MONITORING THE EFFECTS OF POLLUTION ALONG SARONIKOS GULF

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Abstract

The paper deals with the simultaneous use of "biological effects" techniques in mussels (lysosomal destabilization, micronuclei frequency, stress on stress test), associated with the traditional one of the bioaccumulation of metals, in order to evaluate pollution impact on biota along Saronikos Gulf (Greece). Results were in accordance with the known pollution gradient in Saronikos gulf.

Keywords : Bio-accumulation, Bio-indicators.

Although the domestic effluents of the metropolitan area of Athens are the main pollution source of Saronikos gulf, chemical effluents from a variety of industrial installations scattered along its north coasts and the harbor of Piraeus contaminate sea water of Saronikos gulf.

The determination of contaminants in biota provides a good indication of ambient contaminant concentrations, while information about the biological harm of pollutants to the living organisms at the sub-cellular level,either the molecular or the chromosome level can be estimated with the use of "biological effects" biomarkers. The lysosomal membrane stability evaluation (LMS) has become widely accepted as a sensitive index of cellular health [1], while the increased frequency of micronuclei (MN) provide an indication of accumulated genetic damage throughout the life span of the cells and is suitable to monitor genotoxic damage [2]. Although both tests are considered as general, they are sensitive to metal impact. The stress on stress test (SOS) is based on the concept that stress is expressed by a reduced capacity of the individual to adapt to further environmental variations as the mortality in air that would presumably occur more rapidly in pollutant pre-stressed individuals than in animals coming from unpolluted areas [3].The aim of the present study was to evaluate pollution impact on biota along Saronikos, combining different methodologies: LMS, MN, SOS and the traditional one of metals bioaccumulation.

Mussels were collected seasonally from 4 localities seasonally (March 2000 to December 2004). Station C3 located in Elefsis bay is consequently more contaminated than the others. Stations C8A and C8B are located at both sides of the wastes outfall; the station C8B presenting very variable status with temporally aggravating environmental conditions, contrary to C10 that is included as reference area.

For LSM and MN tests the haemolymph was sampled from the posterior adductor muscle of 10 specimens, mixed with saline, dispensed on a slide and incubated in order to allow the cells to attach. Following that for the LMS calculation, neutral red dye was added and the slides were then examined microscopically at 15 minutes intervals. The time at which 50% of the lysosomes in the haemocytes have leached out neutral red in the cytosol was determined for each slide and a mean value was derived for each sampling site. For micronuclei the slides were fixed in methanol: acetic acid (3:1), washed in distilled water, air dried and stained with Giemsa. Two thousand cells with preserved cytoplasm were scored per mussel (per slide) to determine the frequency of micronuclei. For the evaluation of stress on stress, 30 individuals were cleaned carefully in order to eliminate epiphytes and epizoans. The sample was then partitioned in two groups of 15 individuals, enveloped in humidified straining paper, put into plastic containers with cover and subsequently kept in chambers of constant temperature of 10 °C. Survival was assessed daily and dead animals were recorded and removed until 100% mortality was reached. The LT50 was calculated graphically. Finally the determination of metals the samples, during each sampling occasion, 6 pooled samples were performed from the soft parts of 20 individuals each. The analyses of the samples comprised lyophilisation, homogeneisation and digestion of about 1.5 g of dry tissue with 12 ml of HNO_3under pressure using a microwave furnace CEM MDS 2100. Metal measurements were effectuated by atomic absorption spectrophotometry.

The LMS stability varied during time according to sampling locality. At stations C3 and C8A lower neutral red retention levels were found, indicating that mussel populations are subjected to a higher environmental stress at these sampling areas. The low number of analyses accomplished for micronuclei scoring (MN) provided limited information and mainly at station C10 for which only one analysis was made. TheT_{50} survive period during the mussels aerial exposure (SOS) showed that mussels from the reference area C10, survived longer than the specimens from the other sampling stations, while those from C3 shorter. ANOVA showed significant differences in environmental stress among stations only for SOS. However we can observe a tendency of mussels from C3 and C8A to be more stressed than those from C8B and C10 (Fig.1) presenting lower retention times, lower aerial survive and higher micronuclei frequency. In the case of MN the difference between C10 and C3 was not clear due to the limited data(only one analysis in C10). These findings were in accord with the known pollution gradient in Saronikos gulf [4]. Additionally metal bioaccumulation showed higher metal levels in C3 and lower at the reference area C10. Bioaccumulated metals in C8A and C8B were intermediate.

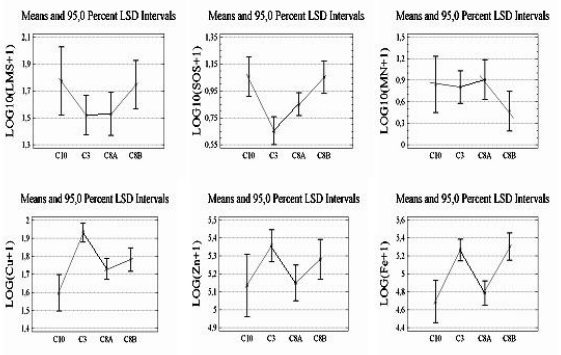

Fig. 1. Differences in studied parameters among mussels collected at the 4 sampling stations.

In conclusion, the results from the biological effects techniques applied in mussels from Saronikos gulf seem to describe well the environmental state along its coasts. Moreover these techniques are faster and consequently they are suitable for the early detection of unfavorable changes in the marine environment.

References

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