Antioxidant Mechanisms of the Nereidid *Laeonereis acuta* (Anelida: Polychaeta) to Cope with Environmental Hydrogen Peroxide

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ABSTRACT

Hydrogen peroxide (H_2O_2) is a naturally occurring prooxidant molecule, and its effects in the macroinvertebrate infauna were previously observed. The existence of a gradient of antioxidant enzymes activity (catalase [CAT], glutathione peroxidase [GPx], superoxide dismutase [SOD], and glutathione-S-transferase [GST]) and/or oxidative damage along the body of the estuarine polychaeta *Laeonereis acuta* (Polychaeta, Nereididae) was analyzed after exposure to H_2O_2. Because this species secretes conspicuous amounts of mucus, its capability in degrading H_2O_2 was studied. The results suggest that *L. acuta* deal with the generation of oxidative stress with different strategies along the body. In the posterior region, higher CAT and SOD activities ensure the degradation of inductors of lipid peroxidation such as H_2O_2 and superoxide anion ($O_2^{\cdot-}$). The higher GST activity in anterior region aids to conjugate lipid peroxides products. In the middle region, the lack of high CAT, SOD, or GST activities correlates with the higher lipid hydroperoxide levels found after H_2O_2 exposure. Ten days of exposure to H_2O_2 also induced oxidative stress (lipid peroxidation and DNA damage)

in the whole animal paralleled by a lack of CAT induction. The mucus production contributes substantially to H_2O_2 degradation, suggesting that bacteria that grow in this secretion provide this capability.

Introduction

Hydrogen peroxide (H_2O_2) occurs in rainwater, freshwater lakes and streams, and coastal and oceanic waters (Herut et al. 1998). According to Abele-Oeschger et al. (1997), maximum H_2O_2 water concentrations range between 3 and 5 μM. These concentrations vary in function of the trophic status of the water (eutrophic or oligotrophic), location (open sea, coastal, estuarine), water depth, and diurnal cycle (Herut et al. 1997). It is believed that the major pathway for H_2O_2 formation in surface waters is photochemical, involving dissolved organic matter (DOM), oxygen, and UV light (Cooper et al. 1994), although H_2O_2 production can also occur in the sediment from the fungi action during the degradation process (Schlegel 1992). Because H_2O_2 is an uncharged active oxygen species, it can diffuse through cell membranes, being a precursor of more deleterious reactive oxygen species (ROS) such as hydroxyl radicals ($^{\cdot}OH$), the most reactive ROS that can oxidize biomolecules (lipids, proteins, DNA), leading to the loss of their functional properties (Abele-Oeschger et al. 1994; Halliwell and Gutteridge 1999).

All aerobic organisms, including marine invertebrates, possess well-developed antioxidant defense systems (ADS) to cope with ROS. The ADS comprises both enzymatic and nonenzymatic compounds (Halliwell and Gutteridge 1999), which have the overall task of preventing ROS formation, intercepting ROS, and repairing oxidized biomolecules (Sies 1993). Between the enzymatic defenses, the enzymes catalase (CAT) and glutathione peroxidase (GPx) degrade H_2O_2, superoxide dismutase (SOD) degrades superoxide anion ($O_2^{\cdot-}$), and the enzyme glutathione-S-transferase (GST) conjugates lipid hydroperoxides with glutathione (Storey 1996; Leaver and George 1998). Several works have demonstrated the induction of the ADS in some marine invertebrate species after H_2O_2 exposure (Abele-Oeschger et al. 1994, 1997; Abele et al. 1998; Storch et al. 2001; Cavaletto et al. 2002).

To cope with ROS, not only enzymatic antioxidant defenses but also several nonenzymatic antioxidant compounds, some of them specific to the life habits of the organism, exist. For

example, Abele-Oeschger and Oeschger (1995) hypothesized about the antioxidant properties of the mucous layer of egg cocoons in the polychaeta *Phyllodoce mucosa*. These authors concluded that this developmental stage relies on nonenzymatic antioxidant defenses probably present in the cocoon, contrary to juveniles and adult worms that have their protection based on the antioxidant enzymatic system.

Several polychaeta species are in close contact with the sediment, exposed to daily variation of several parameters such as Po_2, hydrogen peroxide, and sulphide. Interestingly, Abele et al. (1998) observed a correlation between an external gradient of Po_2 and pH and an internal gradient of ADS of the capitellid worm *Heteromastus filiformis*. In this animal, SOD activity was higher in the posterior region, where the animal has parapods adapted to gas exchange and is exposed to higher Po_2. This situation should lead to this region being prone to suffer oxidative stress if no antioxidant defenses are present in higher concentration.

The nereidid polychaeta *Laeonereis acuta* Treadwell 1923 is a common infaunal species in estuaries along the Atlantic cost of South America (Omena and Amaral 2001). It has been characterized as a selective deposit feeder and, therefore, living in close contact with the sediment (Bemvenuti 1998). This animal was previously utilized as an animal model in metal toxicity assays and environmental monitoring studies (Geracitano et al. 2000, 2002). In previous laboratory experiments it was verified that *L. acuta* secretes a conspicuous mucous layer, which led us to investigate its possible antioxidant properties. As mentioned above, Abele-Oeschger and Oeschger (1995) considered that the mucous layer in eggs of *P. mucosa* should limit the diffusive entry of H_2O_2 to the eggs. Also, these same authors speculated that the mucous secretion could contain antioxidants that degrade H_2O_2. Therefore, if the mucus has these properties in the eggs of that polychaeta species, it can probably exert the same function in adult worms that produce mucus in significant amounts such as *L. acuta*.

In light of the facts cited above, the objective of this study was to verify the effects of H_2O_2 exposure, a known prooxidant, in terms of the parameters of the ADS system of the polychaeta *L. acuta* and in terms of oxidative damage (lipid peroxidation and DNA damage). We also evaluated the possible antioxidant gradient along the body of *L. acuta* and the effects of H_2O_2 exposure in these parameters. Another objective of this study was to verify the contribution of the mucous layer secreted by this animal in the protection against H_2O_2.

Material and Methods

Collection and Maintenance of Worms

Laeonereis acuta adult worms, weighting approximately 90 mg, were collected in two salt marshes around Rio Grande City (southern Brazil) and acclimated for 10 d, following the procedure depicted by Geracitano et al. (2002). The maintenance conditions were saline water 10‰, pH 8.0, and 20°C in the presence of sediment. Animals were fed ad lib. with frozen *Artemia salina* every 2 d, and the water was then renewed.

After that period, animals were individually transferred to glass dishes (200 mL) containing saline water with the same characteristics cited above but without sediment. They were acclimated for 4 d before the beginning of the experiments.

H_2O_2 Exposure Assay

In the first experiment *L. acuta* specimens were individually exposed for 10 d to 10 and 50 μM of H_2O_2 in the same conditions of the acclimatization process. The water was renewed daily, and the mucous secretion of *L. acuta* was scratched from the glass dish bottom every 24 h. After the exposure period, the animals were stored ($-80°C$) for posterior analysis (CAT, GPx-Se, and GST activities; lipid hydroperoxide content). Other organisms of all treatments were employed immediately for determination of DNA damage through comet assay (see "Single Cell Gel Electrophoresis [Comet] Assay").

In the second experiment, worms were exposed for 7 h to the same H_2O_2 concentrations. A control group was maintained in the same conditions. After the exposure period, animals were subdivided in three regions: anterior region (first 20 setiger segments), middle region (next 20 setiger segments), and posterior region (the rest of body) for posterior analysis (antioxidant enzymes and lipid peroxides content).

Tissue Homogenization

For enzymatic assays, animal (or body region) pools were homogenized (1 : 3 w/v) in cold buffer (20 mM Tris; 1 mM EDTA; 1 mM dithiothreitol [DDT]; 500 mM sucrose and 150 mM KCl), with pH adjusted to 7.60. The homogenate was centrifuged for 9,000 g at 4°C for 45 min. The supernatant was utilized as the enzyme source. In another experiment both the worms and the mucous secretions were homogenized (50 mM phosphate buffer plus 2.5% NaCl, 7.5 pH) and centrifuged at 800 g for 10 min. The supernatant was employed for catalase measurements.

Enzymatic Assays

Total protein content of homogenate extracts was determined using a commercial diagnostic kit (Doles reagents, Brazil), based on the Biuret reagent, done in triplicate (550 nm), using bovine serum albumin as standard. The activity of the enzyme CAT was analyzed according to Beutler (1975), measured by following the initial rate of 50 mM H_2O_2 decomposition at 240 nm. The results were expressed in CAT units/milligram protein, where one unit is the amount of enzyme that hydrolyzes 1 μmol of H_2O_2 per minute and per milligram of protein at 30°C and pH 8.00. The H_2O_2 degradation by catalase-like proteins
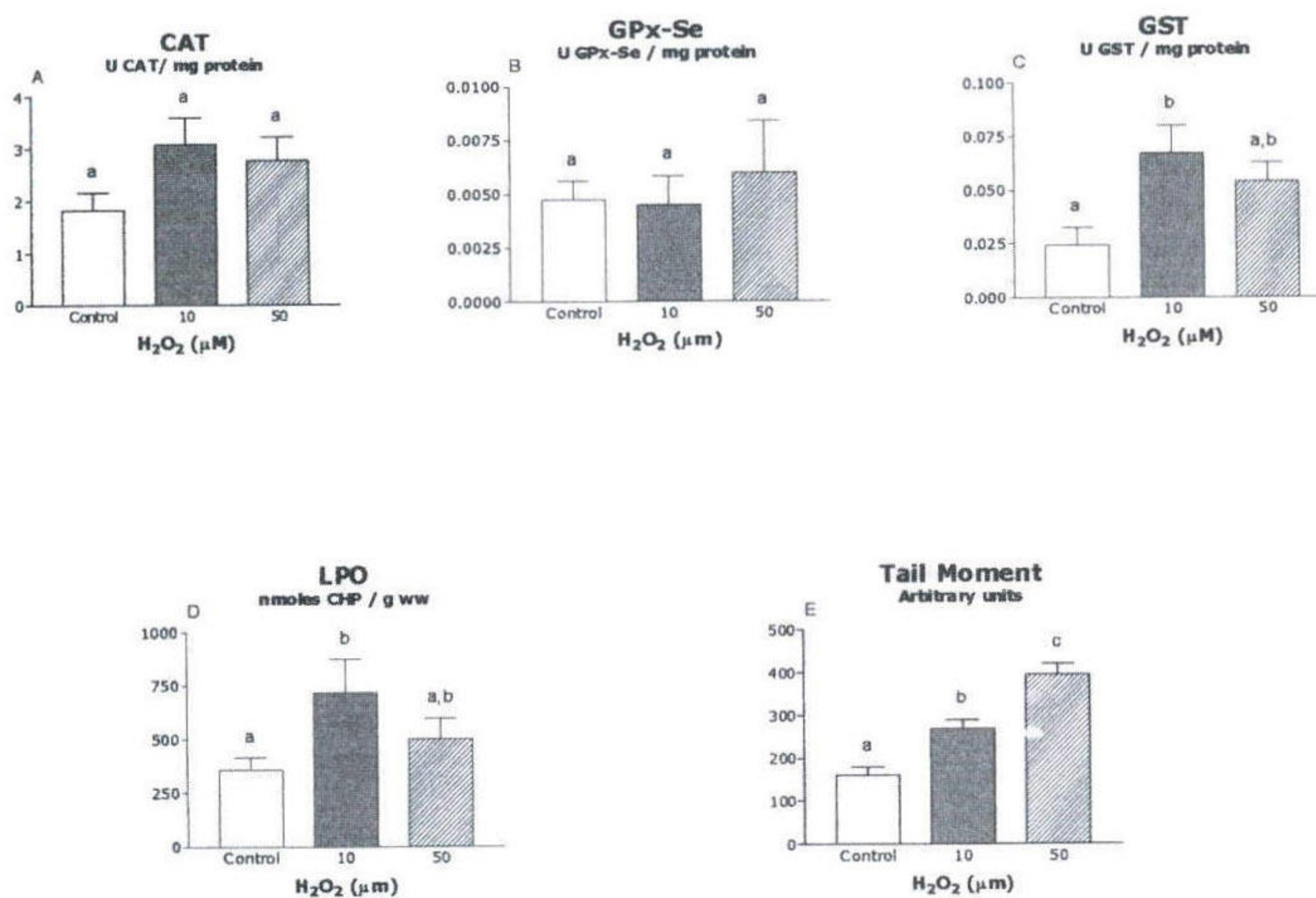
Figure 1. Antioxidant enzymes activities and oxidative damage in *Laeonereis acuta* submitted to hydrogen peroxide exposure for 10 d. *A*, Catalase (*CAT*); *B*, glutathione peroxidase (*GPx-Se*); *C*, glutathione-S-transferase (*GST*); *D*, lipid peroxides (*LPO*) content; and *E*, DNA damage (tail moment). Data are expressed as means $\pm$ 1 SE ($n = 5$). Equal letters indicate absence of significant difference ($P > 0.05$) between treatments. *CHP* = cumene hydroperoxide employed as standard; *ww* = wet weight.

in mucous secretion was done in the presence and absence of sodium azide (1 mM), a known inhibitor of the activity of this enzyme type (Saint-Denis et al. 1998).

The other H_2O_2 metabolizing enzyme, GPx, was measured according to Arun and Subramanian (1998). The assay measured NADPH oxidation at 340 nm in presence of excess glutathione reductase, reduced glutathione, hydrogen peroxide, and aliquots of the homogenate. The results are expressed in GPx units/milligram protein, where one unit is the amount of enzyme necessary to oxidize 1 μmol of NADPH per minute and per milligram of protein at 30°C and pH 7.20.

SOD activity was determined according to McCord and Fridovich (1969). In this assay, superoxide anion is generated by the xanthine/xanthine oxidase system and the reduction of cytochrome *c* monitored at 550 nm. Enzyme activity was expressed as SOD units, where one unit is defined as the amount of enzyme needed to inhibit 50% of cytochrome *c* reduction per minute and per milligram of protein at 25°C and pH 7.80.

GST activity was measured by monitoring at 340 nm the formation of a conjugate between 1 mM glutathione and 1 mM 1-chloro-2,4-dinitrobenzene (CDNB; Habig et al. 1974; Habig and Jakoby 1981). The results were expressed in GST units/ milligram protein, where one unit is defined as the amount of enzyme that conjugates 1 μmol of CDNB per minute and per

milligram of protein at 30°C and pH 7.40. All the reagents employed in the enzymatic assays were supplied by Sigma.

Lipid Peroxidation Assay

Lipid peroxidation was measured according to Hermes-Lima et al. (1995) and Monserrat et al. (2003), using the ferric/xylenol orange reaction. Whole body (or the different regions) was homogenized in methanol (10% w/v) and centrifuged at 1,000 g for 10 min. Lipid hydroperoxides (LPO) were determined using $FeSO_4$ (0.25 mM) prepared immediately before use, H_2SO_4 (0.25 mM), and xylenol orange (0.1 mM). Sample absorbance (580 nM) was measured in a microplate reader after 1 h of incubation at room temperature. LPO values were expressed in terms of cumene hydroperoxide (CHP) equivalents, used as standard (5 nmol/mL).

Single Cell Gel Electrophoresis (Comet) Assay

The comet assay was performed according to Singh et al. (1988) and Steinert et al. (1998), with minor modifications. Before the start of the experiments, fully frosted microscope slides were covered with 1% normal melting point agarose diluted in TAE solution (0.04 M Tris-acetate and 1 mM EDTA), wiping the
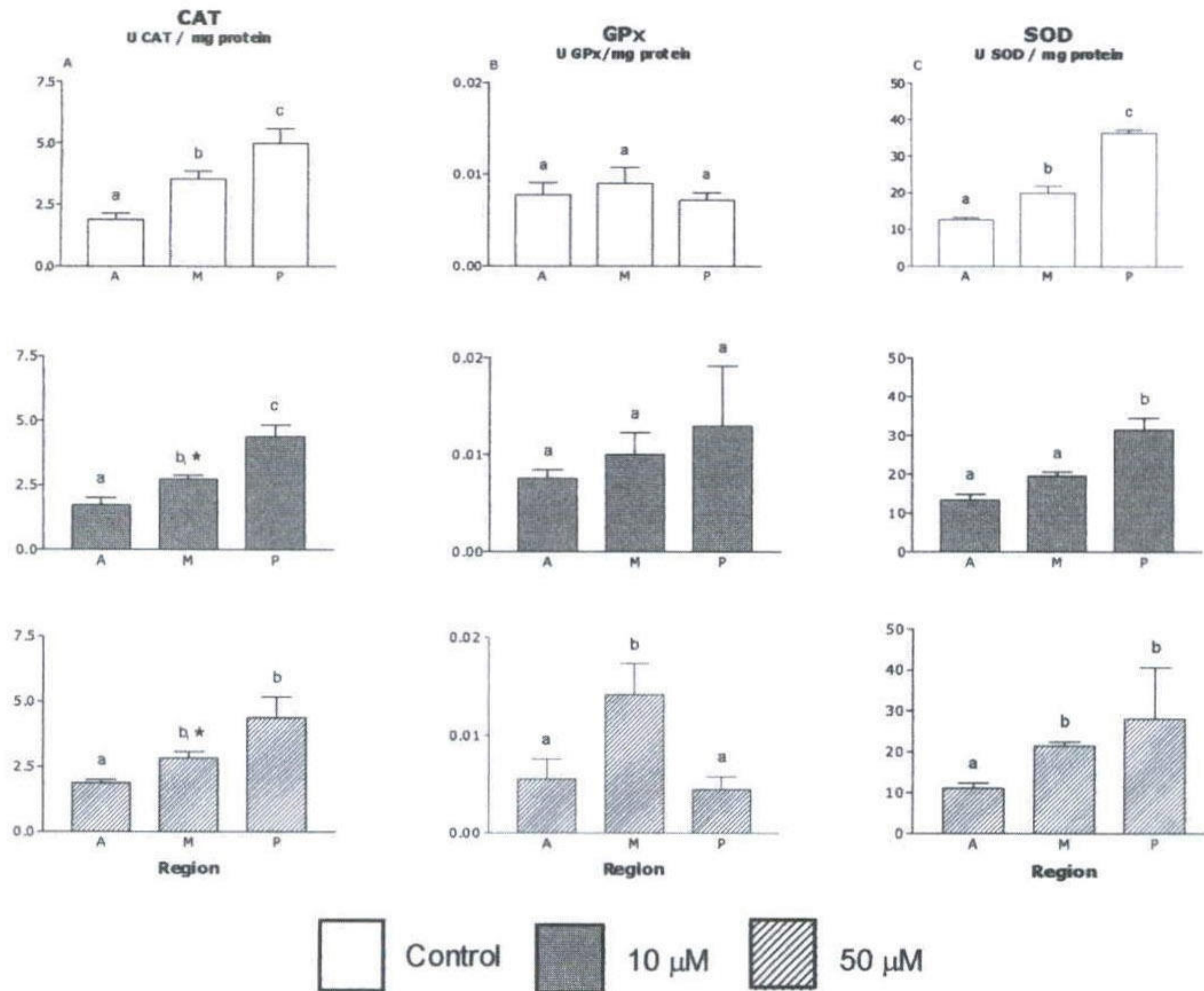
Figure 2. Antioxidant enzymes activities in three different body regions (A = anterior, M = middle, P = posterior) of the polychaeta *Laeonereis acuta* after 7 h of exposure to hydrogen peroxide (10 and 50 μM). A, Catalase (*CAT*); B, glutathione peroxidase (*GPx*); and C, superoxide dismutase (*SOD*). In all cases, data are expressed as means ± 1 SE (n = 5). An asterisk indicates significant difference between the same region from the control group. Equal letters indicate absence of significant difference ($P > 0.05$) between the body regions of the same treatment.

rear side with tissue and then air drying. Three animals per group were assayed. Animals were immediately homogenized in petri dishes at the end of the 10-d experiment. The homogenates were then filtered (pore: 145 μM) for cell separation. The cellular preparation was diluted (1 : 25 v/v) in Kenny's salt solution (0.4 M NaCl, 9 mM KCl, 0.7 mM K_2HPO_4, 2 mM $NaHCO_3$, pH 7.50). The diluted cell preparation was again diluted (1 : 7.5 v/v) in 0.65% low–melting point agarose (diluted in Kenny's salt solution) and added to the prepared frosted slide and covered with a cover slip. Then the slides were submitted to lysis solution (2.5 M NaCl, 0.1 M EDTA, 0.01 M Tris-HCl, 10 % dimethyl sulfoxide, 1% Triton X-100, and 1% sodium sarcosyl) and kept at 4°C overnight. For unwinding DNA strands, slides were transferred into chambers filled with electrophoresis and unwinding buffer (10 N NaOH and 200 mM EDTA) for 15 min. After that, electrophoresis was carried out for 20 min at 25 V and 280 mA. Slides were washed (0.4 M Tris, pH 7.5) and then stained with 80 μL of ethidium bromide. Fifty randomly selected cells per slide were observed and photographed (400 magnification) in an epifluorescence

Zeiss-Axioplan microscope. Images were analyzed by the public domain NIH-Image program (Helma and Uhl 2000) to determine the tail moment, defined as the product of the tail length of comet and the percent of DNA present in the tail.

Water H_2O_2 Degradation Assay

Hydrogen peroxide (50 μM) was spiked to dishes containing only saline water (10‰, pH 8.0), saline water and worms (23.83 ± 4.21 mg of dry weight), or saline water and the mucus secreted during 24 h by one worm in each dish (5.96 ± 0.38 mg of dry weight). The same process was followed utilizing filtered (pore size: 0.20 μm) saline water. In both experiments, five replicates for each treatment were performed. The content of H_2O_2 was determined at 0, 2, and 4 h after spiking using the ferric/xylenol orange reaction, as described above, with a modification: the methanolic tissue extract was substituted by 100 μL of the water sample. The degradation rate ($\Delta[H_2O_2]/$h) was calculated by the slope estimated from regression curves
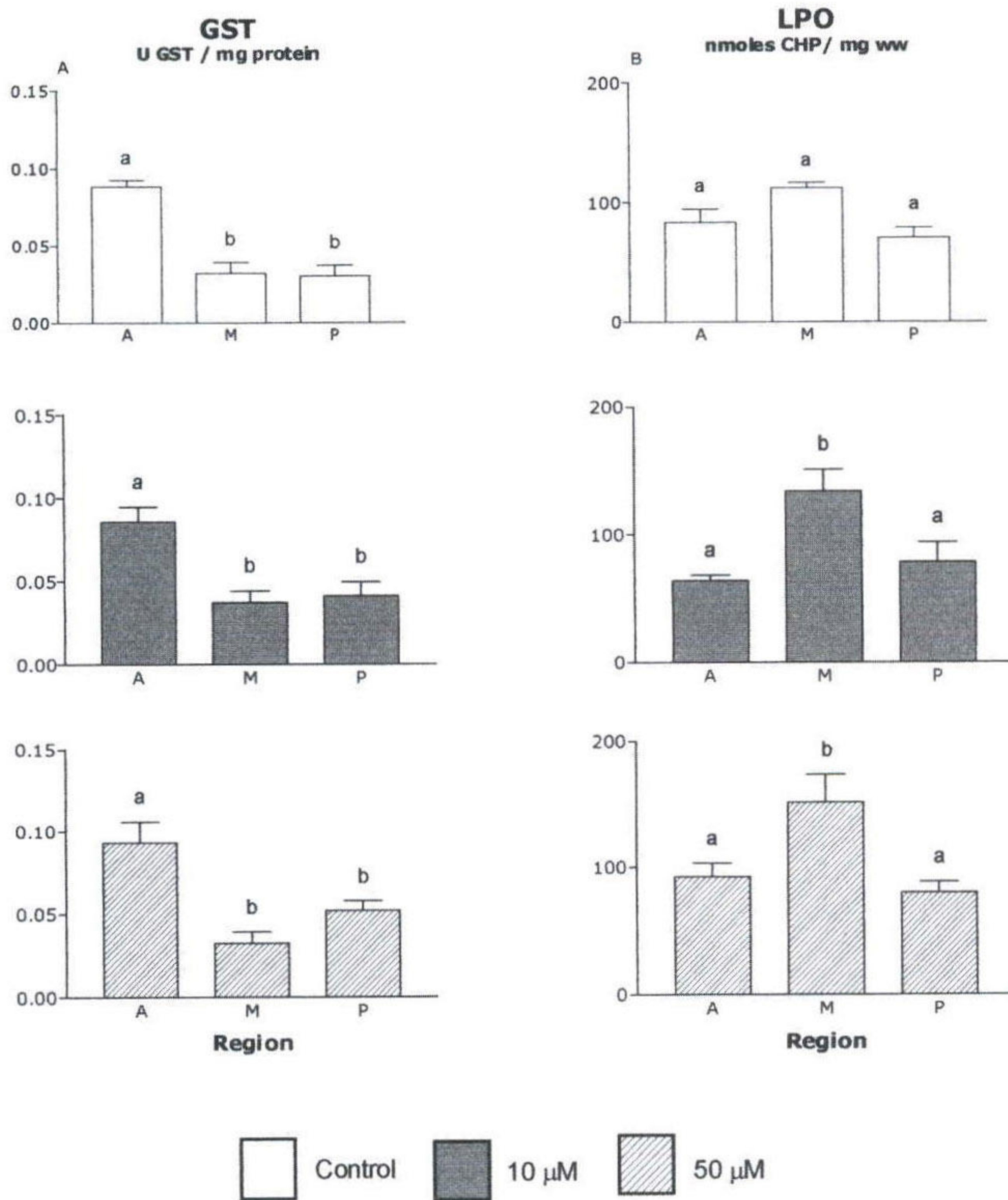
Figure 3. Glutathione-S-transferase (*GST*) activity (*A*) and lipid peroxides (*LPO*) content (*B*) in three different body regions (*A* = anterior, *M* = middle, *P* = posterior) of *Laeonereis acuta* after 7 h of exposure to hydrogen peroxide (10 and 50 μM). Data are expressed as means $\pm$ 1 SE (n = 5). Equal letters indicate absence of significant difference ($P > 0.05$) between the body regions of the same treatment. *CHP* = cumene hydroperoxide; *ww* = wet weight.

adjusted to each replicate of each treatment. Results were expressed as micromoles of H_2O_2 degraded per hour.

Water and Mucous Bacteria Identification

Bacteria present in the experimental water and in the mucus were counted under epifluorescence microscope (Zeiss Axioplan) using blue light excitation filter set (47709) after staining with acridine orange. Bacteria were observed at 1,000 $\times$ final magnification.

Histological Analysis

Worms were fixed in cold Bouin solution and stored for 24 h at 4°C and then transferred to 70% ethanol. Tissue pieces were dehydrated and included in Paraplast. Sections of 7 μm were stained with Masson Trichromic. Observations were done on a light microscope at 400 $\times$. Measurements of cuticle and epithelium width were conducted in anterior and posterior regions of the worms. Only transversal sections, showing longitudinal and circular muscle tissue, were employed for measurements.
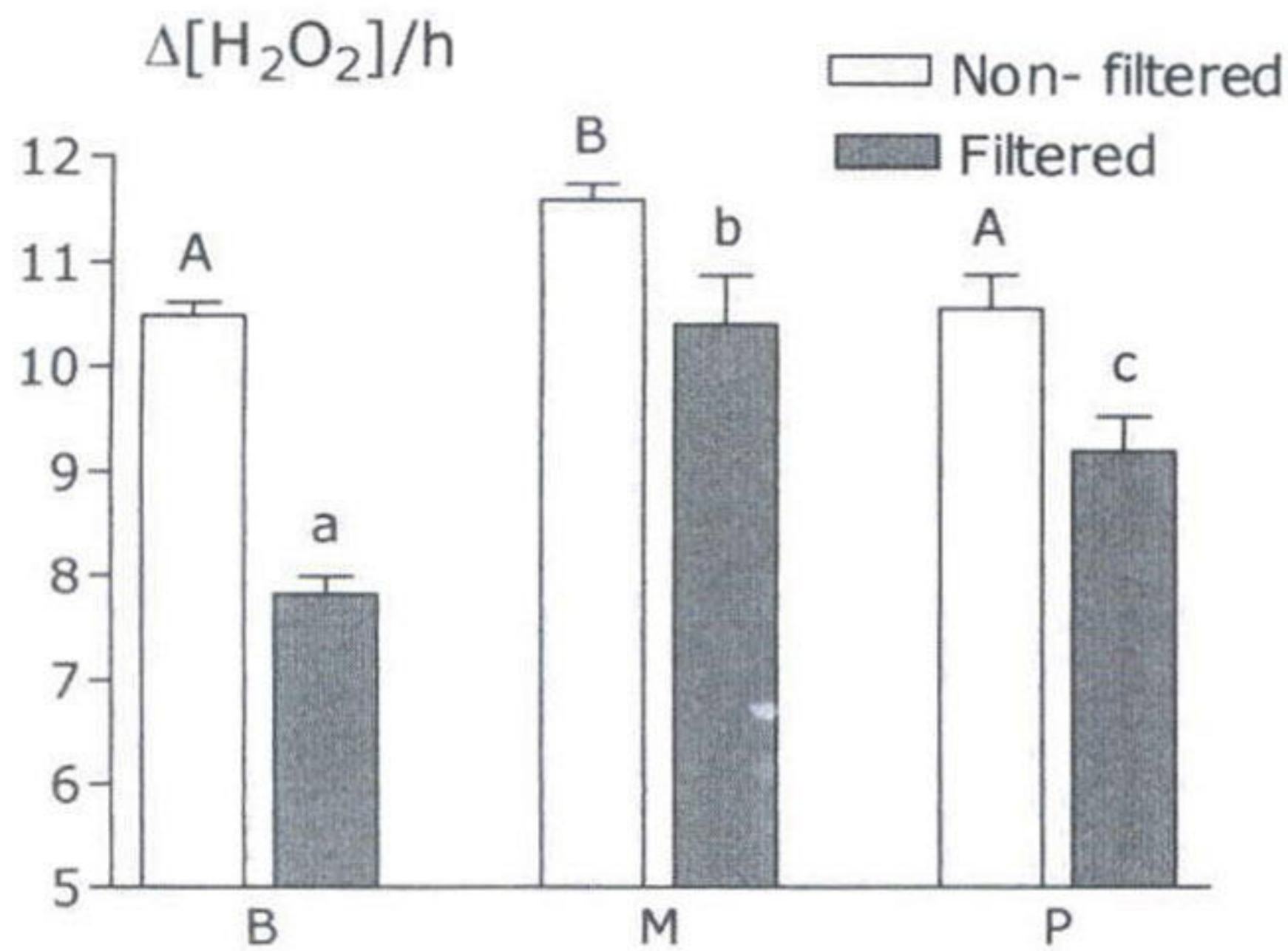
Figure 4. Hydrogen peroxide degradation rates ($\Delta[H_2O_2]$/h) in filtered (pore size: 0.20 μm) and nonfiltered saline water (10‰ salinity, pH 8.0) under different experimental conditions: blanks (B), with polychaeta only (P), and with mucous secretion only (M). Data are expressed as means $\pm$ 1 SE ($n = 5$). Equal letters indicate absence of significant difference ($P > 0.05$) between treatments for each condition (filtered or nonfiltered).

Statistical Analysis

Values in all determinations were computed as means $\pm$ 1 SE. When we analyzed data from the comet assay, the median value of the tail moment from 50 measurements from each worm of each treatment was determined. Statistical analysis was performed through ANOVA, followed by a Newman-Keuls test ($\alpha = 0.05$).

Results

In animals exposed to hydrogen peroxide for 10 d, no induction of CAT and GPx activities was observed (Fig. 1A, 1B). However, augmented GST activity was verified in worms exposed to 10 μM when compared with the control group ($P < 0.05$; Fig. 1C).

In terms of oxidative damage, LPO levels were higher in animals exposed for 10 d to 10 μM with respect to the control group ($P < 0.05$; Fig. 1D). A dose-response relationship for DNA damage was verified, with the higher tail moment registered in animals exposed to 50 μM ($P < 0.05$; Fig. 1E).

When we analyzed data from the 7-h exposure experiment, differences of the antioxidant defenses between the body regions were observed. Control animals showed a gradient of CAT activity, and it was lowest in the anterior region (A), intermediate in the middle region (M), and highest in the posterior (P; $P < 0.05$; Fig. 2A). No gradient of enzyme activity was observed for GPx ($P > 0.05$; Fig. 2B). SOD activity showed a similar pattern to that of CAT, being lowest in A, intermediate in M, and highest in P ($P < 0.05$; Fig. 2C).

Exposure to H_2O_2 lowered CAT activity in the M region in the two concentrations assayed with respect to the same region

in the control group ($P < 0.05$). Animals exposed to 50 μM H_2O_2 also showed a partially disrupted CAT gradient, with enzyme activity in P similar to that of the M region ($P > 0.05$; Fig 2A). In animals exposed to 50 μM of H_2O_2, higher activity of GPx was observed in M with respect to the A and P regions ($P < 0.05$; Fig. 2B). Animals exposed to 50 μM H_2O_2 showed a partially disrupted SOD gradient, with enzyme activity in the P region similar to that of the M region ($P > 0.05$; Fig. 2C).

GST activity was highest in the A region and lowest in the M and P regions ($P < 0.05$; Fig. 3A). Animals exposed to both concentration of H_2O_2 maintained the body gradient of GST activity.

Concerning oxidative damage, LPO levels were similar in the different body regions of control animals. However, higher LPO content was observed in M with respect to the A and P regions in worms exposed to 10 and 50 μM of H_2O_2 ($P < 0.05$; Fig. 3B).

The mucous secretion presented a higher CAT-like activity (12.71 $\pm$ 3.28 U CAT/mg of protein; $n = 8$) than whole worms (2.44 $\pm$ 0.19 U CAT/mg of protein; $n = 4$). The presence of sodium azide, a CAT inhibitor, inhibited hydrogen peroxide degradation in 65.95% $\pm$ 9.7% ($n = 4$). The degradation curves reveal that after 4 h, the initial concentration of 50 μM of H_2O_2 decayed to levels below 10 μM H_2O_2 (Fig. 4). The water employed in the assays presented high capability for H_2O_2 degradation, with this effect attenuated after filtration. The dishes with mucous secretion presented the highest H_2O_2 degradation rates ($P < 0.05$). Those dishes containing only polychaeta showed a superior rate with respect to blanks only when filtered water was used ($P < 0.05$; Fig. 4).

The microorganisms found in mucous samples were bacteria of rod shapes with 2 μm length and 0.5–1.0 μm width, with a mean abundance of 0.03 cells/μm^2. A Large number of bacteria had endospores, typical of the *Bacillus* group. In the unfiltered water the cell density was 1.59 $\pm$ 0.67 × 10^6/mL, and the filtration process removed more than 90% of the cells (0.11 $\pm$ 0.14 × 10^6 cell/mL). Finally, the histological data revealed that the anterior region possessed a thicker cuticle than the posterior region ($P < 0.05$), whereas no difference was observed in the epithelium width between the two regions (Table 1).

Table 1: Cuticle and epithelium width in anterior and posterior regions in *Laeonereis acuta*

	Anterior Region (μm)	Posterior Region (μm)
Cuticle	5.03 $\pm$ .14	2.46 $\pm$ .06*
Epithelium	19.60 $\pm$ 3.78	14.23 $\pm$ 1.95

Note. The anterior is the first 20 setiger segments, and the posterior is the rest of body after the first 40 setiger segments. Data are presented as means $\pm$ 1 SE ($n = 4$).

* Significant differences ($P < 0.05$) between regions.

Discussion

The worm *Laeonereis acuta* did not induce the H_2O_2 detoxifying enzymes CAT and GPx after 10 d of H_2O_2 exposure. The same absence of response was observed in previous studies (C. E. da Rosa, A. Bianchini, and J. M. Monserrat, unpublished data), when this worm was exposed for 4, 7, and 10 d to the same concentrations. In both cases, oxidative damage, in terms of lipid peroxidation or DNA damage, was observed. It is known that H_2O_2 diffuses through cell membranes (Abele-Oeschger et al. 1994) and can react with electron donor molecules (via Haber-Weiss reaction), liberating the hydroxyl radical ˙OH (Halliwell and Gutteridge 1999). This ROS is the highest reactive, capable of acting against all cellular macromolecules, including DNA and fatty acids (Storey 1996).

In marine invertebrates (i.e., polychaeta, molluscs, and crustaceans) the most common response is the induction of antioxidant defenses, mainly CAT activity when exposed to H_2O_2 (Abele-Oeschger et al. 1994, 1997; Abele et al. 1998; Storch et al. 2001; Cavaletto et al. 2002). Previous studies showed that when *L. acuta* was chronically (14 d) exposed to copper (31.25 and 62.50 μg/L), higher CAT and SOD activities in exposed animals were observed with respect to the control group (Geracitano et al. 2002). It is known that the combination of superoxide anion ($O_2^{˙-}$), H_2O_2, and transition metals (i.e., copper and iron) produces hydroxil radicals (˙OH) via Haber-Weiss reaction (Storey 1996; Pourahmad and O'Brien 2000). Therefore, these previous findings indicate that the worms try to avoid the deleterious effects of the ˙OH generation by degrading the precursors (H_2O_2 and $O_2^{˙-}$) through CAT and SOD activity.

The absence of CAT response observed in our study resulted in oxidative stress, as evidenced by the higher levels of LPO and DNA damage in H_2O_2 exposed animals. The augmented H_2O_2 degradation in water containing mucous secretion can at least partially explain this lack of response. In polychaeta species, the epidermis secretes a mucous layer that covers and protects the cuticle. This secretion is used for locomotion, lubrication, and protection against foreign particles (Ruppert and Barnes 1996). However, Abele-Oeschger and Oeschger (1995) suggested antioxidant properties in the mucous layer of *Phyllodoce mucosa* eggs. This fact leads us to investigate the antioxidant properties of the mucus secreted by *L. acuta* worms, after observing a great amount of CAT-like activity not totally inhibited by sodium azide. This capacity can be in part from the great abundance of bacteria living in this secretion because it is known that all organisms that live in aerobic environments must be able to cope with ROS (Storey 1996). This ability is given by the ADS system, also present in microorganisms, including antioxidant enzymes (Baker et al. 1997; Wood and Sørensen 2001). Previous studies reported high CAT activity in some *Bacillus* species (Dowds 1994; Engelmann and Hecker 1996) that confers to these microorganisms high H_2O_2 degradation competence. Also, it can be considered that the higher

degradation rates of H_2O_2 in the water containing the mucous secretion can be due to the liberation of antioxidant enzymes by the mucous secretion cells, as cited for extracellular enzymes and pheromones of annelids (Ruppert and Barnes 1996). However, the existence of other molecules present in the mucous secretion adding in the degradation of hydrogen peroxide cannot be discarded. New studies using sterilized seawater in order to isolate the role of bacteria and other molecules present in the mucus (iron, oxidizable substrates, etc.) should be performed. A similar approach can also be considered to evaluate the H_2O_2 degradation in water.

In sum, the mucous secretion effectively confers an antioxidant protection that should be sufficient to degrade environmental H_2O_2, and this can help explain the absence of responsiveness in terms of antioxidant enzymes induction when *L. acuta* is exposed to this prooxidant. The H_2O_2 degradation assay indicates that, in fact, during the experiments, animals were exposed to pulses of hydrogen peroxide (an initial concentration of 50 μM H_2O_2 was reduced to almost 10 μM in 4 h). It is important to emphasize that during the 10-d exposure experiment, the mucous layer was removed daily and then H_2O_2 was added. So the pulse of H_2O_2 was given when the animal lacked its mucous secretion, leaving it more susceptible to oxidative stress, as evidenced by the higher LPO levels and DNA damage registered.

It is known that environmental factors like Po_2 can influence the antioxidant defense system of animals (Storey 1996; Abele et al. 1998; Wilhelm Filho et al. 2000). Abele et al. (1998) observed that the activity of SOD of *Heteromastus filiformis* inhabiting burrows varied according to a decreasing Po_2 gradient from top to bottom. They reported that in the most oxygen-exposed region of the animal (with gill-like parapods), there is increased SOD activity. The same pattern of response was observed for *L. acuta* in terms of CAT and SOD activity. In the posterior region of this animal, where the cuticle is thinnest (Table 1), the highest CAT and SOD activities were observed. Thus, these regions probably possess higher Po_2 and/or allow easier diffusive environmental H_2O_2 entry, requiring higher antioxidant defenses to cope with the generation of ROS. The differences in antioxidant enzyme activity along the body indicate that *L. acuta* deals with oxidative stress employing different strategies among these body regions. In the P region, higher CAT and SOD activities can eliminate H_2O_2 and $O_2^{˙-}$ (Fig. 1A, 1C), which are known precursors of the hydroxyl radical, an inductor of LPO (Halliwell and Gutteridge 1999). The A region possesses higher levels of GST activity, the phase II detoxifying enzyme that can remove lipid hydroperoxides (Storey 1996), indicating that in this region the strategy could be not to avoid the occurrence of oxidative stress but to remove products derived from this process. In the M region, the lack of high CAT, SOD, or GST activities correlates with higher LPO levels observed after H_2O_2 exposure. At this point, the augmented GPx activity in animals exposed for 50 μM seems to

be a minor response and seems to be insufficient to prevent lipid peroxidation.

In conclusion, *L. acuta* presents differential antioxidant enzymes activities in the anterior, middle, and posterior region of its body. Hydrogen peroxide can disturb this gradient (i.e., lowering CAT activity in the M region), generating an oxidative stress situation (higher LPO levels in the M region). Finally, it can be inferred that in the environment, *L. acuta* can deal with daily H_2O_2 variations, employing its mucous secretion as an antioxidant defence.

Acknowledgments

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