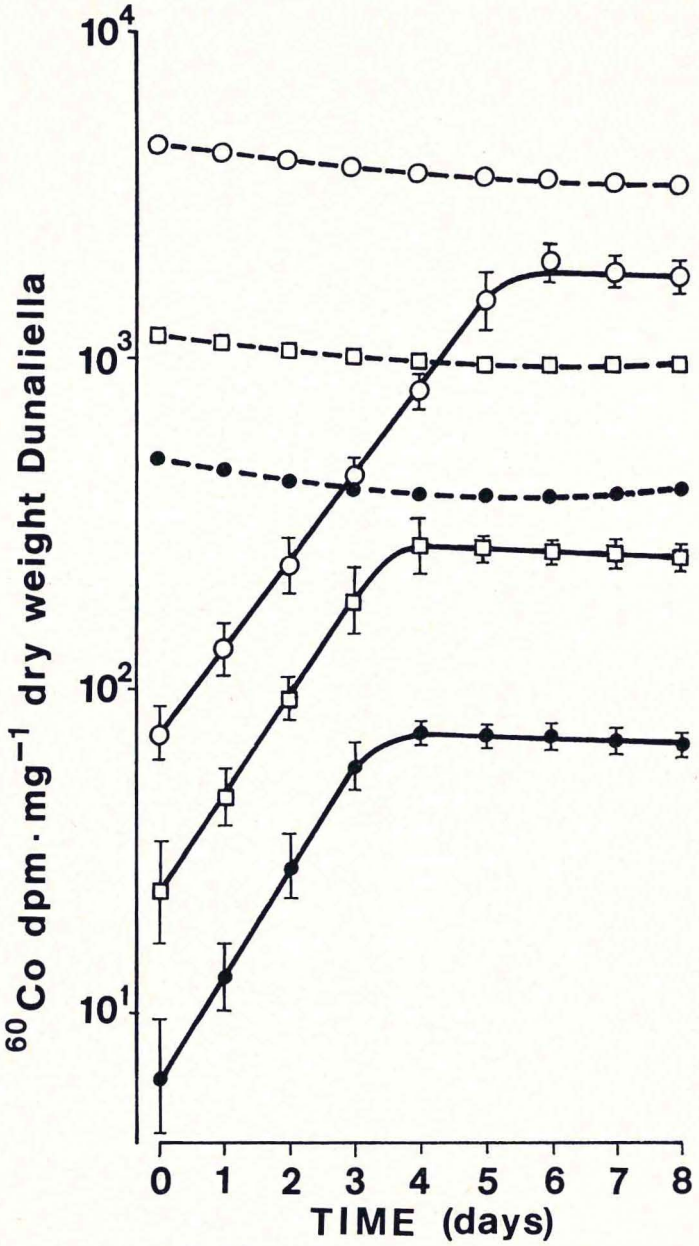

Fig. 2 - Uptake of ^{60}Co by *Dunaliella salina* and its variation in the medium at three concentrations (0.2, 0.5, 2 μCi/l).
Continuous lines: uptake of ^{60}Co by *Dunaliella salina* (dpm mg^{-1} dry weight) with standard deviation (n = 3). Dashed lines: variation of ^{60}Co in the medium (dpm ml^{-1}). Each point represents the mean of three tests in which the variation has been $\leqslant$ 10% in dpm ml^{-1}. Filled circles: 0.2 μCi/l, squares: 0.5 μCi/l, open circles: 2 μCi/l.

DISCUSSION

The first fact we like to underline points to the absence of any gross toxic effect of the radiocobalt on the algae at the doses used, as growth curve of the experimental cells matches that one of the control cells. The cells can take up cobalt, as shown by the data reported in Fig. 2, when it is added as radioactive cation to the culture, although it is not clear yet the way it enters. The uptake at its maximum value varies according to the dosages with an increase of 11, 13, 27 folds when concentration in the culture medium passes from 0.2, 0.5, 2 μCi/l respectively. Such an increase however does not parallel the ^{60}Co concentration that augments 10 times vs 2.5 times of the uptake increase if the minimal and maximal dosages are compared. Smilarly the concentration factor (CF)

$$CF = \frac{\text{Radioactivity of species (dpm/mg dry weight)}}{\text{Radioactivity of sea water (dpm/μl)}}$$

calculated at the 7th day of the culture varies from 170 to 520 with a 3 folds increase vs 10 folds increase of ^{60}Co in the culture medium. These data show that the accumulation of radioactive cobalt into the algae is dose and time dependent, although, under the present experimental conditions, the algae can partly regulate the uptake as the CF values indicate. Cobalt is an essential metabolic element which the algae cannot dispense with, while its toxic effect remains to be demonstrated. Therefore the existence of a subtle regulatory mechanism of the uptake by algae was expected for the concentrations used. Variable CF value of ^{60}Co uptake are reported by NAKAHARA et al. (1975) for different algae, subjected to higher doses of the tracer. In spite of the species specificity for differential cobalt uptake, clearly evidenced in the paper of NAKAHARA et al. (1975) (see also VAN WEERS, 1975), the presence of regulatory mechanisms operating at unknown steps is to be assumed.

The rate of ^{60}Co uptake at three different doses shows an initial rapid increase followed by a shorter period of uptake deceleration and a final stage of steady state. The exponential uptake, which lasts 3 and 5 days in the lower two doses and the higher dose respectively, fits well with a kinetics of first order ($y = a.e^{bx}$) with an exponential value of $e^{0.80x}$ and $e^{0.60x}$ for the former two and the

latter one respectively. The comparison of the ^{60}Co uptake at the two lowest concentrations with the growth pattern of the algae shows a good agreement among the three curves (Fig. 1 and Fig. 2, the two lower curves), which might be purely coincidental. However, the ^{60}Co uptake at the highest dosage which reaches the steady state about 2 days later with respect to the corresponding stage at lower doses, and with a lower exponential value, does not parallel the growth curve. This fact indicates that the rate of ^{60}Co uptake can be different according to the doses since these are the sole variable in the three experimental cultures. It coud be that by increasing the ^{60}Co concentration of ten folds, and consequently that of the carrier, in the algae-culture: (1) the physicochemical state of cobalt may be so modified that the rate of uptake by the cells comes to vary when compared to the uptake at lower doses; or (2) the expenditure in energy, due to the two contrasting forces of passive accumulation and regulatory mechanisms within the cells, results in a slow down of the uptake rate that also delays the reaching of the steady state. Regardless the uptake kinetics, should the radioactive cobalt be considered as a relatively good monitor of total cobalt uptake (NAKAHARA et al., 1975), then one would conclude that *Dunaliella* is able to concentrate cobalt at higher level than that its metabolic need requires (Fig. 2), at least up to its stationary phase. The steady state, corresponding to a saturation state different for each dose, shows a small decrease with time. This « leaking » occurring at all three doses is evidenced also by the concurrent increase of radioactivity in the cell-free fluid. This phenomenon may be ascribed again to regulatory mechanisms tending to diminish the amount of cobalt in the cell or to a lower uptake by cells at the stationary phase which does not compensate the release of the cation caused by the cell death. According to our results, *Dunaliella* has to be considered a good laboratory tester for environmental cobalt pollution and it may provide to be an useful tool for research of radioactive cobalt through the trophic chain.

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