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Lysossomal Membrane Stability of the brown mussel *Perna perna* (Linnaeus) (Mollusca, Bivalvia) exposed to the anionic surfactant Linear Alkylbenzene Sulphonate (LAS)

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Abstract. The effects of the Linear Alkylbenzene Sulphonate (LAS) were evaluated on the mussel *Perna perna* (Linnaeus, 1758), using a cellular level biomarker. The Neutral Red Retention Time (NRRT) assay was used to estimate effects at cellular levels. Significant effects were observed for the NRRT assay, even in low concentrations. The effects at cellular level were progressive, suggesting that the organisms are not capable to recover of such increasing effects. Additionally, the results show that the levels of LAS observed for Brazilian coastal waters may chronically affect the biota.

Keywords: Ecotoxicology; Biomarkers; Detergent; Effect; Marine Pollution; Mussel.

Resumo. Estabilidade de membranas de lisossomos de mexilhões *Perna perna* (Linnaeus) (Mollusca, Bivalvia) expostos ao surfactante Alquilbenzeno Sulfonato de Sódio Linear. Os efeitos do Alquilbenzeno Sulfonato de Sódio Linear sobre o mexilhão *Perna perna* (Linnaeus, 1758) foram avaliados por meio do uso de um biomarcador celular, o ensaio do tempo de retenção do vermelho neutro (NRRT). Efeitos significativos foram observados para o ensaio do NRRT, mesmo em baixas concentrações. Os efeitos ao nível celular foram progressivos, sugerindo que os organismos não conseguem se recuperar dos efeitos crescentes da exposição. Os resultados ainda mostram que níveis de LAS presentes na costa brasileira podem afetar cronicamente a biota.

Palavras-chave: Ecotoxicologia; Biomarcadores; Detergente; Efeito; Poluição Marinha; Mexilhão.

Detergents are often present in industrial and domestic effluents, due to their multiple uses. Such compounds are used worldwide as cleaning agents, participating also in the production of oil and textile products, in mineral extraction and exploration and being used as dispersants during oil spill episodes (Lewis 1991). These substances have surfactant properties, *i.e*. the molecules present a polar and a non polar regions, which allow them to reduce the surficial tension of any solution, to concentrate onto surfaces and to form ions aggregates and micelles (Abel 1974).

In the last 25 years, the anionic surfactant Linear Alkylbenzene Sulphonate (LAS) has been the most used in the composition of domestic detergents (Larson & Woltering 1995). This compound started to be produced during the 1960's decade, substituting the Alquilbeneze sulfonates (ABS) (Holman & Macek 1980). The LAS is nowadays widely spread in many different aquatic environments, where it has an important pollutant potential (Ainsworth 1992). Since the 1970's decade, investigations on the toxic effects of the LAS have been conducted with aquatic organisms, as fish (Swedmark *et al.* 1971, Barbieri *et al*. 2002, Rocha *et al*. 2007), crustaceans (Swedmark *et al.* 1971, Lewis & Suprenant 1983, Singh *et al*. 2002) and mollusks (Swedmark *et al.* 1971, Marin *et al.* 1994, Da Ros *et al*. 1995, Hansen *et al*. 1997), especially in Europe and North America.

The main effects already appointed as a result from the exposure of aquatic organisms to anionic detergents are cellular, histological and physiological damages, which comprise alterations

in the fish gill tissues (Swedmark *et al.* 1971, Abel 1974, Supriyono *et al.* 1998), lysossomal disturbances and enzymatic inhibition or stimulation (Drewa 1988, Da Ros *et al.* 1995), growth reduction (Hansen *et al.* 1997) and alteration of the cardiac activity (Swedmark *et al.* 1971).

The aim of this study was to evaluate the LAS effects on the brown mussel *Perna perna* (Linnaeus, 1758), by using a biomarker at cellular level, in order to contribute to the comprehension of its toxic mode of action and to the management of the LAS environmental levels. To achieve that, the neutral red retention time assay (NRRT) was used.

Adult healthy individuals of *P. perna* (41.2 – 77.3 mm shell length) were collected from a mussel farm, located at Cocanha Beach, Caraguatatuba, North Shore of São Paulo, Brazil. According to the State Environmental Agency, this site is considered clean and its waters present good quality (CETESB 2007). After collection, the animals were acclimated to laboratory conditions for 24 hours, in tanks containing filtered seawater, and kept under intense and continuous aeration, constant temperature (25 ± 2º C) and natural conditions of light.

The LAS used in this investigation consisted in molecules containing linear 12 carbon chain (according to the information provided by the producer). A 1000 $mg.L^{-1}$ LAS stock solution was prepared by the addition of pure LAS salts (Merck, PA) in distilled water. The LAS test-solutions were prepared by diluting a stock solution (50 $mg.L^{-1}$ LAS) in filtered sea water. The following LAS concentrations were prepared: 0.50; 0.25; 0.13; 0.06 and 0.03 $mg.L^{-1}$. Filtered (100 μm acetate membrane) sea water was used as control.

The experiments consisted in exposing the animals for 24h to the LAS dilutions, and the further observation of sub-lethal effects. The physical-chemical characteristics of the different tested solutions were monitored in all the experiments.

The Neutral Red Retention Time (NRRT) assay followed the method described by Lowe *et al.* (1995), using the blood of ten animals of each treatment. The neutral red retention time was obtained by estimations of the proportion of haemocytes which released the neutral red dye to the citosol and/or by anomalies in the size and color of lysosomes and the cells shape. When 50% or more cells exhibited alterations, the time interval was recorded. The mean retention time was calculated for each concentration and the comparison to the control was made by analysis of variance (ANOVA) followed by the Dunnett test (Zar 1984).

In the experiment, the physical-chemical characteristics of the tested dilutions showed very small variations: salinities ranged between 33 and 35 ‰, temperatures ranged from 23 to 25 ºC and pH ranged between 7.60 and 8.08.

The mean NRRT was significantly lower ($p < 0.05$), in comparison to the control, for the organisms exposed to 0.5; 0.25; 0.13 and 0.06 mg l^{-1} LAS (Fig. 1). The decreasing of the dye retention time was directly related to the increasing of the LAS concentration, indicating that this compound damages the membranes. For the smaller concentration (0.03 mg l^{-1}), the NRRT was not significantly different from that observed in the control.

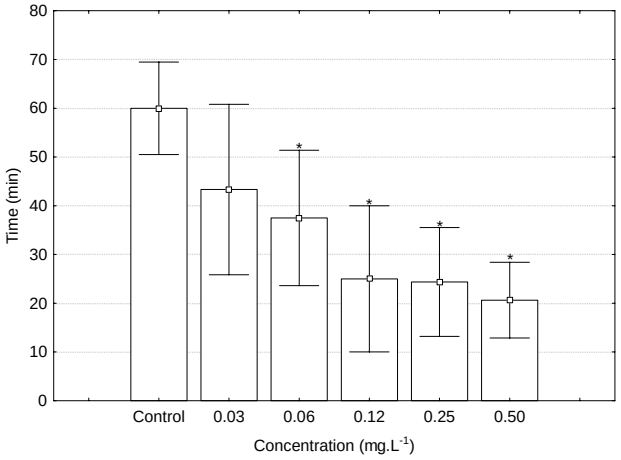

Figure 1. Mean NRRT (minutes) in haemocytes lysosomes of the mussel *P. perna* (Error bars indicate standard deviations and * indicates significant differences, $p \leq 0.05$).

The fragility of lysosomal membranes is quantitatively related to the level of stress induced by the xenobiotics, once the lysosomes are involved in many essential cellular processes, representing the main organelle responsible to remove toxic compounds from the citosol (Moore 1982, 1985). In mollusks, lysosomes play a key role in the process of mediating enzymes for the catabolism of endogenous and exogenous material, and in the regulation of the cell homeostasis, by fagocitosis, digestion, depuration and excretion of xenobiotics (Moore 1985, Viarengo *et al.* 1987, McVeigh *at al.* 2006). As consequence of the accumulation of contaminants in the lysosomes, alterations and damages in the lysosomal membranes may occur, releasing thus the digestive enzymes to the citosol and producing increased autolytic activity (Moore 1985, McVeigh *at al.* 2006).

Due to the tensoactive properties of detergents, the biological membranes can be considered main targets of such compounds; becoming thus useful models to evaluate the sub-

lethal effects of this kind of substance, in special if the membrane stability is considered.

The results obtained in the present study may be explained, at least partially, by the incorporation and accumulation of the detergent into the lysosomes (Bragadin *et al*. 1996). As consequence of the depuration process, free species of oxygen can be produced; consequently, alteration in the concentration of antioxidant enzymes may occur, as observed by Da Ros *et al*. (1995) in mussels exposed to LAS. The resulting lipoperoxidation may affect the stability of the biological membranes (Torres *et al*. 2002, Gorinstein *et al.* 2003), as observed in the present study.

Another factor which could be involved in the fragilization of the cell membranes is the structural modification of their proteins and phospholipids, caused by the anionic surfactant. The detergent is a molecule capable to bind to lipids and form micelles, thus it can bind to the phospholipids and destabilize and/or break the biological membranes. This process is known as *binding membranes* (Cserháti *et al.* 2002).

However, the increasing responses as result of LAS exposure are not always observed. Marin *et al*. (1994), in a study on the effects of LAS contaminated sediments on the mussel *Mytilus galloprovincialis* (Lamarck, 1819), observed a not significant increase in the respiratory rate of the exposed organism, whereas other studies with fishes evidenced the occurrence of effects in the animals exposed to low LAS concentrations (Barbieri *et al*. 2000, Barbieri *et al.* 2002). Depledge & Andersen (1990) and Depledge *et al*. (1995) mentioned the capability of marine organisms to compensate, temporarily or partially, the effects of contaminants by physiological mechanisms. However, if the alterations in biochemical, cellular and histological levels keep growing or remain constant for longer periods, the animals frequently cannot maintain such compensatory mechanisms, and then the physiological effects become apparent (Drewa 1988, Hofer *et al*. 1995, Swedmark *et al*. 1973).

In Brazil, minor importance has been given to the presence and discharges of detergents in the aquatic environment, although the use of such compounds is increasing all along the country. The lacking of information on their toxic effects on the biological communities represents a problem to the establishment of regulations and to the determination of maximum concentrations for the effluents discharged into the environment.

The results obtained in the present study showed that the significant LAS effects on mussels occurred in concentrations lower than those found by Mastroti *et al*. (2001) for sea urchin embryos; in that study, significant effects were observed from 0.2 mg L^{-1} LAS. Mastroti *et al*. (1998) reported that the levels of LAS in estuarine and marine waters of São Paulo ranged between 0.03 and 2.08 mg L^{-1} with exceptional values reaching 8.47 mg L^{-1}, thus many already found values exceed the concentrations capable to produce significant effects, according to data obtained in the present study. Therefore, more attention must be given to the presence of surfactants in the environment, especially in marine and estuarine waters, once these compounds are toxic, present low degradation in salt waters (Mastroti *et al*. 1998) and are widely discharged into the coastal waters. The maximum permitted concentration of LAS in salt waters should be at least 0.03 mg.L^{-1} (or lower), in order to avoid sub-lethal effects and long-term ecological disturbances. However, further studies with more species are needed to establish legal standards for LAS in marine and estuarine Brazilian waters.

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