

## Effect of Mercury on marine bacterial population

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The importance of the adaptation of aquatic microbial communities in response to the exposure to certain pollutants or stress factors is largely accepted. However, this adaptation may be due to three different mechanisms: induction of specific enzymes, genetic changes that produce new physiological abilities or selection of specific organisms. In general, these changes produce a lower diversity in the population, some microbial groups disappearing whereas the population is enriched in groups with high tolerance to the environmental stress. Mercury has been one of the most studied pollutants in relation with microbial communities in recent years (1,3). The aim of this work was to study the response of a bacterial population to mercury

## Material and Methods.

We reported the effect of the addition of different concentrations of mercury (50 and 500 ppb), in the form of  $HgCl_2$ , to non-polluted seawater obtained from Málaga Bay (Spain), on the viability of the marine microbial population and the mercury resistance levels of this heterotrophic bacterial population, kept in darkness conditions, at 20°C for 48 h. Total count of viable cells was done on seawater agar. Resistant bacteria count was done on the same medium to which 10 mg/l or 30 mg/l of  $HgCl_2$  were added. The plates were incubated at 20°C, for 5 days. This experiment was repeated five times and average values of log c.f.u./ml ( $X \pm S/2$  of five experiments) at 0, 10, 24 and 48 h are shown in Figures 1-4. We used the plate count method because of its simplicity, although it has been heavily criticised as a method for estimating the number of viable bacteria in the marine environment. However, some authors (2), have demonstrated that plate count of mercury resistant bacteria is a representative index of these populations.

## Results and Discussion.

In Figure 1, it can be seen that in normal conditions, with a virtual lack of mercury in seawater, the aquatic bacterial population remained stable during all the assay, both in the total number of bacteria, and in the number of resistant bacteria. On the other hand, in these conditions of lack of the selective agent in water, approximately 10% of the bacterial population were mercury-resistant, growing in culture media with 30 mg/l of  $HgCl_2$ , and 20-30% were resistant to 10 mg/l of  $HgCl_2$ .

As seen in Figure 2, the addition of mercury leads to an important alteration in the microbial flora, producing a remarkable initial decrease in the total viable bacterial population, after the addition of 500 ppb of mercury. Although the population goes through an initial adaptation, it quickly recovers, so after 48 h, counts of more than 10 times higher than those obtained in non-stressed medium are attained. Figure 3 shows the behaviour of the mercury-resistant bacterial population when the stressing agent is added to seawater. A clear increase in the resistant bacterial population is produced, and at 48 h this exceeds the resistant bacterial population count in seawater without mercury almost by two orders of magnitude.

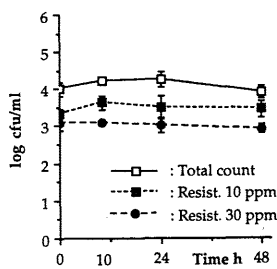


FIG. 1: SEAWATER (0 ppb Hg).

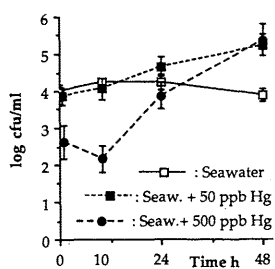


FIG. 2: TOTAL COUNTS.

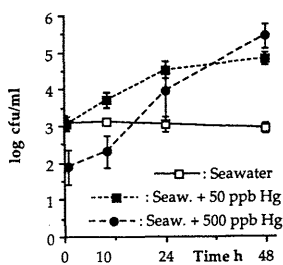


FIG. 3: Hg RESISTANT COUNTS (30 ppm).

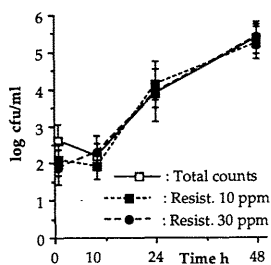


FIG. 4: SEAWATER + 500 ppb Hg.

When the population is subjected to a stress by the addition of mercury (especially when 500 ppb of mercury were added), a selection of mercury-resistant microorganisms is produced in the short adaptation phase, and then, although only 10% of initial bacterial population is resistant (as we can see in Figures 1 and 4), after 10 hours, all the heterotrophic bacterial population is mercury-resistant (Figure 4). These results seem to confirm that there is an adaptation of the microbial population in response to the external selective pressure of mercury, through the selection of the bacteria resistant to this agent, with an increase of this population from approximately 10% to 100%.

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Glutathione protects the Unicellular Marine Alga *Acetabularia* against Cadmium Toxicity

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The giant unicellular marine alga *Acetabularia acetabulum* is used in many laboratories as a model for experimental research in the field of fundamental and applied ecotoxicology (Arapis et al., 1988).

In recent studies we have found that cadmium, at concentrations  $> 0.9 \mu M$ , provoked a strong (> 50%) inhibition of cellular differentiation (cap formation) in both whole and enucleated cells (van der Ben et al., 1988a,b; Bonotto et al., 1989; Qiu et al., 1989). This response of *Acetabularia* to cadmium intoxication prompted the search for protective substances against this toxic metal.

The tripeptide glutathione, which has a protective effect against ionizing radiations (Bonotto and Netrawali, 1969) and oxidation damages, was supplied to *Acetabularia*, alone (0.1 mM) or in combination with cadmium (0.9 and 1.8  $\mu M$ ). In addition, glutathione was added to the algae two days after cadmium, to reveal whether the effects of this metal could be reversed.

All together, the results showed a strong protective effect of glutathione against cadmium toxicity. Moreover, parallel experiments with the gamma emitting isotope  $^{109}Cd$ , in combination with glutathione, suggested that the protective mechanism probably involved both extracellular and intracellular processes, leading respectively to a decrease of cadmium penetration into the cell and to a reduction of its availability for toxic interactions with cellular constituents.

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