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EFFECTS OF GREEN SYNTHESIZED PHOSPHONOAMIDE AND PHOSPHONOCAPROLACTAM COMPOUNDS IN THE CLAMS *RUDITAPES DECUSSATUS*

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Abstract: Derivatives of phosphonoamide and phosphonocaprolactam are well-known for their extensive uses in industrial and medical settings. This has raised worries regarding their toxicity to aquatic life as well as their fate in the ecosystem. Thus, for seven days, biomarkers of oxidative stress, neurotoxicity, and metal accumulation were assessed in clams *Ruditapes decussatus* exposed to 20 and 40 µg/L of N-(2-(diphenylphosphoryl)-2-(thiophen-2-yl) ethyl) acetamide, N-(2-(diphenylphosphoryl)-2-(furan-2-yl) ethyl) acetamide, N-(2-(diphenylphosphoryl)-2-phenylethyl) acetamide, and 7-((diphenylphosphoryl) (phenyl)methyl) azepan-2-one. The results of the metals content analysis indicate that phosphonoamide and phosphonocaprolactam caused metal dyshomeostasis in the digestive gland and gills of clams, with greater levels of zinc and copper detected in the digestive glad following exposure. Phosphonoamide and phosphonocaprolactam derivatives activated biomarkers of oxidative stress, such as GSH, CAT, and MDA, in an organ, concentration, and biomarker dependent manner, exhibiting distinct antioxidant patterns in the digestive glands and gills. By the time the exposure period for both types of phosphorus compounds came to a conclusion, neurotoxic effects were evident in the form of AChE inhibition. Our results demonstrate that filter-feeding species should be taken into consideration when assessing the overall toxicological impact of phosphorus compounds in the aquatic environment. Overall, this exploration was anticipated to yield insights into the distinct responses triggered by these compounds and offer a deeper understanding of the toxicity mechanisms associated with phosphonoamide and phosphonocaprolactam. Additionally, the study highlights the necessity for continuous monitoring of potentially toxic phosphorus compounds in edible marine species to avoid possible repercussions of seafood consumption on human health.

Keywords: Phosphonoamides; phosphonocaprolactams; metals accumulation; clams *Ruditapes decussatus*; Biomarkers.

1. INTRODUCTION

Sentinel species such as *Ruditapes decussatus* are used in marine biomonitoring programs (Amiard-Triquet et al., 2015; Amiard-Triquet & Berthet, 2015). The bivalve *Ruditapes decussatus* is a member of the Venus clam family (Veneridae). The above description emphasizes this marine species' ecological and economic relevance, especially given its tight relationship to the Mediterranean Sea's water column and sediment. Due to the species' high nutritional and economic value, it is commercially bred and used in aquatic toxicity research. This species is frequently found in coastal habitats, which act as the main holding tanks for organic contaminants like pesticides and medications. Background data on pharmaceuticals and medications found in bivalves

shows that the dispersion of these substances in marine environments has increased significantly. The significance of comprehending the possible effects of these organic contaminants on marine animals and the larger ecosystem is emphasized by this knowledge. In order to lessen the possible negative impacts of these contaminants on the species and the marine ecosystem, it also emphasizes the necessity of continuing research and monitoring. For instance, the discovery of azithromycin and hydroxychloroquine in aquatic systems during COVID-19 has posed a significant risk to hospital waste management. As a result, researchers emphasized the necessity of studying how COVID-19 affects aquatic organisms by looking at the dangers of face masks for wildlife (Patrício Silva et al., 2021) as well as how COVID-19 affects pharmaceutical drugs used in aquatic systems (Kumari & Kumar, 2022), such as the aquatic varieties' toxicological evaluation of azithromycin and hydroxychloroquine (Mendonça-Gomes et al., 2021; Luz et al., 2021). Since it was discovered that amides might be utilized to block the SARS-CoV nsp5 protease, researchers have recently developed a greater interest in researching the effects of amides (Chen et al., 2022).

This study presents the synthesis of certain phosphonoamide and phosphonocaprolactam derivatives via the Beckmann rearrangement of γ-phosphonyloximes, which is facilitated by diethyl chlorophosphate, as reported by Wahbi et al. (2015). The well-known, extensive uses of phosphonoamides in business and medicine account for these chemicals' high value. Indeed, phosphonoamides have been one of the most researched and utilized substances in the chemical and pharmaceutical sectors for more than a century in an effort to enhance societal development and quality of life (Solodenko & Kukhar, 1989; Gutman & Freiberg, 1972). Numerous chemical and pharmaceutical products contain phosphonoamides, which are widely recognized for their antiviral, antibacterial, and insecticidal properties (Solodenko et al., 1989; Polyak et al., 1987; Gutman & Freiberg, 1970; Gutman & Freiberg, 1972). Some are also helpful as antiwear and friction-reducing additives for lubricants and liquid hydrocarbon fuels (Howie et al., 1982), as well as plasticizer agents (McConnell et al., 1959). Phosphocalactams, in fact, are well-known for their applications as modifying additives in the manufacture of polyamide-6, a phosphorus-containing material with enhanced flame retardancy, thermo- and thermooxidative resistance (Mateva et al., 1993). In the aquatic and marine environments, the prevalence of organophosphorus compounds (OPs) and their metabolites began to rise (Nowack, 2003).

This enhancement hinders the growth of algae and leads to the eutrophication of water (Wang et al., 2019; Wang et al., 2020). It also remobilizes heavy metals. The aquatic environment is home to several organophosphorus chemicals, and this study is the first to report on the impacts of phosphonoamides and phosphonocaprolactams. According to Hernandez et al. (2019), these organophosphorus compounds (OPs) are found in both municipal and industrial sewer water after it is released from sewer water treatment. According to earlier research, organophosphorus compounds (OPs) have a strong propensity to adsorb onto sediments and suspended debris and remobilize metals, which is why they are not quantified (Wang et al., 2019; Saidani et al., 2021). Saidani et al. (2019) reported an experimental test where doses ranging from 20 to 40 μg/L demonstrated the activation of an oxidative system mechanism. Marine life may be impacted by the variable ambient hazardous product's effects on an animal's physiological and biochemical state (Saidani et al., 2021).

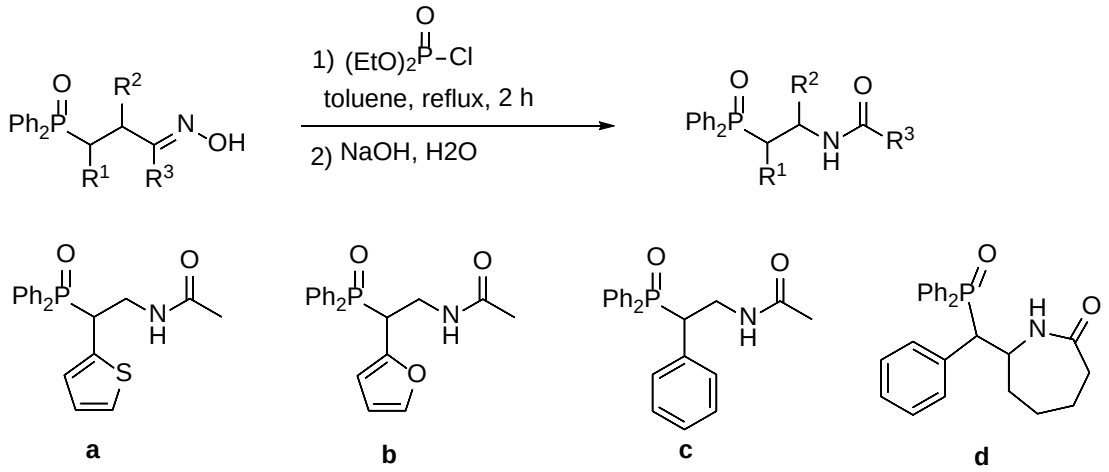
For testing the toxicity of organophosphorus compounds (OPs), *Ruditapes decussatus* are appropriate bioindicators in the coastal environment of Tunisia due to their wide distribution, lengthy life cycle, and filter-feeding behavior. Previous studies in our lab have demonstrated that γ-aminophosphonates, γ-aminophosphine oxides, and certain OPs have extraordinary sensitivity in *Ruditapes decussatus* (Sellami et al., 2015a; Aouani et al., 2017; Saidani et al., 2021).

While previous studies have provided insights into the effects of organophosphorus on aquatic organisms, there is still a substantial amount of information lacking about the possible effects of phosphonoamide and phosphonocaprolactam counterparts. The goal of the current work was to address this gap by examining *Ruditapes decussatus*'s reaction to exposure to phosphonoamide and phosphonocaprolactam derivatives. The effects were assessed using metal content, oxidative stress biomarkers, membrane cell damage, and neurotoxicity in order to provide a systematic study.

2. MATERIALS AND METHODS

2.1. Chemistry

Using the method described by Wahbi & Touil (2015), four phosphonoamide and phosphonocaprolactam molecules (a, b, c, and d) were synthesized (Scheme 1). By mussel tissue (CRM 278) as a reference, the metallic contents (Cu, Zn, Ni, and Co) in the mussels after they were exposed to phosphonoamide and phosphonocaprolactam were examined by flame atomic absorption spectrometry (Thermo Scientific ICE 3300 AA Spectrometer).



Scheme 1. Synthesis of phosphonoamide and phosphonocaprolactam derivatives (a-d)

2.2. Experimental Design

Individual Mediterranean clams (*Ruditapes decussatus*) of similar shell length (i.e., 3 ± 0.5 cm) were collected manually in Mars 2023 from Menzel Jemil at Bizerte lagoon, in North Tunisia (37°13′19.26″N 9° 55′46.24″E). Clams were kept for a week on a 12-hour light/dark cycle after being immediately acclimated in aerated 3-liter glass tanks. Five replicates of five clams per tank were set up in five tests following the acclimatization phase. The conditions are as follows: a1 and a2 represent clams exposed to 20 and 40 µg/l of N-(2-(diphenylphosphoryl)-2-(thiophen-2-yl) ethyl) acetamide; b1 and b2 represent clams exposed to 20 and 40 µg/L of N-(2-(diphenylphosphoryl)-2-(furan-2-yl) ethyl) acetamide; c1 and c2 represent clams exposed to 20 and 40 µg/l of N-(2-(diphenylphosphoryl)-2-phenylethyl) acetamide; and d1 and d2 represent clams exposed to 20 and 40 µg/L of 7-((diphenylphosphoryl)(phenyl)methyl) azepan-2-one. The concentrations under consideration were chosen based on the environmental relevant phosphonate concentrations reported by Wang and al. (2019) as well as on earlier research carried out by Saidani et al. (2022). For a week, both treated and untreated clams were subjected to consistent amounts in saltwater. The setup of the environment followed the guidelines provided by Sellami et al., (2015a) and Saidani et al. (2021).

2.3. Metal content determination following exposure

To find the dry weight, specimens (n = 5) were dehydrated in an oven at 80 °C until they reached a consistent weight. Each sample was treated to a mixture of 69% HNO_3, 30% H_2O_2, and H_2O during the digesting process. The digesting process was run in a microwave. The samples were diluted with ultrapure water once they had cooled. Mussel's tissue (CRM 278) was used as a reference sample. The tissue samples were subjected to analysis for zinc (Zn), copper (Cu), cobalt (Co) and nickel (Ni) using inductively coupled plasma atomic emission spectroscopy, carried out with a Varian Vista MPX instrument. To ensure quality, standard solutions were measured after every 15 samples. The limits of detection (LODs) were 0.15 mg/kg for Co, 0.22 mg/kg for Cu, 0.18 mg/kg for Ni, and 0.03 mg/kg for Zn. The limits of quantification (LOQs) 0.5 mg/kg for Co, 0.73 mg/kg for Cu, 0.6 mg/kg for Ni, and 0.1 mg/kg for Zn.

2.4. Biochemical parameters measurement

One week later, the digestive gland and gills' proteins were removed, and measurements and calculations were made for the antioxidant activity, lipid peroxidation, and neurotoxicity. Aebi (1974) provided the methodology for measuring CAT activity. The GSH levels were measured using the Benke et al. (1974) technique.

Using the Buege and Aust (1978) approach, the malondialdehyde content (MDA) was expressed in terms of thiobarbituric acid reactive species (TBARS). At 535 nm, the absorbance was measured. Using the Ellman et al. (1961) methodology, acetylcholinesterase (AChE) activity was assessed by measuring the rise in the sample's absorbance at 412 nm.

2.5. Statistical Analysis

Standard deviation (SD) ± mean was used to express the data. STATISTICA 8 was used to conduct the statistical analysis. One-way ANOVA was used to examine the differences in biochemical parameters across treatments in comparison to the control group. A 95% confidence interval was used to determine significance at a probability level of $p < 0.05$.

3. RESULTS

3.1. Metals Content in gills and digestive gland

Chemical tests revealed that, following a week of exposure, all compounds release fewer metals from the gills and digestive gland, with larger quantities of zinc found in the gills of clams treated with (a1), (d1), and (d2) (Table 1), as well as in the digestive gland of clams treated with compound b, as depicted in Figure 1. Cu concentration has a similar pattern, with the digestive glands of clams exposed to compound (c) registering the highest concentration.

3.2. Biomarkers responses in clams exposed to phosphorus compounds

Data on biomarker responses in clams exposed to phosphorus compounds are summarized in Figures 2, 3, 4, and 5.

Glutathione (GSH) levels were changed significantly only in the gills of clams exposed over one-week to phosphorus compound (Figure 3). In fact, (a_2) and (b_2) increased significantly ($p = 0.0023$ and $p = 0.0038$) the GSH level in gills from 3.92 (control) to 5.86 and 4.05 units per mg protein. GSH level increased also in gills to 5.54 units per mg of protein after exposure to (d_1) and (d_2).

The bioassay with *R. decussatus* revealed a significant ($p = 0.0027$ and $p = 0.0018$) increased value for catalase activity in gills and digestive gland with 1.6 and 2.9 µmol/min/mg protein, respectively, when clams are exposed to a_1. These levels decreased after exposure to the highest concentration in the digestive gland. Conversely, CAT activity increases significantly with the increasing level of phosphorus compounds in both organs, like shown in Figure2.

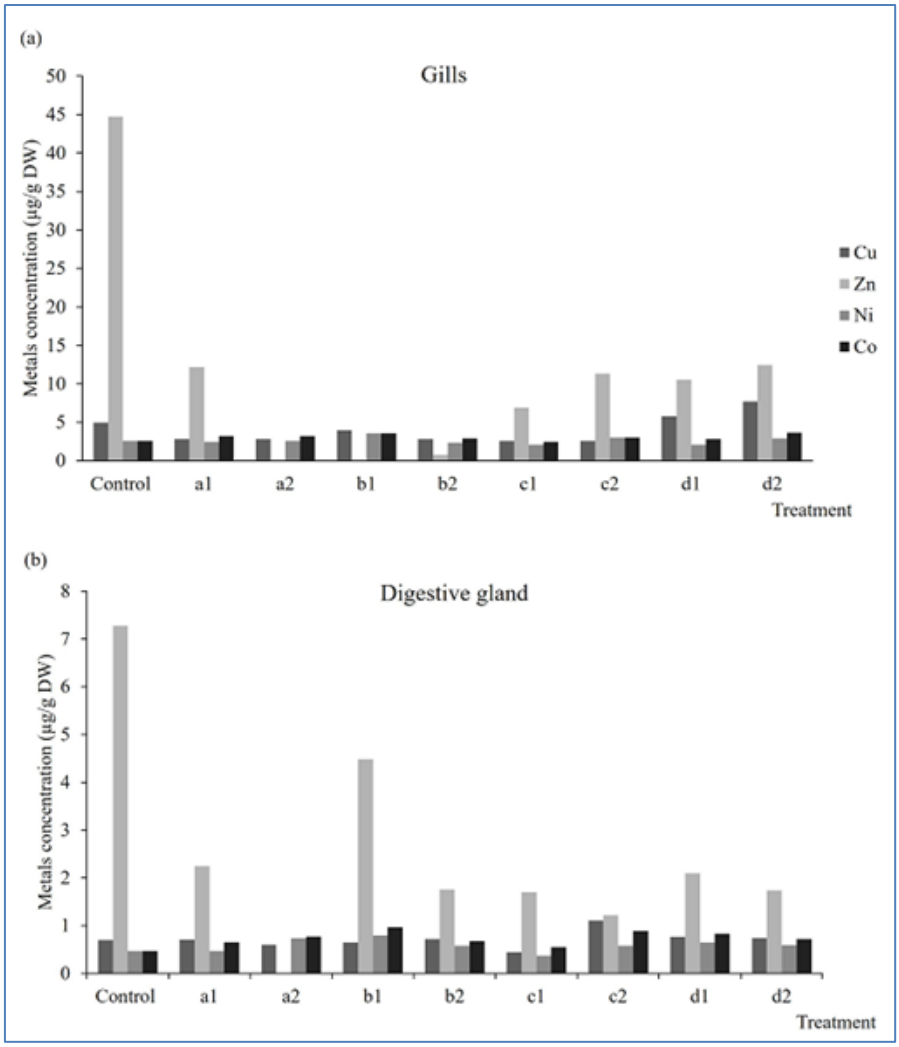


Figure 1. Mean (n = 5) of Zn, Cu, Co and Ni concentration (µg/g DW) in gills (a) and digestive gland (b) of untreated clams and clams treated with 20 and 40 µg/L of Phosphonoamides and Phosphonocaprolactams N-(2-(diphenylphosphoryl)-2-(thiophen-2-yl) ethyl) acetamide, N-(2-(diphenylphosphoryl)-2-(furan-2-yl) ethyl) acetamide, N-(2-(diphenylphosphoryl)-2-phenylethyl) acetamide and 7-((diphenylphosphoryl)(phenyl)methyl) azepan-2-one.

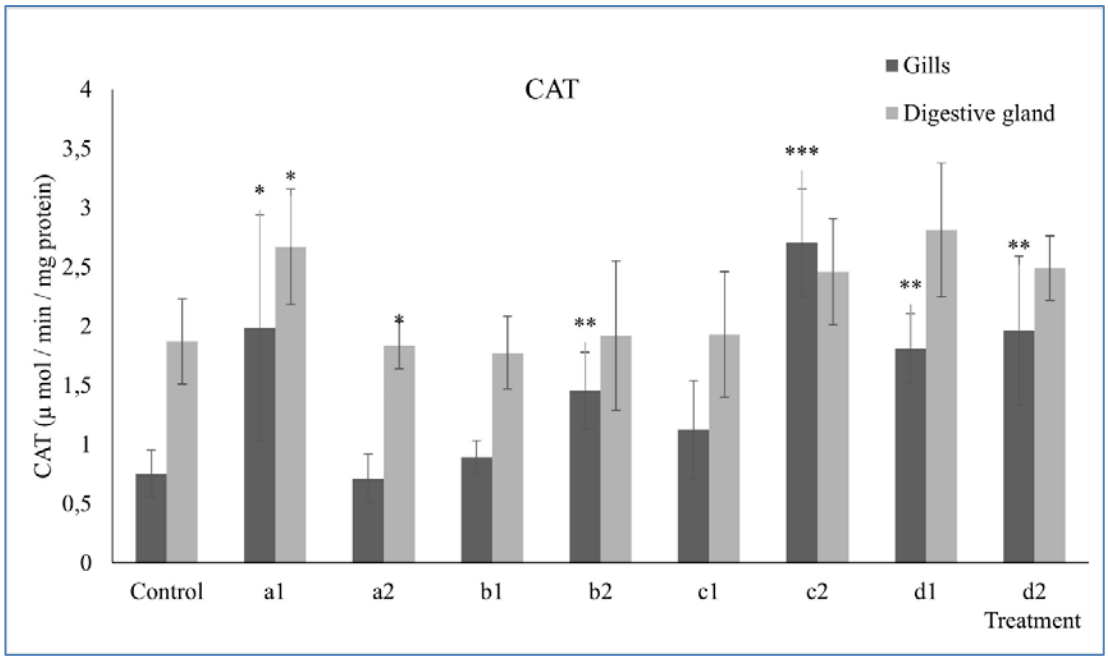


Figure 2. Catalase (CAT) activity in gills and digestive gland of untreated and treated clams with 20 and 40 µg/l of Phosphonoamides and Phosphonocaprolactams N-(2-(diphenylphosphoryl)-2-(thiophen-2-yl) ethyl) acetamide, N-(2-(diphenylphosphoryl)-2-(furan-2-yl) ethyl) acetamide, N-(2-(diphenylphosphoryl)-2-phenylethyl) acetamide and 7-((diphenylphosphoryl)(phenyl)methyl) azepan-2-one. Values are means ± SD (n = 5). *are significantly different at p<0,05 compared to relative controls (ANOVA, post-hoc, Tukey HSD test, STATISTICA s 8.0).

Table 1. Heavy metal concentrations in fish and surface water samples

Digestive gland	Control	a1	a2	b1	b2	c1	c2	d1	d2
Cu	0,69	0,70	0,60	0,64	0,72	0,45	1,11	0,76	0,74
Zn	7,27	2,25	0.01	4,48	1,75	1,70	1,22	2,09	1,74
Ni	0,47	0,46	0,74	0,79	0,58	0,37	0,58	0,65	0,59
Co	0,47	0,65	0,77	0,96	0,67	0,55	0,89	0,83	0,72

Acetylcholinesterase (AChE) activity decreased significantly ($p < 0.05$) in both organs after exposure to (a) compound, then increased in gills of clams exposed to a_2 compared to control and to a_1 from 5.45 and 3.08 µmol/min/mg protein (Figure 5). Similar AChE activities were also registered when testing both (b) and (c) compounds. Conversely, neurotoxicity bioassay with *R. decussatus* showed that (d) compounds affected AChE activity when this enzyme decreased from 6.39 to 2.35 µmol/min/mg protein in gills ($p = 0.00018$) and from 4.59 µmol/min/mg protein in clams exposed to (d_1) to 3.86 µmol/min/mg protein in digestive gland ($p = 0.0025$).

Malondialdehyde (MDA), used as a lipid peroxidation marker, the results show that a one-week exposure to (a) and (b) compounds has no effect on MDA level in both gills and digestive gland. At the lowest (d) compound concentration, MDA level has significantly changed only in the digestive gland following the same temporal period. MDA level decreased significantly from 1.73 units per mg of protein in the control group to 1.32 units per mg of protein in clams treated with c_1 to 1.16 units per mg of protein exposed to d_2. MDA level has significantly ($p = 0.0016$) changed only in gills after one week of exposure to the (c) compound at 20 mg/L by decreasing from 1.8 units per mg of protein to 0.75 units per mg of protein and no significant trend ($p = 0.35$) for this biomarker in digestive gland (Figure 4).

4. DISCUSSION

This study created and approved an in vivo assay to track the activity of phosphonates in marine water clams. Using these species, four candidate compounds are screened for their plasticizing agents, pesticide applications, and inhibition of bacterial and viral activities.

The utilization of clams, particularly *Ruditapes decussatus*, is widespread in ecotoxicological research and diverse surveillance initiatives. The effects of several phosphonoamide and phosphonocaprolactam

derivatives, specifically N-(2-(diphenylphosphoryl)-2-(thiophen-2-yl) ethyl) acetamide (a), N-(2-(diphenylphosphoryl)-2-(furan-2-yl) ethyl) acetamide (b), N-(2-(diphenylphosphoryl)-2-phenylethyl) acetamide (c), and 7-((diphenylphosphoryl) (phenyl)methyl) azepan-2-one (d) are evaluated in this study using the Mediterranean clams *Ruditapes decussatus*.

According to Pearson's 1963 theory, ligands based on S, Se, and Te are more attracted to "soft" metals like Co, Cu, Fe, and Zn, but phosphonates and phosphine oxides with oxygen as a donor atom are

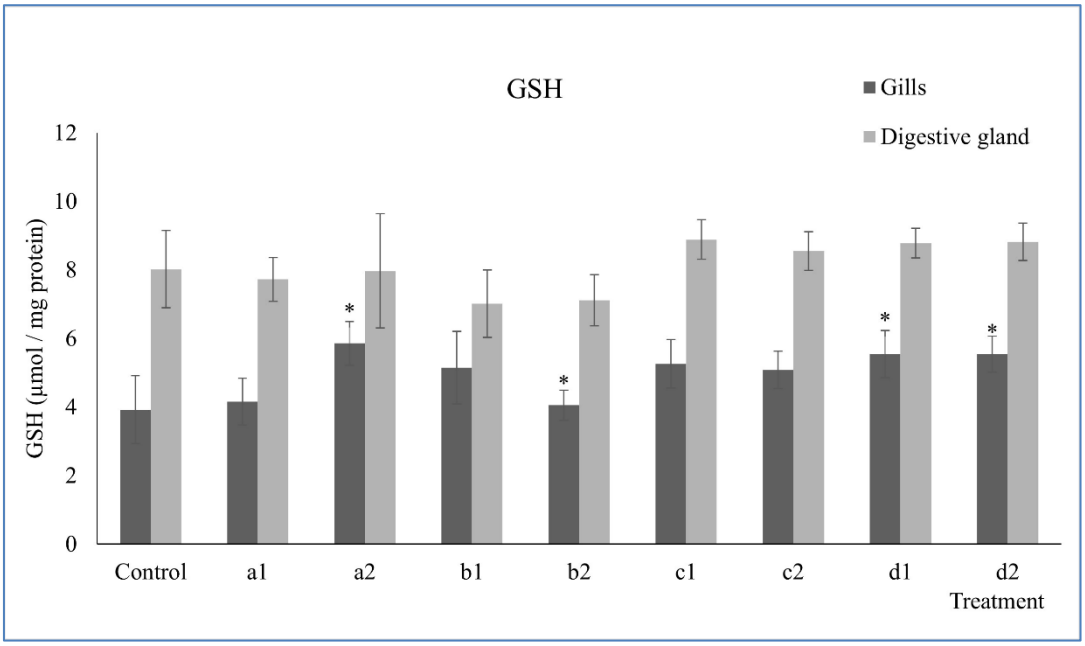


Figure 3. Thiol gluthatione GSH content in gills and digestive gland of untreated and treated clams with 20 and 40 µg/l of Phosphonoamides and Phosphonocaprolactams N-(2-(diphenylphosphoryl)-2-(thiophen-2-yl) ethyl) acetamide, N-(2-(diphenylphosphoryl)-2-(furan-2-yl) ethyl) acetamide, N-(2-(diphenylphosphoryl)-2-phenylethyl) acetamide and 7-((diphenylphosphoryl)(phenyl)methyl) azepan-2-one. Values are means ± SD (n = 5). *are significantly different at p<0,05 compared to relative controls (ANOVA, post-hoc, Tukey HSD test, STATISTICA s 8.0).

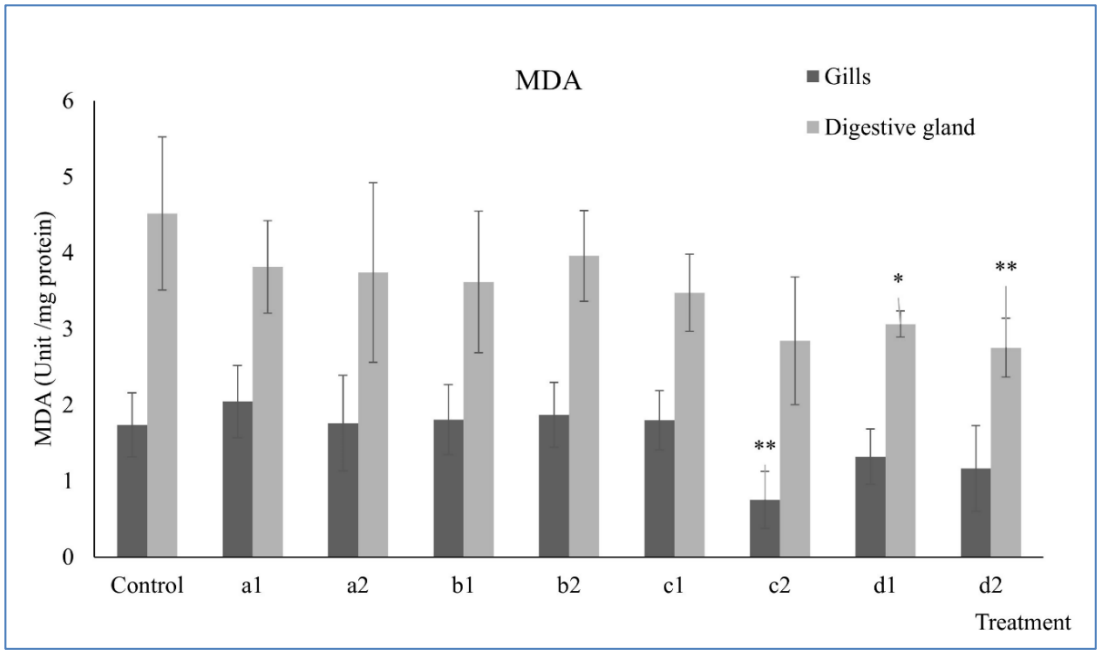


Figure 4. malondialdehyd (MDA) content in gills and digestive gland of untreated and treated clams with 20 and 40 µg/l of Phosphonoamides and Phosphonocaprolactams N-(2-(diphenylphosphoryl)-2-(thiophen-2-yl) ethyl) acetamide, N-(2-(diphenylphosphoryl)-2-(furan-2-yl) ethyl) acetamide, N-(2-(diphenylphosphoryl)-2-phenylethyl) acetamide and 7-((diphenylphosphoryl)(phenyl)methyl) azepan-2-one. Values are means ± SD (n = 5). * are significantly different at p<0,05 compared to relative controls (ANOVA, post-hoc, Tukey HSD test, STATISTICA s 8.0).

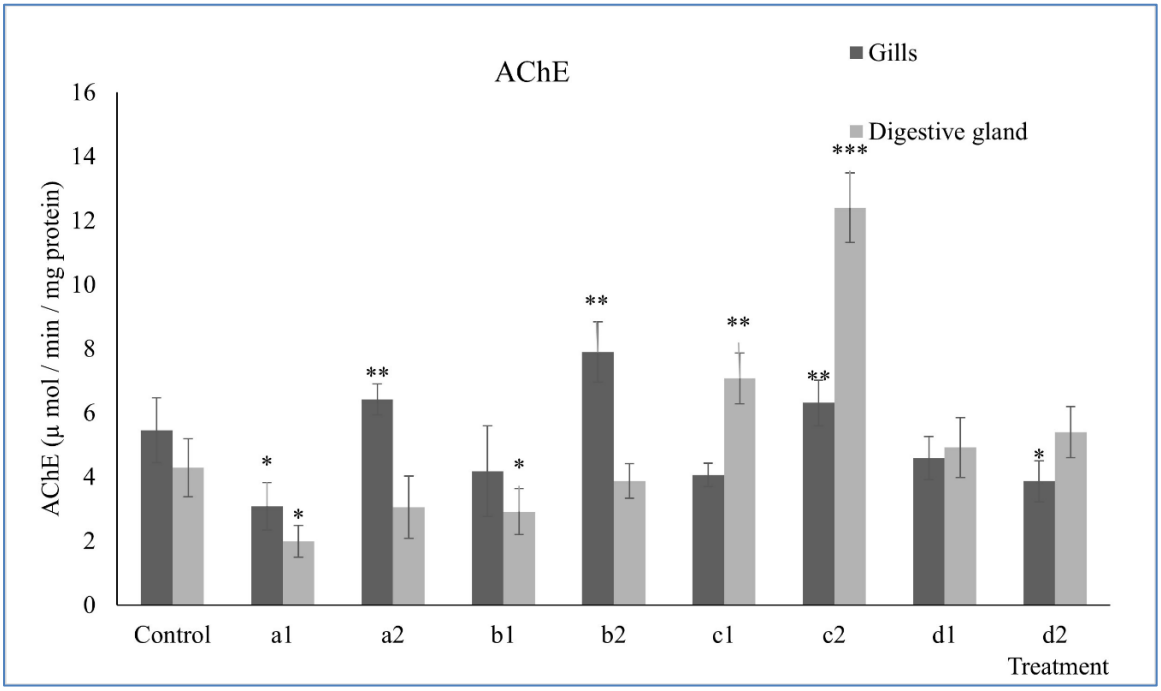


Figure 5. Acetylcholinesterase (AChE) activity in gills and digestive gland of untreated and treated clams with 20 and 40 µg/l of Phosphonoamides and Phosphonocaprolactams N-(2-(diphenylphosphoryl)-2-(thiophen-2-yl) ethyl) acetamide, N-(2-(diphenylphosphoryl)-2-(furan-2-yl) ethyl) acetamide, N-(2-(diphenylphosphoryl)-2-phenylethyl) acetamide and 7-((diphenylphosphoryl)(phenyl)methyl) azepan-2-one. Values are means ± SD (n = 5). *are significantly different at p<0,05 compared to relative controls (ANOVA, post-hoc, Tukey HSD test, STATISTICA s 8.0).

more attracted to "hard" metals like those same metals. This study demonstrates that in polluted clams, the examined organophosphorus ligands' complexation rate with metals is comparatively lower than in untreated clams. The acquired results demonstrate that phosphonoamide and phosphonocaprolactam derivatives under study show a significant inhibitory tendency in the digestive gland and gills, and that phosphocaprolactam (d) has a protective effect against damage to cell membranes.

An eight-membered chelate sustained by electrostatic bonds between the metal ion and the two oxygen atoms or a more stable six-membered chelate stabilized by electrostatic bonds between the metal ion, the phosphoryl oxygen, and the nitrogen atom would be formed by a phosphono-amide/ caprolactam ligand. On the other hand, they would act like monodentate ligands and result in a polymer chain that is stabilized by electrostatic bonds that form between the metal ion, the oxygen of phosphoryl, and the oxygen of an additional amide or phosphoryl motif.

The complexation affinity for phosphonoamide and phosphonocaprolactam ligands with different metals (Co, Cu, Fe, and Zn) in the digestive gland is significant when compared to non-treated clams, according to our data from the atomic absorption analysis. In fact, it is observed that the phosphorus-containing ligands' complexation rate was proportionate to their quantity in both organs. The phosphono-amide/caprolactam ligands would preferably form a six-membered chelate supported by electrostatic bonds between the metal ion, the phosphoryl oxygen, and the nitrogen atom.

The exposure to phosphonoamide/ caprolactam notably affected the activity of multiple biochemical indicators (CAT, GSH, MDA, and AChE) in the digestive gland and gills of *R. decussatus*. All examined substances, whether phosphonoamide or phosphonocaprolactam derivatives, are proven to be bioavailable through metal analysis and the presence of biochemical indicators in the gills and digestive glands. To support this theory, additional bioaccumulation research is necessary. It is commonly recognized that phosphonates can enhance the generation of reactive oxygen species (ROS), such as superoxide anion radicals, which the enzyme superoxide dismutase converts to oxygen and hydrogen peroxide (H_2O_2). The catalase enzyme (CAT) will detoxify excess H_2O_2 (Lassoued et al., 2023). The outcomes corroborate the earlier findings presented by Saidani et al. (2021) and Lassoued et al. (2023). Figure 1 demonstrated a notable rise in CAT activity in the clams' digestive gland and gills in response to a1, indicating that this substance produces ROS. The clams' gills treated by c1, c2, and b2 show the same outcomes. The tendency in CAT activity is consistent with Hanna & Shekha (2024), who found

that organophosphates pesticides increased CAT activity. Similarly, Serdar et al. (2023) observed an increase in the CAT activity and related this response to increased ROS formation occurring due to organophosphate toxicity. Similar trends were observed by Zhong et al. (2024) on the mussel *Mytilus coruscus* exposed to organophosphate. The authors observed an increase in CAT activity following exposure and related this response to the increased oxidative stress that indicates that organophosphate could cause adverse effects on mussels. The tissue-specificity of the catalase reaction is demonstrated by this investigation. The identical outcomes were noted by Sellami et al. (2015b) and Regoli & Principato (1995) for *Mytillus galloprovincialis* exposed to copper and *Venerupis decussata* subjected to anthracene, respectively. Since there was no discernible difference between the CAT enzyme levels and the control group ($p > 0.05$), the clams' effective defensive mechanism may be indicated by their lower CAT enzyme levels; however, the current study did not thoroughly examine this state because the ROS levels were not examined. GSH is an antioxidant that, according to Cnubben et al. (2001) and Peña-Llopis et al. (2003), is crucial for scavenging reactive oxygen species (ROS). These results demonstrate that the primary barrier against hazardous products is the gills, and that none of the investigated substances from the phosphonoamide group have any effect on the digestive gland. The information demonstrates that no type of amide utilized can impact clam defense. Indeed, the tissues of aquatic species have significant quantities of GSH, an endogenous antioxidant. According to this research, phosphonocaprolactam (d) only considerably ($p <0.05$) raised the GSH level in the gills; no discernible change was seen in the digestive gland. This rise suggests that phosphonocaprolactam toxicity is being avoided by activating the antioxidant defense mechanism. Our results are in good agreement with Boughoula et al. (2024), who suggested that the monitoring of stress biomarkers (GSH) confirms the activation of antioxidant defense systems to eliminate organophosphorus to tolerate stressful conditions. Authors confirm this hypothesis since it has been observed that all the molecules induce a cellular response marked by an increase in the level of GSH according to increasing organophosphorus concentrations. The projected ecotoxicity of caprolactam, in contrast, demonstrated that there was no appreciable danger associated with the compound's toxicity to fish, algae, and daphnids (Kim et al., 2021). However, only when clams are exposed to the maximum concentration of phosphonoamide (c) and phosphonocaprolactam (d) in their gills can the reduced level of MDA in the digestive gland be observed. The protective effect of phosphonocaprolactam against xenobiotics found in natural seawater may account for this outcome. The recent findings validate and corroborate this theory. Thus, it is conceivable to speculate that capsrolactams and phosphono-amides likely have antioxidant defense and harm. Additionally, as a sign of phosphonoamide and phosphonocaprolactam contamination, the neurotoxic effect was evaluated. Organophosphates are known to be neurotoxic because they reduce the activity of cholinesterase enzymes and cholinergic synapses (Boughoula et al., 2024). In addition, inhibition of AChE has predominantly been associated with the toxic effect of organophosphorus (Cenov et al., 2024). Our data are in good agreement with Boughoula et al. (2024), who suggested that liposolubility of organophosphorus makes them pass through the cuticle and digestive walls of the target organisms to reach the nerve centers leading to AChE inhibition. Belhassen et al. (2024) have also documented that organophosphate significantly impairs the neurotransmission process, as evidenced by the inhibition of acetylcholinesterase (AChE) activity. The data show that exposure to phosphonoamides for one week greatly increases the activity of AChE, primarily in the gills. Schallreuter et al. (2004) and Lassoued et al. (2023) corroborated these findings, indicating that an increase in ROS levels in aquatic species is accompanied by a decrease in AChE. Results for metal levels indicate that AChE activity is impacted by osmotic stress.

5. CONCLUSION

Derivatives of phosphonoamide and phosphonocaprolactam showed harmful effects in clams that were mainly linked to oxidative stress; however, this could have been largely explained by ROS overproduction after acute exposure. The type of phosphorus utilized determines the different antioxidant efficacy, metal concentration, and MDA induction in clam tissues, which reflects the different metabolic functions of the two tissues. All things considered, this work adds to the body of knowledge regarding the toxicity of phosphonoamide and phosphonocaprolactam to bivalve species, emphasizing their ability to cause oxidative stress and their disparate behaviors in various organs. To completely understand whether the observed oxidative stress and neurotoxicity are only attributable to the free ions dissolved from the phosphonoamide and phosphonocaprolactam, or to a combination with the intrinsic effects of these forms, more research is necessary to fully address phosphorus-induced toxicity. Additionally, it implied that phosphonocaprolactams might be helpful in biomonitoring programs since they have a protective effect against damage to cell membranes. Further

investigation, including immunotoxic, histopathological, and genomic effects at different concentrations and different exposure times with different aquatic species to phosphonoamide and phosphonocaprolactam, is needed to better understand the environmental impact of these chemicals on the marine ecosystem.

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