Trace metals and the marine Bivalve *Venus verrucosa* : détection in the gills, using secondary ion mass spectrometry (SIMS)

Colette CHASSARD-BOUCHAUD* and Victor AXIAK**

*Centre de Microanalyse appliquée à la Biologie(CNRS - INSERM) Laboratoire de Biophysique, Faculté de Médecine, CRETEIL (**France**)
Marine Ecotoxicology Laboratory, Dpt.of Biology, University of Malta, MSIDA (Malta**)

Mediterranean waters are exposed to pollution by trace metals. Mussels and other bivalves readily accumulate toxicants in their tissues which reflect ambient levels of contaminants. The Mussel *Mytilus edulis* is widely used in pollution monitoring programs (e.g."Mussel Watch"). But when this species is not found in a given area, such as Malta, it is necessary to evaluate the possibility of considering another bivalve as an indicator species. The scope of the present paper was to investigate a sublittoral species,*Venus verrucosa* (Mollusca: Bivalvia), already studied concerning accumulation of petroleum hydrocarbons (1).

Animals were collected by divers from coastal waters of Malta. Gill which is the main exchange organ, was investigated using SIMS, which enables the détection and visualization of elements on histological sections (Fig.1), with a high sensitivity (2). Low mass and high mass resolution spectra (to make the distinction between mono and polyatomic ions) were obtained, indicating the presence of fluorine 19, aluminium 27, sulfur 32, iron 56 (Fig. 2 & 3), copper 63, bromine 81, silver 107 (Fig. 4 & 5), and the rare earth elements : lanthanum 139 and thulium 169.

These preliminary data have to be followed by further investigations on other tissues of *V.verrucosa*, using SIMS and the Electron Microprobe (EMP) which allows elemental détection at the ultrastructural level.

From our present data, it appears that *Venus verrucosa* could be used as sentinel organism for monitoring trace metal pollution and, moreover, may be of ecological significance in those Mediterranean coastal zones characterised by relatively low productivity.

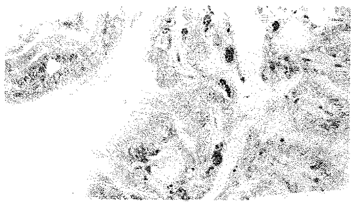

Fig.1. Venus verrucosa, gill. Photon micrograph of semithin section of filament showing epithelial cells with cilia. X 800.

Fig.2. Venus verrucosa, $^{40}Ca^{+}$ ion image showing the topography of the section with gill filaments. X 800.

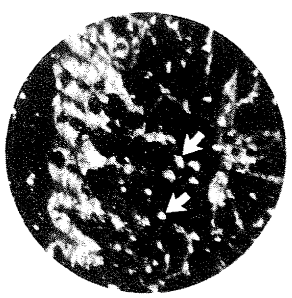

Fig.3. Venus verrucosa, $^{56}Fe^{+}$ ion image obtained from the same area as Fig.2, showing high iron emission from numerous white points of epithelial cells (arrows), X 800.

Fig.4. Venus verrucosa, $^{40}Ca^{+}$ ion image showing the topography of the section with gill filaments. X 800.

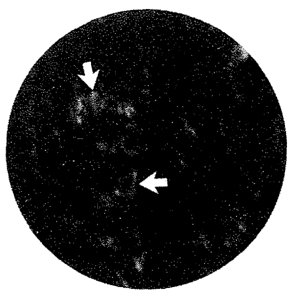

Fig.5. Venus verrucosa, $^{107}Ag^{+}$ ion image obtained from the same area as Fig.4, showing silver emission from white areas of epithelial cells (arrows), X 800.

These investigations were carried out in the framework of MED POL and a MED TRUST FUND contribution was received through the Food and Agriculture Organization of the United Nations (contract FRA 53). We thank F. KLEINBAUER for her technical assistance.

REFERENCES

AXIAK V., GEORGE J.J. and MOORE M.N., 1988. - Petroleum hydrocarbons in the marine bivalve, *Venus verrucosa* : accumulation and cellular responses. *Mar.Biol..* 97: 225-230.

CHASSARD-BOUCHAUD C., 1991. - Microanalytical techniques in toxicological investigations. In : *Ecotoxicology and the Marine Environment* (Abel PD, Axiak V eds) Ellis Horwood, New-York: 176-200.

In situ toxicity of oil and dispersants in phytoplankton communities

U. CHRISTAKI, E. PAPATHANASSIOU, E. CHRISTOU and A. MILONA

National Centre for Marine Research, Ag. Kosmas, HELLINIKON (**Greece**)

Most ecotoxicological studies quantify the effects of oil dispersants performing an analogous test with individual species in the laboratory. The extrapolation of the results from the laboratory to the actual field situation is often based on misleading results. This study, taking into account the model of a coastal ecosystem, evaluates the effects of oil and oil dispersants on phytoplankton at *in situ* conditions.

The experiment took place in June 1990 in a coastal area of East Saronikos Gulf. Three plastic enclosures (3.5m-depth X 1.2m-diameter), one with control (C1), one with oil (Arabian light- 500ml) (C2) and one with oil added dispersant (Arabian light 500 ml + BP 1lOOX at a ratio 1:lO)(C3) were used. The experiment was based on the general idea of KUIPER (1984). Sampling was done every other day. Analysis of phytoplankton and chlorophyll determinations were made while diversity and dominance indices were calculated; the concentration of oil in each sample was also determined. Univariate analysis, descriptive plotting and multivariate (MDS) analysis was used.

The salinity was almost constant while the temperature ranged from 20.9 to 22.3 °C. The oxygen measurements on selected days showed that the concentration was always near the saturation level. The total oil in water concentrations in the three enclosures ranged from 0.02 to 0.12 in C1, 3.8 to 6.1 in C2 and 64.0 to 24.5 (ppm) in C3, always descending with time. The differences in the oil concentration could be attributed to the fact that C1 is the control, in C2 the oil remained only in the surface, while in C3 the oil has been distributed in the whole water column. Chlorophyll-a ranged between 0.144 μgr/lt (C2, 13-6-90) and 0.588 μgr/lt (environment, 15-6-90). Chlorophyll-b and Chlorophyll-c values fluctuated proportionally to those of chlorophyll-a. The pigment index in polluted enclosures showed some extremely high values ranging from 1.33 to 23.91 in the C2 and from 1.44 to 83.41, in the C3. The analysis of variance of chlorophyll-a values for the four series of samples (Environment,Cl,C2, C3) showed that not significant difference occurs between the control, polluted and environment samples.

Diversity indices ranged from 0 (C3, 13/6/90) to 3.096 (C1, 20/6/90) while dominance value ranged from 41.55 (Environment, 9/6/90) to 100 (C3, 13/6/90, 22/6/90). The very high/low dominance/ diversity values were due to a coccolithophore bloom (*Coccolithophore* sp.) and it should be attributed to the pollution effects in combination with the environmental factors and the prevailing winds.

Phytoplankton abundance fluctuated in a wide range from some thousands (2,8x103 cells/lt) to some millions (12,4x106 cells/lt). The phytoplankton cells in C1 were decreasing with time, which is characteristic of a confined population. The decrease of the number of species observed in C3was more pronounced than in C2 and C1. In C1 *Clyptomonas* sp., *Gymnodinium* spp. and *Flagellates* spp. were the dominant species.

The k-dominance curves revealed the instability of this coastal ecosystem (CHRISTAKI, 1990; CHRISTOU, 1991) and showed that C3 is dominated by one species; two days later a considerable decrease of species number was observed in C2 while the situation in C3 remained the same (Fig. 1).

Chlorophyll-a values in C2 and C3, fluctuated in a quite narrow range (from 0.1 to 0.58 μgr/lt), which suggests that heterotrophic forms (nanoflagellates, bacteria) apparently dominated the population and could be attributed to the duration of the experiment. The above observation is more pronounced in the case of C3, when cell concentrations of $12X10^6$ cells/l gave relatively low (0.365 μg/lit) chlorophyll-a values. The pigment index in polluted enclosures showed some extremely high values which can be attributed to the presence of oil in the samples in connection to the species poor to chlorophyll-a.

Phytoplankton diversity and species composition changed, in oil mesocosms immediately after the start of oil additions; an increase of temperature in the North Sea caused also a parallel increase of phytoplankton biomass (ELMGREN *et al.*, 1980). It should be mentioned, however, that relevant experiments in the North Sea and the Baltic may give differentiated results. This is expected since different environmental factors exist between northern and southern European seas. Finally visual observations after treatment of the oil with dispersant showed that turbulence was the key factor in the dispersion procedure. Thus, the oil and the mixture of oil+dispersant had an effect on phytoplankton communities and the most interesting feature of phytoplankton succession in the enclosures was the shifting of the population towards monospecific blooms which was evident and was more pronounced in the oil+ dispersant mixture.

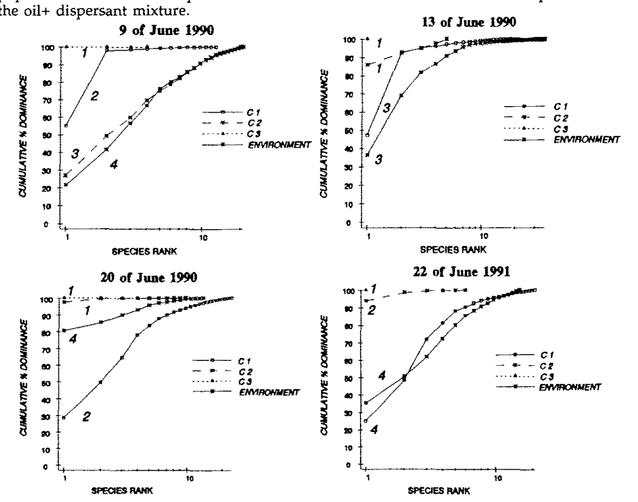

Figure 1 K-dominance curves including the species dominance. *1-Coccolithus* sp. *2-Gymnodinium* sp., *3=Flagellates* sp., *4=Cryptomonas* sp.

REFERENCES

CHRISTAKI U., 1990. - *Annual cycle of phytoplankton in a coastal area of Saronikos gulf.* M. Sc. Thesis, pp. 143 (In Greek).

CHRISTOU E., 1991. - Fluctuations of zooplankton during spring and summer in a coastal area of Saronikos gulf. *III Hellenic Symp. Oceanogr. Fish.*: 513-520.

ELMGREN R., VARGO G.A., GRASSLE J.F., GRASSLE J.P., HEINLE D.R., LANGLOIS G. and VARGO S.L., 1980. - Trophic interactions in experimental marine ecosystems perturbed by oil. *In : Microcosms in ecological research.* (edit.J. P. Giesy, Jr.) U.S. Tech. Inf. Centre, US Dept. of Energy, Symposium series 52 (CONF-781101).

KUIPER J., 1984. - The fate and effects of dispersants and dispersant-treated crude oil in marine model ecosystems with different mixing regimes. For : EEC, DG XI. Brussels. Belgium.