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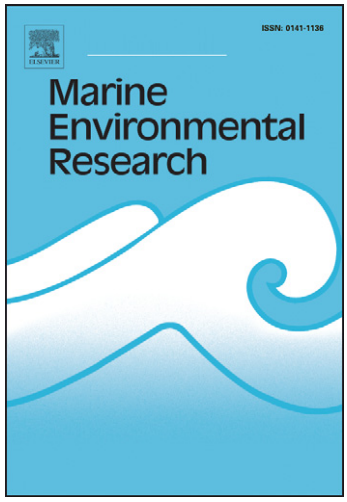

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Microcystin-induced oxidative stress in *Laeonereis acuta* (Polychaeta, Nereididae)

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Abstract

Oxidative stress induced by microcystins was evaluated in an estuarine worm, *Laeonereis acuta* (Nereididae). Ten organisms were exposed to lyophilized cells of a toxic *Microcystis aeruginosa* strain RST9501 (≈2 mg/L microcystins, MC); ten were exposed to lyophilized cells of a nontoxic *Aphanotece sp.* strain RSMan92 and ten were maintained without cyanobacterial cells. Exposure time was 48 h. The enzymatic antioxidant defenses, as well as the oxidative damage, were analyzed. Toxic and nontoxic cyanobacteria lowered catalase activity with no changes in glutathione reductase and glutathione-*S*-transferase activities. This may have led to toxin intracellular accumulation, which should favor oxidative stress generation, observed by the high lipid peroxide and DNA-protein crosslink levels in the group exposed to MC.

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Keywords: Microcystin; *Microcystis*; *Laeonereis*; Oxidative stress; Lipid peroxidation; DNA-Protein Cross-link

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Microcystins (MC), common cyanobacterial hepatotoxins, are known to inhibit PP1 and 2A enzymes and to induce oxidative stress in different organisms leading to cell damage (Pflugmacher, 2004; Pietro et al., 2006).

Anthropogenic pollution has promoted cyanobacterial toxic blooms in water bodies. Several blooms containing toxic *Microcystis aeruginosa* have occurred in the Patos Lagoon (Southern Brazil) over the last two decades with effects on the aquatic life. Physiological and behavioral changes with the death of some species have been observed; this fact may decrease the biodiversity (Yunes et al., 1998; Dewes et al., 2006).

The worm *Laeonereis acuta* has been regarded as an important bioindicator in the Patos Lagoon. Its environmental abundance and physiological characteristics, such as the ability to modulate its antioxidant defense system to several pollutants, make this worm an important tool for ecotoxicological studies (Geracitano et al., 2004).

The present study aimed to evaluate some antioxidant enzymatic parameters in *L. acuta* exposed to acute MC levels, as well as oxidative damage.

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Cells of a toxic *M. aeruginosa* strain (RST 9501) were cultured and employed as a source of MC. Cells of a nontoxic cianobacterium, *Aphanotece sp.* (RSMan92), were cultured in parallel with *M. aeruginosa* cultures.

Specimens of *L. acuta* were collected in an unpolluted area of the Patos Lagoon and immediately transferred to the laboratory in an ice-cold box. After 4 days of acclimation in plastic boxes with sand and water (10‰; pH 8.00), the worms were transferred to individual recipients (10 cm diameter) containing 50 mL water (10‰; pH 8.00) and 10 g sterilized sand at a fixed temperature (20°C) and photoperiod (12L:12D).

Three groups (n=10) were tested; the first one was exposed to lyophilized cells of toxic *M. aeruginosa* (2 mg/mL) reaching ≈2 µg MC/mL, the second one to lyophilized cells of the nontoxic cyanobacterium, *Aphanotece sp.* (2 mg/mL), and the third group was the control. All tests lasted 48 hours.

According to Matthiensen *et al.* (2000), [D-Leu^{1}]MC-LR is the major MC variant produced by this *Microcystis* strain, and it presents an LD_{50} value of 71 µg/kg in mouse bioassay. In virtue of the MC concentrations used, the assays were considered acute.

For protein concentration and enzyme activity analysis, every organism was homogenized (20% w/v) in a buffer [Tris base (20mM), EDTA (1mM), dithiothreitol (1mM, Sigma), sucrose (0.5 mM), KCl (150 mM); pH 7.6]. Samples were centrifuged at 9,000 x g (4 ^{o}C) for 30 min and the supernatants employed as the enzyme source.

The following parameters were measured: activity of glutathione-*S*-transferase (GST) and catalase (CAT) (Geracitano et al., 2004); glutathione reductase (GR) (Nagalakshmi and Prasad, 2001); lipid peroxides levels (LPO) (Geracitano et al., 2004);

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and DNA-protein crosslink levels (Barker, 2005). Data were subjected to ANOVA (α=0.05).

After 48-h exposure, CAT activity was reduced ($p<0.05$) whereas GST and GR activities had no difference when compared to the control group ($p>0.05$; Fig. 1). Only the *Microcystis*-exposed group presented higher lipid peroxides and DNA-protein crosslink levels, when compared to the control group ($p<0.05$; Fig. 2).

Reactive oxygen species (ROS) are produced during several biochemical processes, thus an antioxidant system is necessary to protect the organism. CAT is an enzyme that catalyzes H_2O_2 (precursor of $HO^{\bullet}$, the most toxic ROS) degradation. GST catalyzes the conjugation of oxidized products with reduced glutathione (GSH). GST also catalyzes the detoxification process through the conjugation of MC with GSH, whereas GR catalyzes the regeneration of GSH from GSSG (oxidized glutathione) (Pflugmacher, 2004).

In this work, MC did not activate the antioxidant system; in fact, CAT was diminished. Besides, non-altered GST activity may induce MC intracellular accumulation, leading to an oxidative stress condition. Moreover, high levels of ROS such as superoxide anion may inhibit CAT activity (Kono and Fridovich, 1982). Dewes et al. (2006) have reported the rise of oxidative stress in an estuarine crab orally exposed to MC. Pflugmacher (2004) and Pietro et al. (2006) have observed MC-induced oxidative stress in the aquatic macrophyte *Ceratophyllum demersum* and in the tilapia fish (*Oreochromis* sp.). The absence of antioxidant responses in *L. acuta* led the organism to an oxidative stress condition after acute MC exposure, as evidenced by LPO and DNA-PC damage.

Oxidative stress condition is induced by an unbalance of pro-oxidants and the antioxidant capacity in favor of the pro-oxidant. In this work, high DNA protein crosslinks

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and LPO levels were observed. Although there is no evidence of MC-induced DNA-protein crosslink damage in the literature, Zegura et al. (2004) have observed that the genotoxicity of MC is mediated by the induction of ROS production. High LPO levels caused by MC have been observed in crabs and fish by Dewes et al. (2006) and Pietro et al. (2006). The fact that those variables were altered during exposure to MC indicates that there is a common response triggered by aquatic organisms, and also suggests that not only the accumulation of MC in tissues, but also the biochemical variables should be analyzed in order to estimate the toxicological risk to populations exposed to these toxins.

Acknowledgements

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Figure Captions

Fig. 1. Enzymatic activity after 48-h exposure to 2 mg/mL lyophilized cells of toxic *Microcystis aeruginosa* (≈2 µg/L microcystins), 2 mg/mL lyophilized cells of nontoxic *Aphanotece sp.*, and absence of cyanobacteria (control). (A) catalase (CAT) activity; (B) glutathione-*S*-transferase (GST) activity; and (C) glutathione reductase (GR) activity. Data are expressed in enzymatic units (U) as mean + standard error. Similar letters indicate absence of significant differences ($p<0.05$).

Fig. 2. Induced-oxidative stress damage after 48-h exposure to 2 mg/mL lyophilized cells of toxic *Microcystis aeruginosa* (≈2 µg/L microcystins), 2 mg/mL lyophilized cells of nontoxic *Aphanotece sp.* and absence of cyanobacteria (control). (A) lipid peroxide levels (LPO); (B) DNA-protein crosslink (DNA-PC) levels. Data are expressed as mean + standard error. Similar letters indicate absence of significant differences ($p<0.05$). CHP stands for cumene hydroperoxide, employed as standard for LPO measurements.

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Figure 1.

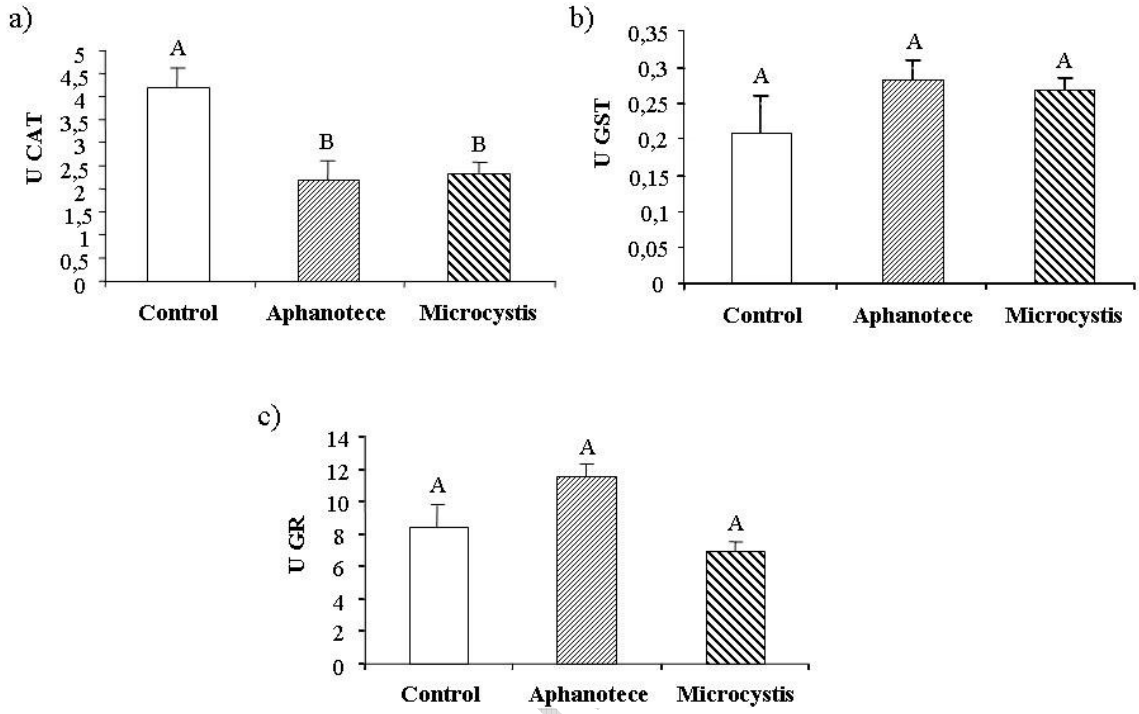

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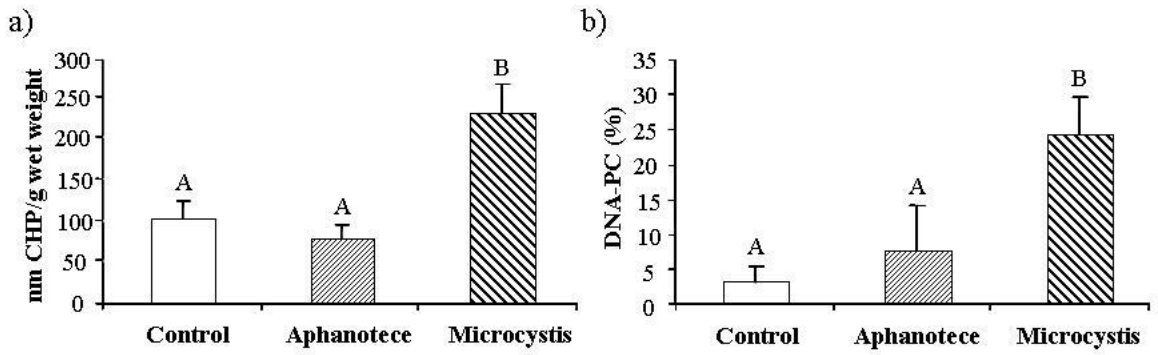
Figure 2.



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