INSTM Bulletin
Marine and Freshwater Sciences

*Vol. 51, 2026, 45–57*

*Research Article*

# Behavioral, biochemical and histological responses of the clam *Ruditapes decussatus* to a triazole fungicide

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**Received**: 25/01/2026; **Accepted**: 04/05/2026; **Published**: 06/05/2026

**Abstract:** Clams (*Ruditapes decussatus*) were exposed for 7 days to a triazole fungicide, Alto Super 330EC (AS330), widely used in agriculture in the Bizerte Lagoon watershed. Its two active substances (propiconazole and cyproconazole) were detected in wastewater discharges at Oued Guenniche. Considering the environmental levels of these compounds, clams were exposed in microcosms under controlled conditions to three concentrations: C1 (25 µg.L$^{-1}$), C2 (50 µg.L$^{-1}$) and C3 (100 µg.L$^{-1}$).

Behavioral (filtration capacity), oxidative and neurotoxic stress and histological damage responses were assessed to evaluate clam health status. Four biomarkers; catalase (CAT), glutathione S-transferase (GST), malondialdehyde (MDA) and acetylcholinesterase (AChE) were measured in gills and digestive gland.

After exposure, the filtration rate was significantly increased at the lowest pesticide concentration. Antioxidant enzyme activities (CAT, GST) and cellular damage (MDA) exhibited organ- and concentration-dependent responses. The AChE activity decreased in a highly significant, dose-dependent manner.

Exposure induced significant histopathological alterations. In gills, malformations at the tips of lamellae and marked hemocyte infiltration were observed. In the digestive gland, extensive degeneration of digestive tubules occurred, characterized by accumulation of cellular debris and necrotic areas within the tubule lumen. These changes indicate a pronounced pathological response to contamination.

**Keywords:** *Ruditapes decussatus*; Alto Super 330EC; Biomarkers; Histopathology. Ecotoxicology; Sea pollution; Behavioral responses; Oxidative stress; Coastal ecosystems; Bizerte lagoon (Tunisia); Mediterranean Sea.

## 1. Introduction

The Mediterranean Sea, one of the most intensively used and valued marine regions worldwide, hosts a wide diversity of marine and coastal ecosystems that provide essential services to coastal populations. However, it is increasingly subjected to anthropogenic pressures and contaminant inputs. The Mediterranean region is characterized by high levels of industrialization, rapid urbanization and intense tourism activity, leading to marine environmental degradation and ecosystem alteration (Katikou et al., 2021). In this context, the introduction of chemical substances may result in the disappearance of animal and plant species and disrupt trophic networks (Stehle and Schulz, 2015).

Among these substances, plant protection products represent a major source of concern. Although pesticides contribute to agricultural productivity, their intensive use may generate significant risks due to their persistence, dispersion and bioamplification potential (Sharma et al., 2019). These compounds contaminate soil and aquatic environments, may persist in crops and enter the food chain, thereby posing a threat to aquatic organisms and potentially to human health.

In response to these pressures, biomonitoring has become an essential tool for assessing ecosystem health and guiding management and remediation strategies. Several approaches can be implemented, including physicochemical, analytical, ecobiological and biochemical monitoring, as well as integrative approaches combining multiple levels of biological organization (Dellali et al., 2021).

Within this framework, Alto Super 330 EC, a fungicide formulated as a mixture of propiconazole and cyproconazole, represents a contaminant of particular interest. Triazoles constitute a major class of fungicides widely used in agriculture to control fungal diseases affecting fruits, vegetables, ornamental plants and cereals (Cui et al., 2021). Some of these compounds are considered emerging contaminants, as they are not routinely monitored despite their potential environmental and human health risks.

Bivalves, such as mussels, oysters and clams, are commonly used as sentinel organisms in ecotoxicological studies due to their sensitivity to contaminants.

Their filter-feeding activity and capacity to accumulate pollutants in their tissues make them excellent bioindicators of aquatic environmental quality (Livingstone, 1993; Mansour et al., 2020).

Several studies have reported toxic effects of triazole fungicides on aquatic organisms. For instance, cyproconazole affects the freshwater alga *Pseudokirchneriella subcapitata* ($EC_{50}$, 72 h: 8.84 mg.$L^{-1}$), the cladoceran *Daphnia magna* ($EC_{50}$, 48 h: 30.90 mg.$L^{-1}$) and zebrafish (*Danio rerio*) embryos ($LC_{50}$, 72 h: 40.20 mg.$L^{-1}$) (Saraiva et al., 2018). In addition, Huang et al. (2025) demonstrated in the alga *Raphidocelis subcapitata* that several azole fungicides strongly inhibit growth by reducing chlorophyll content, induce oxidative stress (increased ROS and MDA levels), and modulate antioxidant defenses through the activation of enzymes such as superoxide dismutase (SOD) and catalase (CAT).

The assessment of contaminant effects can be strengthened using biomarkers. Biomarkers are measurable biological indicators that provide information on exposure, effects or organism sensitivity to chemical substances or environmental stressors. In ecotoxicology, acetylcholinesterase (AChE) is widely used as a biomarker of neurotoxicity, particularly in response to pesticide exposure (Dalmolin et al., 2020), while glutathione S-transferase (GST) is considered a key biomarker of detoxification processes and oxidative stress (Banaee et al., 2024).

The integration of biochemical biomarkers with behavioral parameters and histopathological observations allows a more comprehensive assessment of contaminant impacts.

The objective of the present study was to investigate the behavioral, biochemical and histopathological responses of the marine clam *Ruditapes decussatus* exposed to Alto Super 330 EC. The selection of this xenobiotic is justified by its detection in wastewater discharges transported by Oued Guenniche, which flows into the southeastern part of the Bizerte Lagoon (Grumberger et al., 2024).

## 2. Materials and Methods

### *2.1. Sampling, acclimation and exposure conditions*

Clams (*Ruditapes decussatus*) were collected from the Bizerte Lagoon (northeastern Tunisia), near Menzel Jemil (37°14′19″ N, 9°54′59″ E), and transported to the laboratory in a cooler containing site water. Specimens were acclimated for 7 days in glass microcosms under controlled conditions (temperature: 18 ± 2°C; photoperiod: 12 h light/12 h dark), without feeding. Seawater, collected from Rimel beach (Mediterranean Sea), was renewed every 48 h.

Alto Super 330 EC, a commercial emulsifiable concentrate fungicide, was used for exposure. This systemic triazole fungicide contains 80 g $L^{-1}$ cyproconazole and 250 g $L^{-1}$ propiconazole. Three nominal concentrations were tested: 25 µg $L^{-1}$ (C1), 50 µg $L^{-1}$ (C2) and 100 µg $L^{-1}$ (C3). A total of 65 clams of similar size ( shell length: 38.6 ± 3.4 mm) were randomly distributed into microcosms (5 individuals per aquarium containing 2 L of seawater) and exposed for 7 days. Each treatment, including the control, was conducted in triplicate. Due to the regular renewal of the exposure medium and the short exposure duration, nominal concentrations were assumed to reflect actual exposure levels throughout the experiment.

Three levels of biological response were assessed to evaluate the effects of Alto Super 330 EC on *R. decussatus*: (i) behavioral response, assessed by filtration rate; (ii) biochemical response, based on biomarker measurements; and (iii) tissue-level response, examined through histopathological analysis of gills and digestive glands.

### *2.2. Behavioral response: Filtration rate*

Filtration rate (FR) was determined following Coughlan (1969), based on the decrease of neutral red concentration in the water column under dark conditions. For each treatment, one clam was placed in a beaker containing 100 mL of neutral red solution (1 g $L^{-1}$). Prior to introducing clams, a 10 mL aliquot was sampled from each beaker to determine the initial dye concentration ($C_0$). During the assay, clams were removed at 30 min intervals over a total duration of 2 h and the remaining dye concentration at time *t* ($C_t$) was determined. A calibration curve was established using neutral red standards, and optical density (OD) was measured at 530 nm using a microplate reader.

Filtration rate was calculated as:

$FR = (M / (n \cdot t)) \cdot \ln(C_0/C_t)$

where FR is the clearance rate (L $indiv^{-1}$ $h^{-1}$), M is the total volume of water (L), *n* is the number of clams, *t* is time (h), $C_0$ is the initial dye concentration, and $C_t$ is the dye concentration at time *t*. Blanks (beakers containing dye solution without clams) were run in parallel to account for any non-biological variation in dye concentration.

### *2.3. Biochemical response: biomarker assays*

At the end of the experiment, clams were dissected on ice (4°C). Gills and digestive glands were carefully excised, processed separately, and kept cold throughout. Tissues were homogenized in phosphate buffer (0.1 M, pH 7.5), and homogenates were centrifuged at 9000 rpm for 30 min at 4°C to obtain the S9 fraction. Supernatants were collected in Eppendorf tubes and stored at −80°C until analyses. All enzymatic activities and lipid peroxidation levels were normalized to protein content.

Total protein concentration was determined according to Bradford (1976) by measuring absorbance at 595 nm using a spectrophotometer. Catalase (CAT) activity was measured following Clairborne (1985) by monitoring the decomposition of $H_2O_2$ at 240 nm; activity was expressed as µmol $min^{-1}$ $mg^{-1}$ protein. Glutathione S-transferase (GST) activity was assayed according to Habig et al. (1974); absorbance was recorded at 340 nm and activity was expressed as µmol $min^{-1}$ $mg^{-1}$ protein.

Lipid peroxidation was evaluated using the thiobarbituric acid reactive substances (TBARS) method (Buege and Aust, 1978); absorbance was read at 530 nm and results were expressed as µmol $mg^{-1}$ protein. Acetylcholinesterase (AChE) activity was determined following Ellman et al. (1961) with absorbance measured at 412 nm; activity was expressed as µmol.$min^{-1}$.$mg^{-1}$ protein.

### 2.4. *Histological analysis*

For histological assessment, gills and digestive glands were sampled and separated from surrounding tissues. Tissue processing followed the method of Martoja and Martoja, using an automated workflow performed at the Department of Pathological Anatomy, Regional Hospital of Menzel Bourguiba (Tunisia). Samples were fixed in 5% formalin, dehydrated through a graded ethanol series followed by toluene, embedded in paraffin, and sectioned at 5 µm using a microtome. Sections were mounted on glass slides and stained with hematoxylin and eosin (H&E). Slides were examined under a ZEISS light microscope (×40-100) and photographed using an Axio-Cam 105 camera.

### 2.5. *Statistical analysis*

Data are presented as mean ± standard deviation (SD). Graphs and statistical analyses were performed using STATISTICA 8 software. Variations in behavioral and biochemical responses in clams were analyzed using a one-way analysis of variance (one-way ANOVA) relative to the control group. When significant differences were detected by ANOVA, post hoc comparisons were conducted using Tukey's honestly significant difference (HSD) test. Differences were considered statistically significant at $p < 0.05$.

## 3. Results

### *3.1. Effect of Alto Super 330 EC on the filtration capacity of* Ruditapes decussatus

Exposure of *Ruditapes decussatus* clams to Alto Super 330 EC at the tested concentrations (C1 = 25 µg.$L^{-1}$, C2 = 50 µg.$L^{-1}$ and C3 = 100 µg.$L^{-1}$) for 7 days significantly affected filtration capacity ($F(3,12) = 8.1939$; $p = 0.003$). A significant increase was observed only at C1 (45.85 ± 17.8 mg.$h^{-1}$.$indiv^{-1}$), compared with the control group (8.18 ± 4.22 mg.$h^{-1}$.$indiv^{-1}$) and with C2 and C3 (Fig. 1).

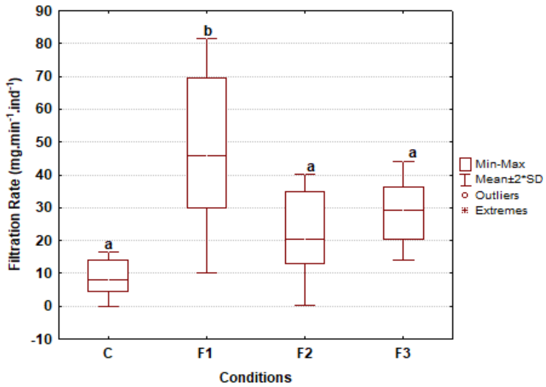


**Figure 1.** filtration rate (FR) in *Ruditapes decussatus* (mg·$h^{-1}$·$ind^{-1}$) exposed to three concentrations of Alto super 330EC (C = control, F1 = 25 µg·$L^{-1}$, F2 = 50 µg·$L^{-1}$, F3 = 100 µg·$L^{-1}$) for 7 days. (a, b: significant differences at the 5% level, ANOVA: Tukey's HSD test).

Regarding the temporal variation in filtration capacity, contamination induced a marked increase in the C1 group (25 µg.$L^{-1}$) compared to the other groups. However, the temporal profile for the control, C1 and C2 groups showed a clear decreasing trend over time, for example in the control group from 14.10 ± 0.09

mg.min⁻¹.indiv⁻¹ at 30 min to 4.60 ± 0.01 mg.min⁻¹.indiv⁻¹ at 120 min. At C3 (100 µg.L⁻¹), the profile displayed a slight peak around 60 min followed by a decrease until the end of the experiment (Fig. 2).

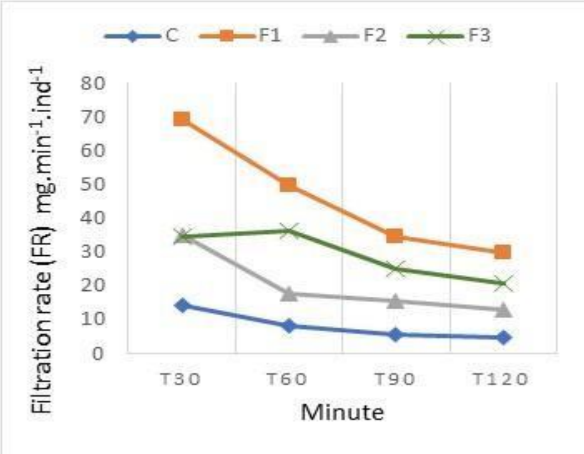


**Figure 2.** Temporal dynamics of the filtration rate (FR) in *Ruditapes decussatus* ($mg \cdot min^{-1} \cdot ind^{-1}$) exposed to three concentrations of Alto super 330EC (C = control, F1 = 25 $\mu g \cdot L^{-1}$, F2 = 50 $\mu g \cdot L^{-1}$, F3 = 100 $\mu g \cdot L^{-1}$).

### *3.2. Effect of Alto Super 330 EC on the oxidative stress of* Ruditapes decussatus

#### *3.2.1. Catalase (CAT) activity*

The effect of Alto Super 330 EC on catalase (CAT) activity in clams exposed for 7 days to the different concentrations (C1 = 25 µg.L⁻¹, C2 = 50 µg.L⁻¹ and C3 = 100 µg.L⁻¹) is shown in Fig. 3A. In gills, CAT activity exhibited a highly significant increase only at C3, rising from 70.57 ± 7.5 µmol.min⁻¹.mg⁻¹ protein in the control group to 136.25 ± 27.74 µmol.min⁻¹.mg⁻¹ protein. In contrast, C1 and C2 induced only slight, non-significant increases compared to the control. A similar pattern was observed in the digestive gland, where CAT activity significantly increased only at C3, from 109.79 ± 27.34 µmol.min⁻¹.mg⁻¹ protein in the control group to 134.49 ± 18.71 µmol.min⁻¹.mg⁻¹ protein.

#### *3.2.2 Glutathione S-transferase (GST) activity*

Exposure of clams to Alto Super 330 EC for 7 days resulted in a similar GST activity profile in gills and digestive glands (Fig. 3B). In gills, GST activity in the control group was 0.94 ± 0.19 µmol.min⁻¹.mg⁻¹ protein. Following exposure, a clear dose-response relationship was observed, with a highly significant increase ($p < 0.001$), reaching 1.63 ± 0.12 µmol.min⁻¹.mg⁻¹ protein at C1 (25 µg.L⁻¹) and 2.06 ± 0.32 µmol.min⁻¹.mg⁻¹ protein at C3 (100 µg.L⁻¹). Similarly, in digestive glands, GST activity significantly increased ($p < 0.001$) from 0.82 ± 0.09 µmol.min⁻¹.mg⁻¹ protein in the control group to 1.73 ± 0.12 µmol.min⁻¹.mg⁻¹ protein at C3.

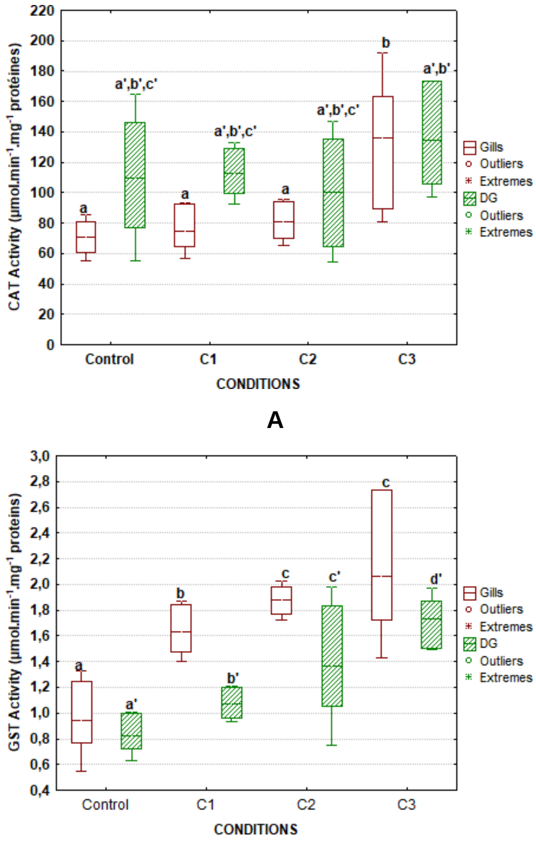


**Figure 3.** catalase (CAT) (A) and glutathione S-transferase (GST) (B), activities ($\mu mol \cdot min^{-1} \cdot mg^{-1}$ protein) in the gills and digestive glands (DG) of *Ruditapes decussatus* exposed to Alto super 330EC in the laboratory for 7 days (C = control, C1 = 25 $\mu g \cdot L^{-1}$, C2 = 50 $\mu g \cdot L^{-1}$, C3 = 100 $\mu g \cdot L^{-1}$). (ANOVA; a, b, a', b', c': significant differences at $p < 0.05$, Tukey's HSD test).

#### *3.3.3. Malondialdehyde (MDA) levels*

For Alto Super 330 EC exposure, malondialdehyde (MDA) levels showed a highly significant increase compared to the control in both gills and digestive glands (Fig. 4). In gills, MDA concentrations increased from 19.07 ± 4.07 µmol.mg⁻¹.protein in the control group to 27.97 ± 2.34 µmol.mg⁻¹.protein at C3 (100 µg.L⁻¹). In digestive glands, MDA levels also increased significantly, from 22.56 ± 1.07 µmol.mg⁻¹.protein in the control group to 30.42 ± 2.09 µmol.mg⁻¹.protein at C3.

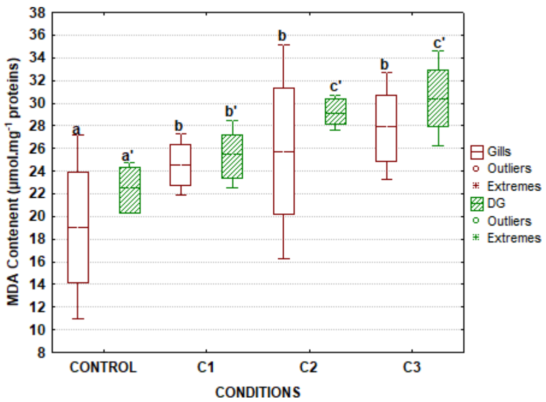


**Figure 4.** content of malondialdehyde (MDA) (µmol·mg⁻¹ protein) in the gills and digestive glands (DG) of *Ruditapes decussatus* exposed to Alto super 330EC in the laboratory for 7 days (C = control, C1 = 25 µg·L⁻¹, C2 = 50 µg·L⁻¹, C3 = 100 µg·L⁻¹). (ANOVA; a, b, a', b', c': significant differences at $p < 0.05$, Tukey's HSD test).

### *3.3. Neurotoxicity: Acetylcholinesterase (AChE) activity*

AChE activity was highest in the control group, reaching 110.16 ± 13.06 µmol.min⁻¹.mg⁻¹.protein in gills (Fig. 5). Exposure to Alto Super 330 EC induced a significant decrease in AChE activity, particularly at C3 (100 µg.L⁻¹), where activity declined to 33.41 ± 1.86 µmol.min⁻¹.mg⁻¹.protein.

In digestive glands, a slight but significant increase was observed at C1 (286.23 ± 5.07 µmol.min⁻¹.mg⁻¹.protein) compared to the control (282.13 ± 12.38 µmol.min⁻¹.mg⁻¹.protein). At higher concentrations (C2 and C3), AChE activity followed a pattern similar to that observed in gills, decreasing from 282.13 ± 12.38 µmol.min⁻¹.mg⁻¹.protein in the control group to 195.10 ± 13.06 µmol.min⁻¹.mg⁻¹.protein at C3. Overall, the results indicate that exposure to Alto Super 330 EC induced a highly significant inhibition of AChE activity, more pronounced in gills than in digestive glands, with a clear dose-response relationship.

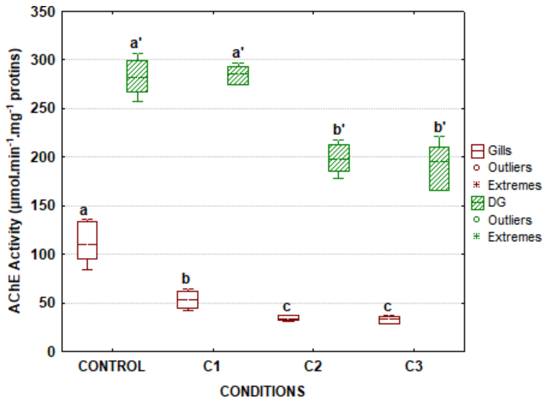


**Figure 5.** Acetylcholinesterase (AChE) Activity (µmol·min⁻¹·mg⁻¹ protein) in the gills and digestive glands (DG) of *Ruditapes decussatus* exposed to Alto super 330EC in the laboratory for 7 days (C = control, C1 = 25 µg·L⁻¹, C2 = 50 µg·L⁻¹, C3 = 100 µg·L⁻¹). (ANOVA; a, b, a', b', c': significant differences at $p < 0.05$, Tukey's HSD test).

### *3.4. Histopathological changes in gills*

In control clams, the gills displayed a normal and well-preserved histological organization. Each gill consisted of two primary gill lamellae folded into secondary lamellae.

The latter were composed of regularly arranged gill filaments with homogeneous length and width. Each gill filament was formed by an epithelium supported by two internal chitinous rods providing structural support. It contained a central hemolymphatic channel and was subdivided into three distinct zones: frontal, intermediate, and proximal. The frontal and intermediate zones were lined with frontal, lateral and latero-frontal cilia, while mucous cells were interspersed among epithelial cells.

The two primary lamellae were connected by loose connective tissue containing lacunae forming hemolymphatic vessels, as well as water tubes separated by intermediate junctions.

These tubes communicated with the pallial cavity through ostia (Fig. 6a).

Exposure of clams to concentration C1 of Alto Super 330 EC induced mild histological changes, mainly of an adaptive nature. The overall gill architecture remained largely intact; however, a moderate reduction in gill filament size, associated with widening of the frontal zone and slight lamellar disorganization, was observed. A moderate hemocyte infiltration within hemolymphatic vessels was also noted (Fig. 6b).

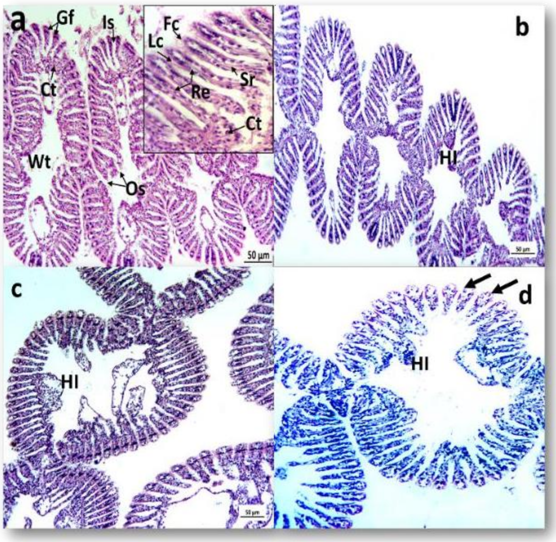


**Figure 6.** Microphotographs of the gills of *Ruditapes decussatus*. Control clams (a), clams exposed to Alto super 330EC at 25 µg·L⁻¹ (b), 50 µg·L⁻¹ (c), and 100 µg·L⁻¹ (d). ((Bc: basophilic cells, Ct: connective tissue, Dc: digestive cells, Hc: hemocytes, L: lumen, Hi: hemocyte infiltration, black arrow: cilia erosion, Gf: gill filament, Wt: Water tubule, Os: ostia, Lc: lateral cilia, Fc: frontal cili, Re: receptory epithelium, Sr: Supporting rod).

At C2 concentration, gills alterations became more pronounced. Gill filaments showed structural deformities, areas of inter-filament fusion, and localized epithelial ruptures (Fig. 6c). The respiratory tissue exhibited multiple lesions, accompanied by partial ciliary erosion and diffuse hemocyte infiltration, indicating a more marked inflammatory response. At the highest concentration C3, histopathological alterations were severe. Gills filaments appeared clearly shorter and wider, with pronounced ciliary erosion compared to controls. Some epithelial cells exhibited clear signs of apoptosis, associated with intense and generalized hemocyte infiltration, indicating major structural and functional impairment of the gills (Fig. 6d).

### *3.5. Histopathological changes in the digestive tract*

In control clams, a major part of the digestive diverticulum consists of a highly convoluted system of primary, secondary, and tertiary tubules. Tertiary tubules, with oval to rounded cross-sections, are the most abundant. The tubule wall, delimited by a basal membrane, is composed of a pseudostratified epithelium comprising two cell types: eosinophilic, vesiculated digestive cells (DCs), and basophilic secretory cells (BCs), pyramidal in shape, intercalated at the base of the wall between digestive cells.

The lumen (L) of the tubules, closed or slightly open, may be narrow or wide depending on the health status and metabolic activity of the animal. Digestive cells account for approximately 60-80% of the tubule wall, with basally positioned nuclei. The digestive tubules are surrounded by connective tissue containing hemocytes and fibrocytes (Fig. 7a). Exposure to 25 µg.L⁻¹ of Alto Super 330 EC induced marked hemocyte infiltration within the digestive tissue, reflecting a pronounced inflammatory response.

Hypertrophy and hyperplasia of basophilic secretory cells were observed, accompanied by tissue disorganization. Several digestive tubules exhibited wall alterations characterized by cytoplasmic vacuolization and partial necrosis of digestive cells. In some areas, the basal membrane appeared ruptured or disrupted, indicating advanced structural damage (Fig. 7b). Exposure to concentration C2 (50 µg.L⁻¹) resulted in more pronounced lesions in the digestive tubules, accompanied by marked dilation of the lumen. Foci of necrosis in digestive cells were observed, characterized by nuclear alterations (Fig. 7c). In individuals exposed to concentration C3 (100 µg.L⁻¹) of Alto Super 330 EC, the digestive tubules displayed a clearly atrophied wall and a markedly dilated lumen. These changes were associated with advanced necrosis of digestive cells, evidenced by the presence of necrotic tissue and cellular debris within the lumen, along with extensive dilation of the tubule lining (Fig. 7d).

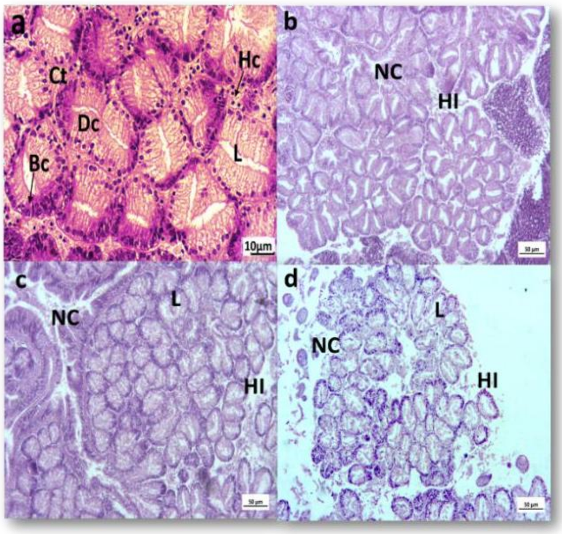


**Figure 7.** Microphotographs of the digestive tubules of *Ruditapes decussatus*. Control clams (a), clams exposed to Alto super 330EC at 25 $\mu g \cdot L^{-1}$ (b), 50 $\mu g \cdot L^{-1}$ (c), and 100 $\mu g \cdot L^{-1}$ (d). (Bc: basophilic cells, Ct: connective tissue, Dc: digestive cells, Hc: hemocytes, L: lumen, Hi: hemocyte infiltration, NC: necrotic cell, black arrow: cilia erosion).

## 4. Discussion

Exposure of *Ruditapes decussatus* to Alto Super 330 EC significantly affected their filtration capacity. The marked increase in filtration rate at the lowest concentration (25 $\mu g \cdot L^{-1}$) suggests a transient stimulation, consistent with an early functional response to moderate chemical stress. At higher concentrations (50 and 100 $\mu g \cdot L^{-1}$), although filtration remained above control levels, it decreased relative to the 25 $\mu g \cdot L^{-1}$ group, indicating a progressive attenuation of this stimulation. Gosiling, (2021) reported that pesticides can significantly disrupt bivalve filtration, with some studies showing an initial increase followed by a decline when exposure is prolonged or intensified. Blaise et al. (2002) also suggested that certain pesticides may elevate the metabolic activity of aquatic organisms, including bivalves, as part of an adaptive strategy to neutralize toxicity and support defense mechanisms. A similar response may occur with triazole fungicides. Consistently, Chmist et al. (2019) observed increased valve opening in the freshwater mussel *Unio tumidus* exposed to tebuconazole, generally associated with enhanced filtration. The reduced stimulation at higher concentrations in our study may thus reflect impairment of branchial function and/or an energetic trade-off, with resources redirected toward detoxification and antioxidant defenses at the expense of basic physiological processes.

Catalase (CAT), a key antioxidant enzyme, protects cells against hydrogen peroxide ($H_2O_2$) toxicity by catalyzing its decomposition into water and oxygen (Jomova et al., 2024). Exposure to pollutants often enhances reactive oxygen species (ROS) production, activating antioxidant defenses, including CAT induction (Regoli and Giuliani, 2011). In the present study, exposure of *R. decussatus* to increasing concentrations of Alto Super 330 EC led to a significant rise in CAT activity in both gills and digestive glands, indicating an adaptive mechanism aimed at limiting $H_2O_2$ accumulation and preserving redox homeostasis. Triazole fungicides, such as propiconazole and cyproconazole, interfere with demethylation processes and may compromise membrane integrity, promoting ROS generation.

The observed CAT induction likely reflects a compensatory response to oxidative stress, consistent with reports in other aquatic organisms (Jin et al., 2011; Sellami et al., 2014; Khazri et al., 2015). However, CAT responses may vary with exposure duration and tissue specificity, with prolonged exposure potentially leading to enzymatic inhibition (Tabassum et al., 2016).

Glutathione S-transferases (GST) play a major role in detoxification by catalyzing conjugation of reduced glutathione (GSH) to electrophilic compounds, enhancing their solubility and elimination, and protecting cells against oxidative damage (Zhang et al., 2019). In our study, GST activity was significantly elevated in exposed clams, indicating activation of biotransformation pathways in response to the fungicide. Previous studies support this interpretation, showing GST induction in fish and bivalves following exposure to triazoles (Egaas et al., 1999; Li et al., 2010).

Malondialdehyde (MDA), a product of lipid peroxidation, serves as a biomarker of oxidative damage to cellular membranes. Increased MDA levels, as observed in our study, indicate intensified lipid peroxidation and membrane injury. Similar elevations have been reported in aquatic organisms exposed to triazoles (Ait Ayat et al., 2010; Zhao et al., 2020; Saha et al., 2025).

Acetylcholinesterase (AChE) is a critical enzyme in neurotransmission, and its inhibition leads to neurotransmitter accumulation and cholinergic overstimulation, potentially causing behavioral and physiological disturbances (Colovic et al., 2013). In our study, exposure to gadoteric acid, Alto Super 330 EC, or their mixture resulted in significant AChE inhibition in gills and digestive glands, suggesting neurotoxic effects or disruption of neuromodulatory processes, consistent with previous observations in fish and bivalves exposed to propiconazole (Pallavi and Ajay, 2015; Formicki et al., 2025).

Histopathological alterations in gills reflected a progressive response to chemical stress. At 25 $\mu g \cdot L^{-1}$, mild morphological changes, including reduced filament size, widening of the frontal zone, and moderate hemocyte infiltration, suggest an adaptive response to maintain gas exchange.

At 50 $\mu g \cdot L^{-1}$, more pronounced lesions filament deformation, epithelial rupture, lamellar fusion, and ciliary erosion indicate impaired structural and functional integrity. At 100 $\mu g \cdot L^{-1}$, gills exhibited severe damage, including shortened and thickened filaments, ciliary erosion, apoptosis, and massive hemocyte infiltration, consistent with overwhelming chemical stress. Similar patterns have been documented in bivalves and fish exposed to pesticides and fungicides (Alesci et al., 2023; Velmurugan et al., 2020).

Overall, these results indicate that Alto Super 330 EC induces dose-dependent alterations in filtration, antioxidant enzyme activity, lipid peroxidation, neurotoxicity, and gill morphology in *R. decussatus*, reflecting the interplay between early adaptive responses and the onset of toxic effects at higher concentrations.

## 5. Conclusion

The present study demonstrates that exposure of *Ruditapes decussatus* to the triazole fungicide Alto Super 330 EC induces a multifaceted toxic response, even at low concentrations. Functional impairment, evidenced by reduced filtration capacity, is accompanied by oxidative stress, as shown by increased activities of catalase (CAT) and glutathione S-transferase (GST) and elevated malondialdehyde (MDA) levels.

Concurrently, significant inhibition of acetylcholinesterase (AChE) indicates potential neurotoxicity affecting cholinergic regulation. Histopathological analyses corroborate these biochemical findings, revealing progressive structural damage in gills and digestive tissues, ranging from mild adaptive changes to severe necrosis and apoptosis at higher concentrations. Collectively, these results suggest that Alto Super 330 EC poses a significant risk to clam health and functionality, highlighting the need for careful monitoring of triazole fungicides in aquatic environments.

The observed responses spanning physiological, biochemical, neurotoxic, and histological endpoints underscore the importance of integrated biomarkers in assessing sublethal and chronic effects of environmental contaminants on bivalves.

**Acknowledgments**:

The authors would like to thank the two anonymous reviewers for their valuable comments and constructive suggestions.

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