

\*Variations in algal sensitivity to metals

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Abstract

A radiotracer study of the accumulation and toxicity of Cd, Zn, Ag, and Hg in four marine phytoplankters showed that varying algal sensitivities to these metals was in part attributable to the varying metal reactivities of the different cell surfaces but primarily attributable to the extent of safe accommodation or storage of metal within the cells.

Résumé

Une étude avec radiotraceurs de l'accumulation et de la toxicité du Cd, Zn, Ag, et Hg pour quatre espèces phytoplanctoniques a montré que les différentes sensibilités algales à ces métaux étaient en partie attribuables aux variations des réactivités métalliques pour les différentes surfaces cellulaires, mais qu'elles étaient dues avant tout à une séquestration, par sécurité, de ces métaux dans les cellules.

There are numerous reports which document the very wide range of sensitivity of phytoplankton - both marine and freshwater - to metals. There is both considerable variation in toxicity of different metals to a given algal cell and in sensitivity of different algal species to a given metal. Previous studies have identified two possible mechanisms for the latter observation: differential metal accumulation between sensitive and resistant cells (see, for example, Foster, 1977) and sequestration of cellular metal in harmless sites within the cell (see, for example, Daniel and Chamberlain, 1981). However, to date there have been no systematic studies to compare the varying sensitivities of different algal species to different metals and at the same time to monitor metal accumulation in these cells. We have therefore conducted a series of laboratory culture experiments, using gamma-emitting radiotracers, to examine the accumulation and toxicity of Cd, Ag, Zn, and Hg in four marine phytoplankters, including a diatom, a green alga, a coccolithophore, and a filamentous blue-green alga (cyanobacterium) (Fisher *et al.*, in press).

The results indicated that accumulation of all metals always proceeded by passive adsorption; in general accordance with Freundlich adsorption

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isotherms. Volume/volume concentration factors (VCFs) computed at equilibrium ranged from  $3 \times 10^2$  for the diatom to  $9.5 \times 10^4$  for the coccolithophore (Table 1). Toxicity, determined by depression of log-linear cell division rate ( $\mu$ ), was examined as a function of external and cellular metal concentration. In both cases, and for all metals and species, toxicity was best described as an exponential function of metal concentration:

$$y = 100 - ae^{bx}$$

where  $y = (\mu_{\text{treated}} \div \mu_{\text{control cells}})(100)$  and  $x = \text{metal concentration (log M for dissolved ambient, log mol cell}^{-1} \text{ for cellular)}$ . When expressed as a function of ambient metal, the order of accumulation and toxicity was  $\text{Hg} > \text{Ag} > \text{Zn} \geq \text{Cd}$ . On a cellular basis, Hg was the most toxic to all species while no clear trend appeared for the other metals.  $\text{EC}_{50}$  values, or those metal concentrations in the cells which depressed  $\mu$  by 50%, ranged from  $7.9 \times 10^{-5}$  M for Hg in the blue-green to 0.2 M for Zn in the green alga. In comparing the algal response to cellular metal (on a weight basis) with metal levels in natural phytoplankton in the Mediterranean and the Pacific, it was found that natural cellular levels of Cd are  $7 \times 10^{-4}$  the lowest  $\text{EC}_{50}$  observed. Ag is  $5 \times 10^{-3}$  the lowest  $\text{EC}_{50}$ , Hg is  $\sim 10^{-2}$  the lowest  $\text{EC}_{50}$ , and Zn is  $\sim 5 \times 10^{-2}$  to  $10^{-1}$  the lowest  $\text{EC}_{50}$ . Thus, only in very contaminated coastal waters would cellular concentrations of these metals be expected to approach levels toxic to marine phytoplankton.

Table 1. Concentration factors (VCFs)( $\times 10^3$ ) and sublethal toxicity ( $\text{EC}_{50}$ ) of four metals in four marine phytoplankters. Cellular  $\text{EC}_{50}$  values are normalized on a cellular volume basis ( $-\log \mu \text{ mol metal } \mu \text{m}^{-3} \text{ cell}$ ).

	Cd		Zn		Ag		Hg	
	VCF	$\text{EC}_{50}$	VCF	$\text{EC}_{50}$	VCF	$\text{EC}_{50}$	VCF	$\text{EC}_{50}$
<u>Thalassiosira pseudonana</u> (diatom)	0.3	16.7	12	16.8	34	16.6	93	18.6
<u>Dunaliella tertiolecta</u> (green)	1.0	16.8	10	15.7	13	16.2	32	16.9
<u>Emiliana huxleyi</u> (coccolithophore)	0.4	16.7	4.6	16.7	24	17.8	95	18.8
<u>Oscillatoria woronichinii</u> (blue-green)	1.0	nd	5.2	16.6	66	18.0	76	19.1

Dunaliella tertiolecta, the green alga, was the most resistant species to Zn, Ag, and Hg. In part, its greater resistance was attributable to metal exclusion, as it had 2 to 5 fold lower VCFs for Ag and Hg than did the other

species (Table 1). However, *D. tertiolecta* was capable of tolerating cellular metal concentrations of up to 10 (Zn) or 100 (Ag and Hg) times those in the other species (Table 1), indicating safe accommodation of cellular-bound metal. This hypothesis of safe accommodation of cellular metal is consistent with the "threshold" response of the cells to cellular metal concentrations, as reflected in the exponential equation which described  $\mu$  vs. metal concentration. Thus, it appears that differential metal sensitivity among algal species is primarily a function of the degree to which the different cells can safely sequester - chemically or physically - the metal within the cell.

Mechanisms of detoxification of cellular metal may include precipitation of metal sulfide, binding of metal by metallothionein-type proteins, and metal inclusion in intracellular vesicles.

#### REFERENCES

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- FISHER, N.S., M. BOHÉ and J.L. TEYSSIÉ. Accumulation and toxicity of Cd, Zn, Ag, and Hg in four marine phytoplankters. *Mar. Ecol. Prog. Ser.* 18: 201-213 (1984).
- FOSTER, P.L. Copper exclusion as a mechanism of heavy metal tolerance in a green alga. *Nature* 269: 322-323 (1977).

#### Discussion

A. BOLOGA: Have you used contaminated phytoplankton for food chain experiments?

N. FISHER: Yes, we frequently conduct experiments in which we feed radio-labelled phytoplankton to marine herbivores, including mussels, crustacean and gelatinous zooplankton. I would refer you to another contribution in this volume - involving mussels - as an example of this sort of work.

F. CARVALHO: Did the metals tested behave similarly to transuranium elements, in particular with respect to adsorption by cells and localization in the cell?

N. FISHER: I did not attempt to localize the sites of deposition in the cells of these metals. However, I can say that the accumulation process appeared to be strictly passive in all cases. You'll recall that all metal-treated cells were washed with  $10^{-4}$  M EDTA, so the metal we detected was either within the cells or very firmly bound to the cells' surfaces.

T. VUCETIC: Have you done some experiments with algal resting stages?

N. FISHER: No, all experiments were conducted with algal cells in the vegetative state.

S. FOWLER: What was the range of concentrations you used and those at the threshold of toxicity?

N. FISHER: The external (i.e. dissolved) range of Cd was  $4.4 \times 10^{-8}$  M to  $4.4 \times 10^{-3}$  M; of Zn from  $1.5 \times 10^{-6}$  M to  $1.5 \times 10^{-3}$  M; of Ag from  $9.3 \times 10^{-10}$  M to  $9.3 \times 10^{-5}$  M, and of Hg from  $5 \times 10^{-10}$  M. Given the different concentration factors of different metals in different species, it is most appropriate to relate toxicity to cellular metal concentrations, and compare these with metal concentrations in natural phytoplankton populations. In so doing, it was found that natural cellular levels of Cd are  $7 \times 10^{-4}$  the lowest EC<sub>50</sub> observed; Ag is  $\sim 5 \times 10^{-3}$  the lowest EC<sub>50</sub>; Hg is  $\sim 10^{-2}$  the lowest EC<sub>50</sub>, and Zn  $\sim 5 \times 10^{-1}$  the lowest EC<sub>50</sub>. Thus, in contaminated waters, one can expect Zn and perhaps Hg to reach levels toxic to sensitive algal species.