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# Genotoxicity, Catalase, and Acetylcholinesterase in the Assessment of the Pollution Status of Some Sites on the Tunisian Littoral

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The marine environment is the ultimate recipient of pollutants produced by natural and entropogenic sources. Accumulation and persistence of some toxicants as metals in the environment represent a threat to biological life, as witnessed by chronic and acute poisoning of aquatic organisms (Langston 1990 and George 1990). The study of the biological response of organisms to different environmental conditions and the quantitative evaluation of their physiological status are being considered as a successful approach for the assessment of environmental quality (Bayne et al. 1986 and Banni et al. 2001). Micronucleus test is a sensitive genotoxic test which could be considered as a biomarker for the detection of heavy metals in mussels (Nepomuceno 1997and Bolognesi et al. 1999).
Antioxydants are induced by the production of oxy-radicals in cells, as a result of oxidant mediated response. Catalase (Cat) appears to have the highest present potential as biomarker , especially in the case of hydrocarbons contamination in mussels (Livingstone;1993 and Murat Kasmas et al. 1999).
Despite their instability, organophosphorus (OP) and carbamate pesticides are a threat to the marine environment. Their main target is acetylcholinesterase (Ache), an essential enzyme for the transmission of the nerve influx (Boquene and Gagliani. 1991).

The purpose of our study was to evaluate the status of four tunisian littoral sites (Bizerte Lagoon: Menzel Jemil; Faroua, Tunis lake and Tunis canal) using micronucleus test, catalase activity and acetylcholinesterase activity as biomarkers in *Ruditapes decussatus* as well in gills, digestive gland and in the total soft body tissue for a better knowledge of the pollution source.

## MATERIALS AND METHODS

Adult specimens of *Ruditapes Decussatus* (major axis mean size 3,5 cm) were obtained from the littoral sites, immediately desiccated (Gills, digestive gland and the total soft body the total soft body tissue) and kept in liquid nitrogen until the day of experiment. A group of ten animals were randomly chosen. Control animals were obtained from a clam rearing and maintained in clean sea water for more than 2 months.

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Littoral sites of, Bizerte lagoon, Tunis lake and Tunis canal were chosen because of their economic importance in shell production.
Bizerte lagoon is a mediterranean lagoon distinguished by halin and thermal seasonal variations. The economically important lagoon is covering an area of 150 $Km^2$ and constitute a weak ecosystem because of its natural instability since water wastes are poured in from neighbouring agglomerations (urban and industrial rejects ; heavy metals, and drained pesticides coming from agricultural areas). Samples were collected from the two sites of Menzel Jemil and Faroua (Khissiba 1999 and Dellali 2000).

Tunis lake is covering an area of 30 $Km^2$ with a sea communication (Crouzet and Belkhir 1977). It is continuously submitted to the urban and industrial (heavy metals) rejects of Tunis city. Tunis canal allows the communication between the Mediterranean sea and Tunis commercial harbour with 10 Km length and an average of 5 m depth (Crouzet and Belkhir 1977). Because of the intense navigation activity along the canal, the contamination is mainly by hydrocarbons.

For determining micronucleus frequency, gills were removed and cells isolated by enzymatic digestion with a solution of 0.1 mg/ml dispase I (neutral protease, grade I, Boehringer, Mannhein, Germany) in modified (20‰) HBSS for 10 min at 37°C. The cellular suspension obtained by filtration was centrifuged at 1000 rpm for 10 min. Aliquots of the cellular pellet of mussel gills were fixed in methanol :acetic acid (3:1) for 20 min and centrifuged at 1000 rpm for 10 min. The resuspended cells were spread on slides, air dried and stained with 3% Giemsa. Four thousands cells with preserved cytoplasm per mussel were scored to determine the frequency of micronuclei observed.

For enzymatic activity studies, tissue was homogenised using a Potter homogenizer (Polytron PCU 8) in four volumes of 0.1 M phosphate buffer, pH 7.5. The homogenate was centrifuged at 9000xg for 20 min at 4°C. Supernatant was recovered and total protein content was evaluated according to Bradford's method (1976) using serum albumin as a standard.

Catalase activity was determined according to Clairbone's method (1984). Reaction mixture (final volume of 1 ml) contained 0.78 ml of 0,1M phosphate buffer (pH 7.5) and 0.2 ml of 0,5mM $H_2O_2$,. After pre-incubation , the reaction was started by the addition of 0.02 mL of the stock solution containing catalases fractions. Catalase activity was determined by kinetic measurement at 20°C using Jenway 6105 spectrophotometer ($\lambda$ : 240 nm). Results were expressed as nmoles hydrogen-peroxide transformed per min and per mg protein.

Acetylcholinesterase activity was determined according to Ellman's method (1961). Reaction mixture (final volume of 1 mL) contained 0.85 ml of 0,1M phosphorus buffer (pH 7.5) , 0.05 ml of 8mM DNTB , Sigma® and 0,05 mL of the stock solution containing acetylcholinesterase fractions. After pre-incubation, the reaction was started by the addition of 0.05 ml of 8,25 mM acetylthiocholine Sigma®. Acetylcholinesterase activity was determined by kinetic measurement at

20°C using Jenway 6105 spectrophotometer (λ : 420 nm). Results were expressed as μmoles thiocholine produced per min and per mg protein.

The standard Anova test was used for statistical analysis and results were expressed as mean values of experiments run independently ± significant deviation.

## RESULTS AND DISCUSSION

Biomarkers were chosen to detect sublethal biochemical and cellular changes in organisms exposed to toxicants. The utility of these methods lies to their ability to provide an early warning of toxicant stress in organisms and of the whole nevironment quality of a marine ecosystem. The genotoxicity in terms of micronucleus frequency was evaluated in gill cells mussel taken directly from littoral sites. Figure 1 represent the comparative frequency of micronucleus between sites. The results indicate higher frequency in Tunis Lake and Bizerte Lagoon with respect to Tunis canal.

The spatial variation of acetylcholinesterase specific activity, is reported in figure 2 , showing lower activities in Bizerte lagoon in comparison with the Tunis Lake and Tunis canal. The comparison of the catalase activity between tissues confirmed that this enzyme is essentially produced in muscles tissues (adductors muscles and intestinal tissues). Catalase specific activity assessment revealed higher values in Tunis lake and Faroua (Bizerte lagoon).

The comparative study of the catalase specific activity between tissues, reported in fig 4, 5 and 6 shows that in Tunis canal higher values are in gills indicating a contamination by hydrocarbon exposure during the period of our investigation. At the opposite in Faroua, higher values were found in the digestive gland indicating food contamination by hydrocarbons.

The assessment of the catalase specific activity in the total soft body tissue gives us a general indication about the drastic hydrocarbon contamination during the period of our investigation without specifying its origin. Ecotoxicological studies *in vivo* permits to agree a large number of biomarkers, supplying only a distorted vision of the aquatic organisms response (Viarengo 1997).

Even thought *in situ* studies doesn't show this disadvantage, two fundamental problems come up. The first is linked to the difficulty to identify the various pollutants in presence and the second concern the interference of the temporal variations of some environmental parameters like background noises (Aissa 2001). Chemical determination of pollutants concentrations in water as well in sediment may not give information about the severity of contamination, especially in the case of sublethal levels. The present study combined the use of biomarkers as sensitive approach to predict potential risk of marine contamination with the *in situ* study of four ecosystems.

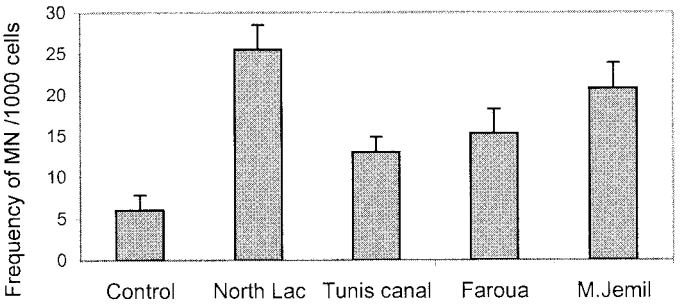


**Figure 1**. Comparison of the micronucleus frequency between sites.

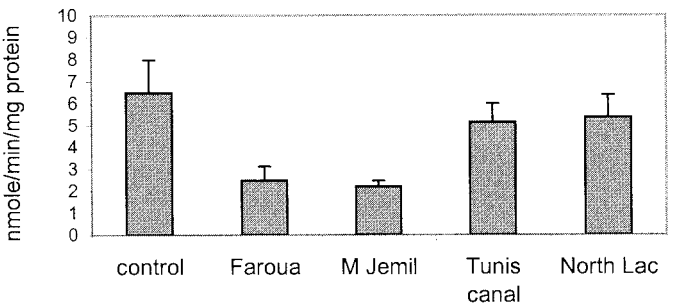


**Figure 2 .** Comparaison of the acetylcholinesterase specific activity between sites (muscles tissues ).

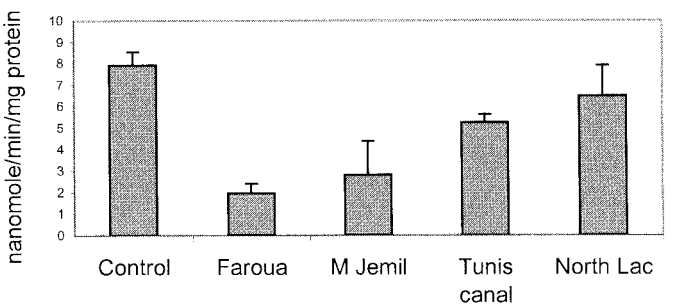


**Figure 3.** Comparaison of the acetylcholinesterase specific activity between sites (total soft body tissue).

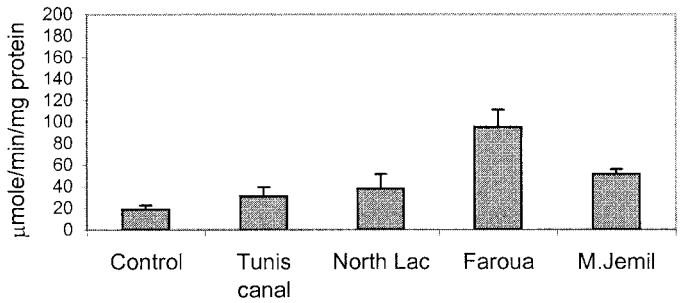


**Figure 4.** comparison of Catalase specific activity in gills

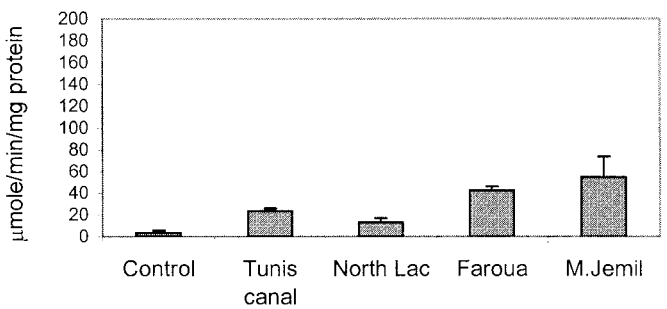


**Figure 5.** comparison of Catalase specific activity in the digestive gland

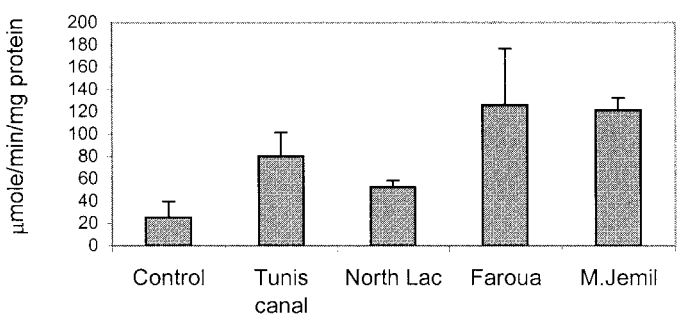


**Figure 6.** Comparaison of catalase specific activity in total soft body tissue.

As expected, the micronucleus frequency was higher in Bizerte lagoon and Tunis lake than in Tunis canal. Indeed, these two sites are continuously submitted to heavy metal contamination issued from neighbouring industry. An elevated micronucleus frequency in gills may be correlated with an increase in single strand DNA breaks as a response to Cu, Cd and Hg higher level contamination's as described *in vivo* by Bolognesi (1999).

ACHe activity seems to be inhibited in Bizerte lagoon when compared to other sites. Previous works reported that Bizerte lagoon was highly influenced by the Tinja "oued" , contaminated by drained pesticides from neighbouring agricultural areas. In addition, Bizerte lagoon is continuously submitted to insect management (Dellali 2001 and Mahamadi 2001).

Differential catalase activity assessment between tissues gives us more indications about the contamination status by hydrocarbons. The results noticed an exposure pollution in Tunis canal confirming the continuous navigation activity in this site. Conversely, Bizerte lagoon seems to be affected at a trophic level.

The major problem we could face in an *in situ* approach for marine contamination detection is the large number of compounds that could reach such ecosystem and could be able to interfere. For example, it was reported that ACHe activity could be inhibited by pesticides as well that by heavy metals (Narbonne 2001). Micronucleus could result from heavy metals contamination as well as by hydrocarbons (Lafaurie 1991).

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