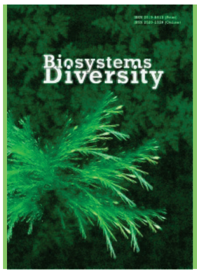

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Assessing the effect of glyphosate on the shrimp *Palaemon adspersus*: Acute toxicity and biomarker responses

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Glyphosate, a widely used agricultural herbicide, poses a risk of aquatic contamination. This study assessed the acute toxicity of glyphosate in the shrimp *Palaemon adspersus* (Decapoda, Palaemonidae). The sublethal (LC$_{10}$ and LC$_{25}$) and lethal (LC$_{50}$ and LC$_{90}$) concentrations were estimated after 24 and 96 hours of exposure. The compound was added to rearing water at LC$_{25}$ and LC$_{50}$ for 96 hours during the exposure phase (24, 48, 72, and 96 hours). Shrimp were then transferred to clean seawater and collected during the recovery phase (24, 48, 72, and 96 hours). Enzymatic activities in shrimp heads and flesh fragments were measured for acetylcholinesterase, gluthathione S-transferase, and malondialdehyde, followed by lipid quantification. Toxicological data indicated the toxicity of glyphosate against shrimp, exhibiting a dose-response effect. Lethal concentrations LC$_{10, 25, 50, 90}$ were 1.15, 1.25, 1.35, 1.59 mg/L after 24 hours and 0.99, 1.06, 1.14, 1.31 mg/L after 96 hours. Two-way ANOVA during the treatment phase showed significant ($P < 0.05$) effects of glyphosate concentration and treatment time on all the biomarkers. During the recovery phase, shrimp compensated for herbicide effects, demonstrating acute toxicity that caused oxidative stress and neurotoxic effects at sublethal concentrations. Careful control is recommended to minimise the negative impacts on non-target aquatic organisms.

Keywords: Decapoda; herbicide; glyphosate; toxicity tests; biomarkers; biochemical responses.

Introduction

The primary environmental challenges facing the Mediterranean Sea coastline include urbanisation, sewage and urban runoff, waste disposal, industrial discharge, maritime transportation, sand erosion and eutrophication (Boukari et al., 2021; Sebbih et al., 2023). Pesticides are crucial tools for improving crop yields in agricultural fields (Zhang et al., 2017), and play an irreplaceable role in disease vector control (Wang et al., 2022). They boost agricultural product productivity and gross output, while reducing crop losses caused by pests, plant diseases, and weeds. However, their long-term and intemperate applications worldwide can potentially disturb the homeostasis of the natural environment and threaten the health of humans and other organisms (Lieshchova et al., 2018; Bilan et al., 2019; Kozak et al., 2020). They also affect macroinvertebrates and microorganisms in aquatic environments (Gull et al., 2019). A study of the impact of pesticides on ponds revealed that, regardless of the pesticides used, the number of applications made, or the rate at which they were applied, there were still significant direct negative effects on various groups of invertebrates, such as amphibians (Ruiz de Arcaute et al., 2020), fish (Bonifacio et al., 2020), and crustaceans (Parlapiano et al., 2021). However, their use also affects human and environmental health.

Glyphosate, also known as N-(phosphonomethyl) glycine, is a systemic herbicide introduced in 1971 (Fabrello et al., 2020). Registered under CAS number 107-83-6 with the chemical formula $C_3H_8NO_5P$, it originated from glycine and encompasses three functional groups: carboxylic acids, phosphonic acids, and amines. Functioning as an active ingredient in non-selective, broad-spectrum herbicides, glyphosate is widely used. This herbicide, penetrating leaves and systemically reaching roots, is a key component in various formulations. Commonly comprised of isopropylamine salt, a surfactant (typically polyethoxylated tallowamine), and water, these formulations contribute to its global application. Glyphosate serves as the primary component in over 750 herbicides, with an annual usage ranging from 0.6 million to 1.2 million tons globally (Rodriguez-Gil et al., 2017). According to findings of Clapp (2021), the application of glyphosate-based herbicides is a common agricultural practice globally. They are generally applied before sowing and as a pre-harvest drying treatment to accelerate and standardise the ripening process (De Carvalho et al., 2020). The herbicidal activity of glyphosate constrains plant growth by inhibiting 5-enolpyruvylshikimate-3-phosphate synthase, a key enzyme in the biosynthesis of aromatic amino acids, such as phenylalanine, tyrosine, and tryptophan. Study of the literature reveals that glyphosate residues and their metabolites have been commonly detected in surface waters and can cause adverse effects on non-target organisms, including fish, molluscs and crustaceans (Robichaud & Rooney, 2021). This is mainly due to the fact that glyphosate is not metabolised in the plant and root systems distribute this herbicide into deep soil layers where microorganism activity is relatively low. Therefore, its persistence and transport in the soil depends on its composition, climatic conditions, and microbial activity (Mirella da Silva, 2018).

The use of biochemical biomarkers to assess toxicity effects under controlled laboratory conditions remains a useful approach to provide indications of xenobiotic toxicity (Santana et al., 2022). Thus, there are biomarkers indicative of neurotoxic responses, such as acetylcholinesterase, a neurotransmitter hydrolase that helps in the transmission of nerve impulses by the hydrolytic metabolism of acetylcholine into choline and acetate (Bernal-Rey et al., 2020). Glutathione S-transferase biomarkers related to oxidative stress or malondialdehyde, a product of lipid peroxidation, have been widely used asa biomarkers of free radical damage in lipid molecules. The former is an important enzyme in the conjugation phase (phase II), as it combines with contaminants and generates compounds that are more easily excreted (Ribeiro et al., 2022). Lipid peroxidation is known to cause cellular injury through the inactivation of membrane enzymes and receptors, depolymerisation of polysaccharides, and cross-linking and fragmentation of proteins. Superoxide dismutase, catalase, glutathione peroxidase, glutathione, and glutathione reductase are oxidative stress biomarkers (Li et al., 2020), whereas metallothioneins are widely used as biomarkers of metal contamination by binding and removing toxic metals. Integrated analysis of these biomarkers may help overcome possible variations in biomarkers and assess polluted sites (dos Santos & Martinez, 2014). Furthermore, multiple scientific studies have confirmed that shrimps are indicators of estuarine health because of their global distribution and sensitivity to most pesticides (Ameur et al., 2022). The genus *Palaemonetes* (Crustacea, Decapoda, Caridea) is a good model for assessing the effects of pollution. The physiological, and toxicological aspects of non-target aquatic species need to be clarified, and the effects of xenobiotics on these organisms determined. The aim of this study was to assess the acute toxicity of glyphosate, an herbicide intensively used in Algeria which can reach the aquatic environment through runoff (Cheloufi et al., 2017). The study was conducted on a non-targeted biological model, the shrimp *Palaemon adspersus* (Rathke, 1837) (Decapoda, Palaemonidae) fished in the El-Mellah Lagoon and is considered to be a good model for monitoring the effects of pesticides. The main objectives of this study were to estimate the sublethal (LC_{10}, LC_{25}) and lethal (LC_{50}, LC_{90}) concentrations of glyphosate, commercial formulation Rondo®, against the shrimp after 24 and 96 hours. Additionally, selected biomarkers acetylcholinesterase, glutathione S-transferase, malondialdehyde and lipid quantities were determined in order to obtain additional information on the toxicity of this product during the treatment and recovery phases.

Materials and methods

Collection and maintenance of organisms. The shrimp *P. adspersus* was collected from the El-Mellah Lagoon, located on the extreme eastern side of Algeria (8°20′ E 36°54′ N), in the constriction zone of the channel that leads to the Mediterranean Sea (Fig. 1). This site is far from any source of pollution and is considered as a relatively clean site away from pollution sources. The shrimp were transported and acclimated to laboratory conditions for one week prior to the start of the experiment. Their rearing in the laboratory was maintained in glass aquaria (100 × 60 × 80 cm) filled with seawater (salinity 37 psu; temperature 22–25 °C; photoperiod 12:12 h light/dark) for 3/4 days. The filtration was performed using a water filter at a flow rate of 180 1/h (Rena 225). The shrimp were fed fresh mussels daily in the afternoon during the experiment. Shrimps of a similar size (length: 25 mm; weight: 850 mg) were used in the experiment.

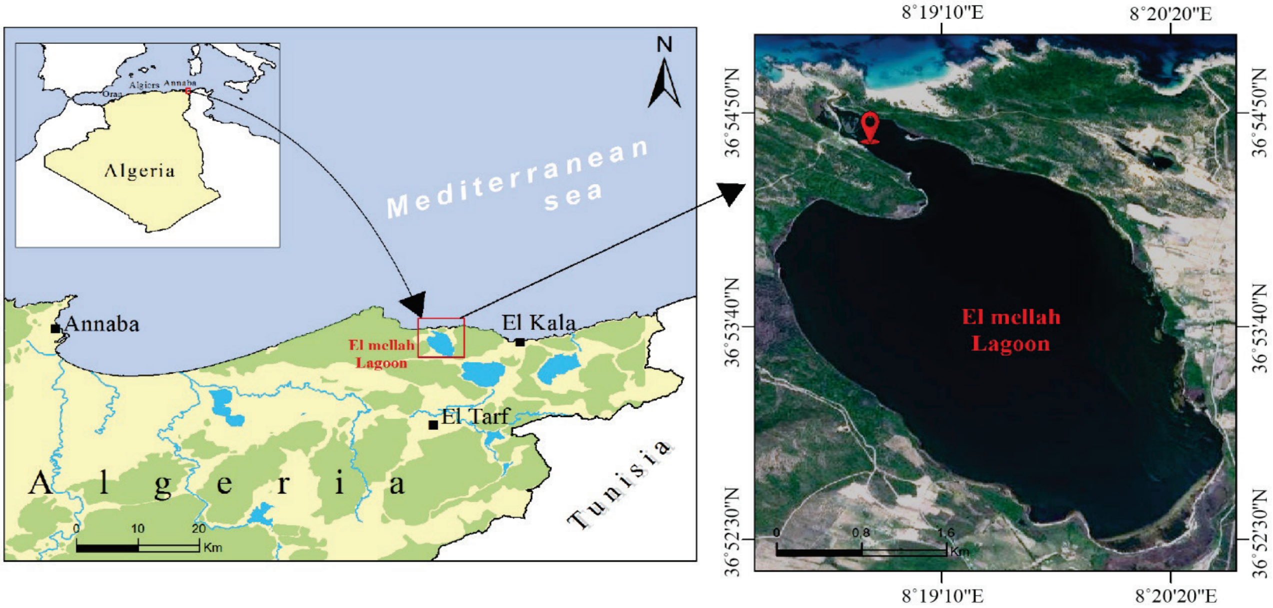

Fig. 1. Geographical location of the sampling site: El-Mellah Lagoon (El-Kala) (ArcGIS 10.3)

Herbicide. Glyphosate is the active ingredient in the commercial preparation of Rondo® (480 mg/L). It is an anionic organophosphate herbicide which is recognised for its potent and non-selective weed elimination properties (Ogunbiyi et al., 2023) with a molecular formula ($C_3H_8NO_5P$).

Acute toxicity test. Acute toxicity tests were performed on *P. adspersus* adults (15 individuals) placed in plastic boxes containing one litre of rearing water with a commercial formulation of glyphosate, Rondo® (480 mg/L) (1.75, 2.00, 2.25, 2.50, 2.75 mL), corresponding respectively to different concentrations (0.84, 0.96, 1.08, 1.20, 1.32 mg/L). The experiment was conducted with five replicates for each concentration. In addition, a control series was conducted in parallel. Mortality was checked daily for 96-hours and assessed considering the cumulative mortality. The percentage of mortality was corrected (Abbott, 1925) and then subjected to angular transformation according to Hendry (1909). The sublethal (LC_{10} and LC_{25}) and lethal (LC_{50} and LC_{90}) concentrations were determined along with their corresponding 95% confidence limits (95% LC), and the slope of the concentration-mortality lines was calculated using a regression probit analysis method.

Treatment and collecting tissues. Glyphosate was added to the rearing water containing mixed-sex *P. adspersus* shrimp at stage A (early postmolt), as described by Roberston et al. (1987). The compound was used at LC_{25} and LC_{50} concentrations obtained from shrimp after 96-hours. Samples (head and flesh) were taken from *P. adspersus* at different exposure times of 24, 48, 72, and 96 hours from the control and treated groups during the exposure period, and then transferred to clean water for 24, 48, 72, and 96 hours for the recovery period.

Biomarker analysis. After dissecting *P. adspersus*, shrimp heads were used to assess acetylcholinesterase activity, while flesh fragments (weight: 49–50 mg) were employed for the quantification of glutathione S-transferase, malondialdehyde, and lipid levels. Assays were conducted on five individuals in both the treated and control groups, spanning a treatment period of 96 hours and a re-covery period of 96 hours.

Acetylcholinesterase activity. Specific activity of acetylcholinesterase in the *P. adspersus* cephalothorax was determined according to the method described by Ellman et al. (1961). The method is based on a coupled enzyme reaction involving acetylthiocholine as the specific substrate for acetylcholinesterase and 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB) as an indicator of the enzyme reaction at 412 nm. The results are expressed as micromoles of thiocholine produced per minute per milligram of protein (µmol/mn/mg of protein).

Gluthatione S-transferase analysis. In this study glutathione S-transferase activity was determined using the method described by Habig et al. (1974), based on the glutathione S-transferase-catalysed conjugation of reduced glutathione with 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate. The increase in chloro-2,4-dinitrobenzene conjugate was monitored at 340 nm, and enzyme activity was expressed in micromoles of chloro-2,4-dinitrobenzene conjugate per minute per milligram of protein (µmol/mn/mg of protein).

Malondialdehyde analysis. Lipid peroxidation was estimated by the quantification of malondialdehyde rates using the method described by Draper & Hardley (1990). Malondialdehyde determination was used as an index of lipid peroxidation. This method is based on measuring the colour produced during the reaction between thiobarbituric acid (TBA) and malondialdehyde. The rate of malondialdehyde was measured at 532 nm and expressed as µmol/mn/mg of protein.

Lipid content essay. The lipid content was estimated according to the method described by Folch et al. (1957). Lipids were extracted using a mixture of chloroform/methanol/water (2/1/0.8). The lipid extract was placed in a pre-weighed screw tube and evaporated under nitrogen flow, and the total lipid content was estimated by the difference in the weight of the tube before and after evaporation. The lipid extracts from the samples were taken in a mixture of toluene/ethanol (4v/1v), which allowed the preservation of lipids at low temperature (–20 °C) without risk of alteration for several months. The absorbance was measured by excitation at 530 nm.

In parallel, enzymatic activity was calculated in terms of the protein content of the sample (Bradford, 1976) using Coomassie Brilliant Blue G250 as a reagent and bovine serum albumin as the standard. Absorbance was measured at 595 nm and reported as µM/min/mg of protein.

Statistical analysis. The results are presented as arithmetic mean ± standard deviation (x ± SD). Normal distribution of data (Shapiro-Wilk's test) and homogeneity of variances (Bartlett's test) were assessed. Data for the bioassay were analysed using nonlinear sigmoid curve fitting, and the activity of the treatment was evaluated in terms of a concentration-dependent response. The goodness of fit of the curve model was evaluated based on the R^2 values. Toxicity statistical analyses were performed using R (version 4.2.1; R Core Team, 2022) and RStudio (RStudio Team, 2022). For all biomarkers, statistical analysis was performed using the SPSS software (V22.0, IBM Corporation, NY, USA). Two-way analysis of variance (ANOVA) followed by Tukey's comparison test with HSD post-hoc analysis was used to assess the differences between the control, treated, and recovered series, with P < 0.05, indicating a statistically significant difference. Pearson's correlation test was used to correlate malondialdehyde with lipid content.

Results

Acute toxicity of glyphosate. As shown in Figure 2, glyphosate at different concentrations (0.84, 0.96, 1.08, 1.20, 1.32 mg/L) was added to the rearing medium of *P. adspersus* and mortality was observed at 24 and 96-hours. The corrected cumulative mortality varied from 8.9 ± 2.2% for the lowest concentration (0.84 mg/L) to 97.8 ± 2.2% for the highest concentration (1.32 mg/L) with dose-response manner. Mortality was not observed in the control group. Statistical analysis indicated a significant effect of the concentration (P < 0.05). The lethal concentrations and the corresponding 95% fiducial limits (95% FL) are listed in (Table 1).

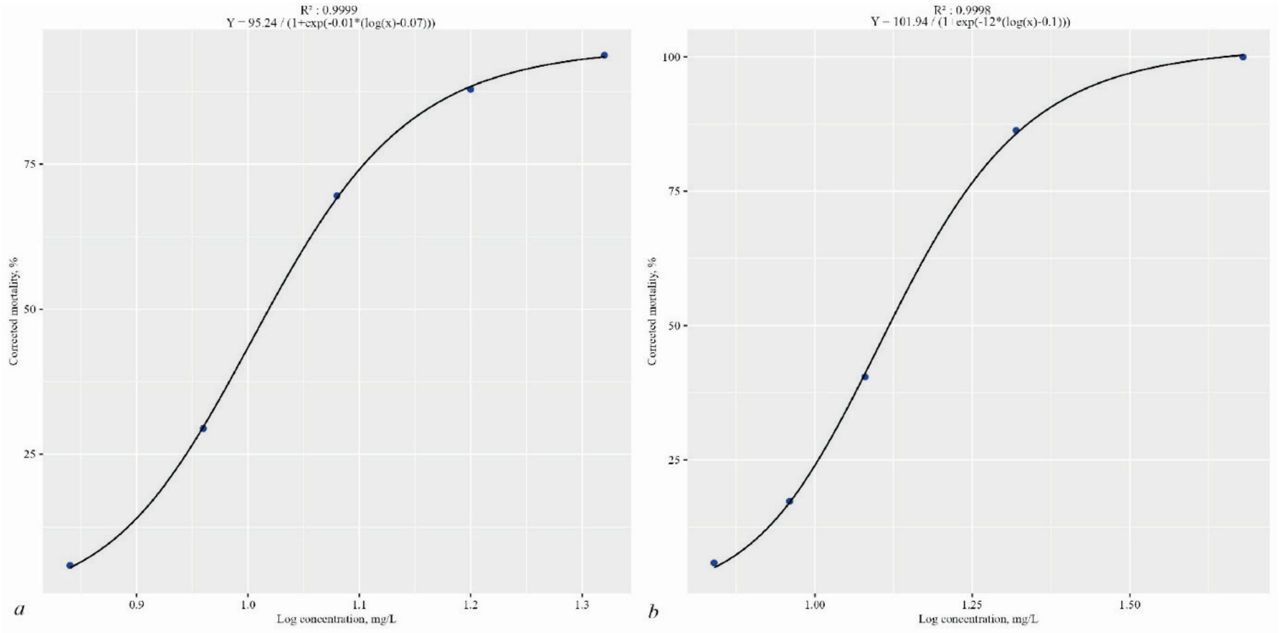

Fig. 2. Effect of glyphosate on adult *P. adspersus* shrimp after 24 (*a*) and 96-hours (*b*): sigmoidal dose-dependent response curve of corrected mortality (%) as a function of the decimal logarithm of concentration

Table 1
Glyphosate lethality parameters in adult *P. adspersus* shrimp after 24 and 96-hours: the data were expressed in terms of lethal concentration (LC, %) together with the corresponding 95% fiducial limits (FL [95%]), coefficient of determination (R^2), and hill slope (n = 5 repeats, each containing 15 individuals)

Time	Concentrations	Values, mg/L	Fiducial limits 95%	R²	Hill slope
24 h	LC_{10}	1.15	0.82–0.90	0.9999	13.63
	LC_{25}	1.25	1.03–1.05		
	LC_{50}	1.35	1.24–1.47		
	LC_{90}	1.59	2.04–2.23		
96 h	LC_{10}	0.99	0.88–1.07	0.9998	15.61
	LC_{25}	1.06	0.99–1.11		
	LC_{50}	1.14	1.09–1.18		
	LC_{90}	1.31	1.22–1.44		

Effects of glyphosate on biomarker measurements during treatment and recovery phases. All treatments were performed on mixed-sex *P. adspersus* at Stage A (early postmolt). The results obtained for the variation in the enzymatic activities of acetylcholinesterase, glutathione S-transferase, malondialdehyde, and lipid content in the treated and control groups after the treatment (24, 48, 72 and, 96-hours) and recovery phases (24, 48, 72, and 96-hours) are presented in (Fig. 3–6).

Changes in acetylcholinesterase activity. The enzymatic activity of acetylcholinesterase in the head fragments of the control series decreased over time, up to 96 hours. Treatment with glyphosate at both LC_{25} and LC_{50} revealed a significant (P < 0.05) dose-dependent decrease in acetylcholinesterase activity after 24 to 96-hours (Fig. 3a). The most significant inhibitory action was recorded at 48 h with LC_{50} (1.14 mg/L), where acetylcholinesterase activity was the lowest compared to the treated series. This activity increased significantly (p) after 96 hours, as compared with 48 and 72 hours. A two-way ANOVA revealed a highly significant time effect ($F_{3, 48}$ = 11.964; P < 0.05), treatment effect ($F_{2, 48}$ = 1428.893; P < 0.05), and time/treatment interactions ($F_{6, 48}$ = 35.160; P < 0.05).

During the recovery phase, acetylcholinesterase activity remained inhibited after 24 and 48 hours, with a significant difference between the treated and control series. However, acetylcholinesterase activity returned to normal levels after 72 and 96 hours, with no significant difference between the treatment (LC_{25} and LC_{50}) and control series (Fig. 3b). A two-way ANOVA revealed a highly significant effect of time ($F_{3, 48}$ = 16.797; P < 0.05), treatment effect ($F_{2, 48}$ = 27.461; P < 0.05) and time/treatment interaction ($F_{6, 48}$ = 7.337; P < 0.05).

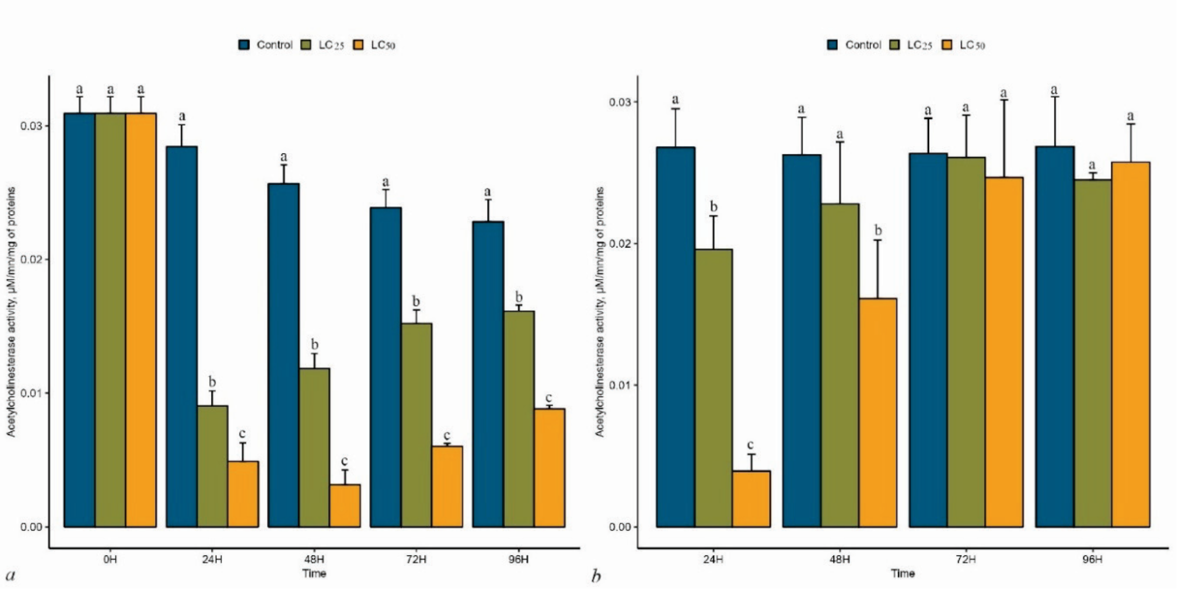

Fig. 3. Effect of glyphosate on acetylcholinesterase in *P. adspersus* during the treatment (*a*) and recovery (*b*) periods ($LC_{25} = 1.06$ mg/L and $LC_{50} = 1.14$ mg/L): different letters indicate differences between dose within one time (hours) of observations (Tukey's test, $P < 0.05$); x ± SD, n = 5

Changes in gluthatione S-transferase activity. Under normal conditions, the enzymatic activity of glutathione S-transferase in flesh fragments increased over time, up to 96 hours. Treatment with glyphosate at both LC_{25} and LC_{50} revealed a significant ($P < 0.05$) dose-dependent induction of glutathione S-transferase after 48 to 96-hours (Fig. 4a). The highest activity was observed at the LC_{50} (1.14 mg/L). A two-way ANOVA revealed a highly significant time effect ($F_{3,48} = 602.007$; $P < 0.05$), treatment effect ($F_{2,48} = 1411.895$; $P < 0.05$), and time/treatment interactions ($F_{6,48} = 152.013$; $P < 0.05$).

During the recovery phase, glutathione S-transferase activity was induced at 24 and 48 hours, with a significant difference between the treated and control series. However, glutathione S-transferase activity returned to normal levels at 72 and 96 hours, with no significant difference between the treated and control series (Fig. 4b). A two-way ANOVA revealed a highly significant time effect ($F_{3,48} = 1508.926$; $P < 0.05$), treatment effect ($F_{2,48} = 1304.721$; $P < 0.05$), and time/treatment interaction ($F_{6,48} = 378.949$; $P < 0.05$).

Changes in molandialdehyde activity. In the control series, malondialdehyde activity in flesh fragments increased over time to 96 h. Treatment with glyphosate at both LC_{25} and LC_{50} revealed a significant ($P < 0.05$) dose-dependent induction of malondialdehyde after 24 to 96-hours (Fig. 5a). The most potent activity was observed at 48 hours, as measured with LC_{50}. A two-way ANOVA revealed a highly significant time effect ($F_{3,48} = 90.835$; $P < 0.05$), treatment effect ($F_{2,48} = 655.325$; $P < 0.05$), and time/treatment interaction ($F_{6,48} = 27.135$; $P < 0.05$).

During the recovery phase, malondialdehyde activity returned to normal levels significantly from 24 to 96-hours (Fig. 5b). A two-way ANOVA revealed no significant effect of time ($F_{3,48} = 1.764$; $P > 0.05$), a significant treatment effect ($F_{2,48} = 8.008$; $P < 0.05$), and no significant time/treatment interaction ($F_{6,48} = 0.039$; $P > 0.05$).

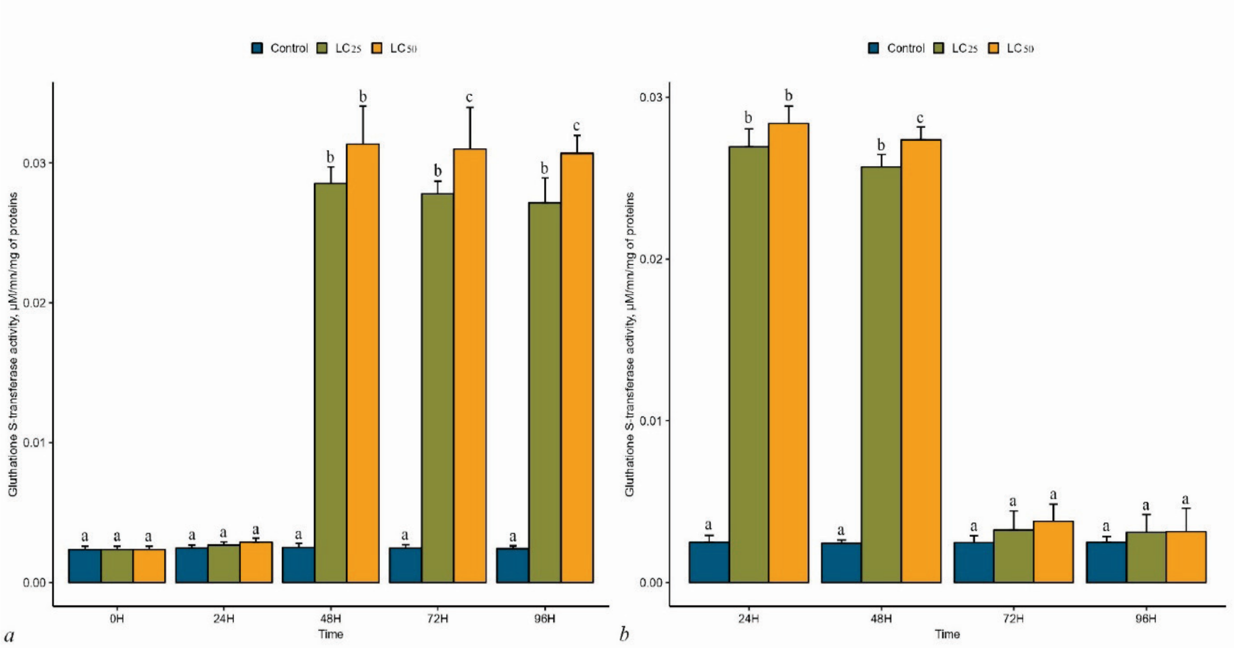

Fig. 4. Effect of glyphosate on glutathione S-transferase in *P. adspersus* during the treatment (*a*) and recovery (*b*) periods ($LC_{25} = 1.06$ mg/L and $LC_{50} = 1.14$ mg/L): different letters indicate differences between dose within one time (hours) of observation (Tukey's test, $P < 0.05$); x ± SD, n = 5

Changes in lipid activity. As shown in the figure, under normal conditions, the lipid content in the flesh fragments decreased over time, up to 96 hours. Treatment with glyphosate at both LC_{25} and LC_{50} revealed a significant ($P < 0.05$) dose-dependent decrease in lipid levels after 24 to 96-hours (Fig. 6a). The most significant reduction was obtained with the LC_{50} at the end of the treatment. A two-way ANOVA revealed a highly significant time effect ($F_{3, 48} = 12.763$; $P < 0.05$), treatment effect ($F_{2, 48} = 379.597$; $P < 0.05$), and significant time/treatment interaction ($F_{6, 48} = 3.814$; $P < 0.05$). During the recovery phase, the lipids were maintained at low levels (Fig. 6b). Two-way ANOVA revealed a significant effect of time ($F_{3, 48} = 17.237$; $P < 0.05$), treatment effect ($F_{2, 48} = 412.549$; $P < 0.05$), and a significant treatment/time interaction ($F_{6, 48} = 3.207$; $P < 0.05$).

Correlation test. The Pearson correlation test, employed to analyse data between malondialdehyde and lipid levels after glyphosate-treatment and recovery phases is displayed in Figure 7. The results showed a highly significant negative correlation ($P < 0.05$) between the two variables during both the treatment and recovery phases.

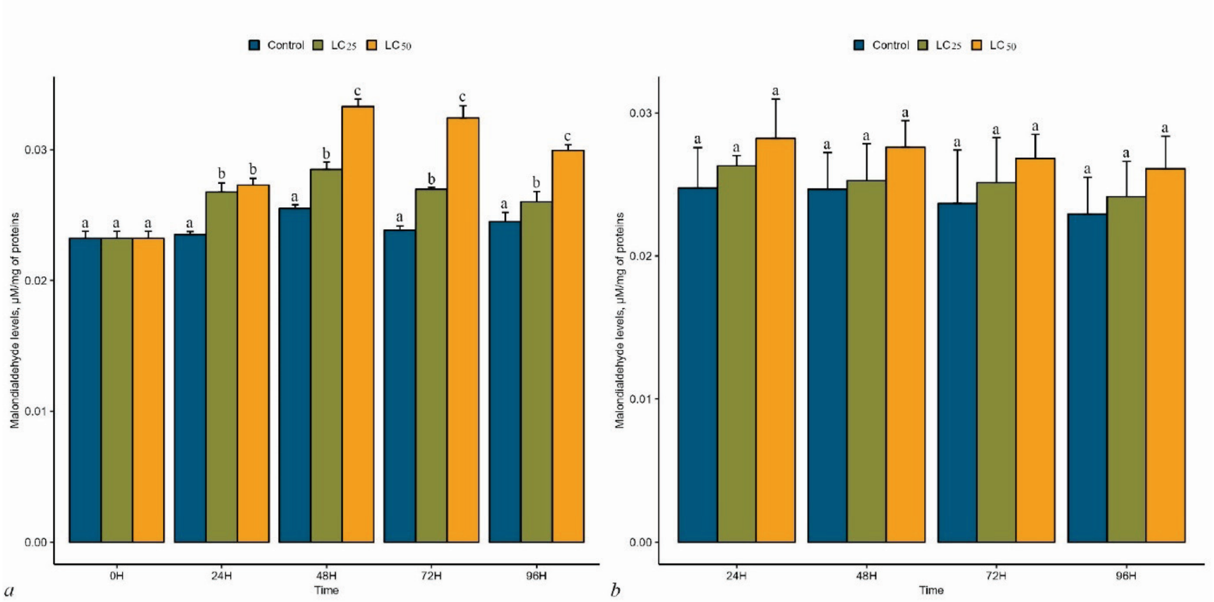

Fig. 5. Effect of glyphosate on malondialdehyde activityin *P. adspersus* during the treatment (*a*) and recovery (*b*) periods ($LC_{25} = 1.06$ mg/L and $LC_{50} = 1.14$ mg/L): different letters indicate differences between dose within one time (hours) of observation (Tukey's test, $P < 0.05$); x ± SD, n = 5

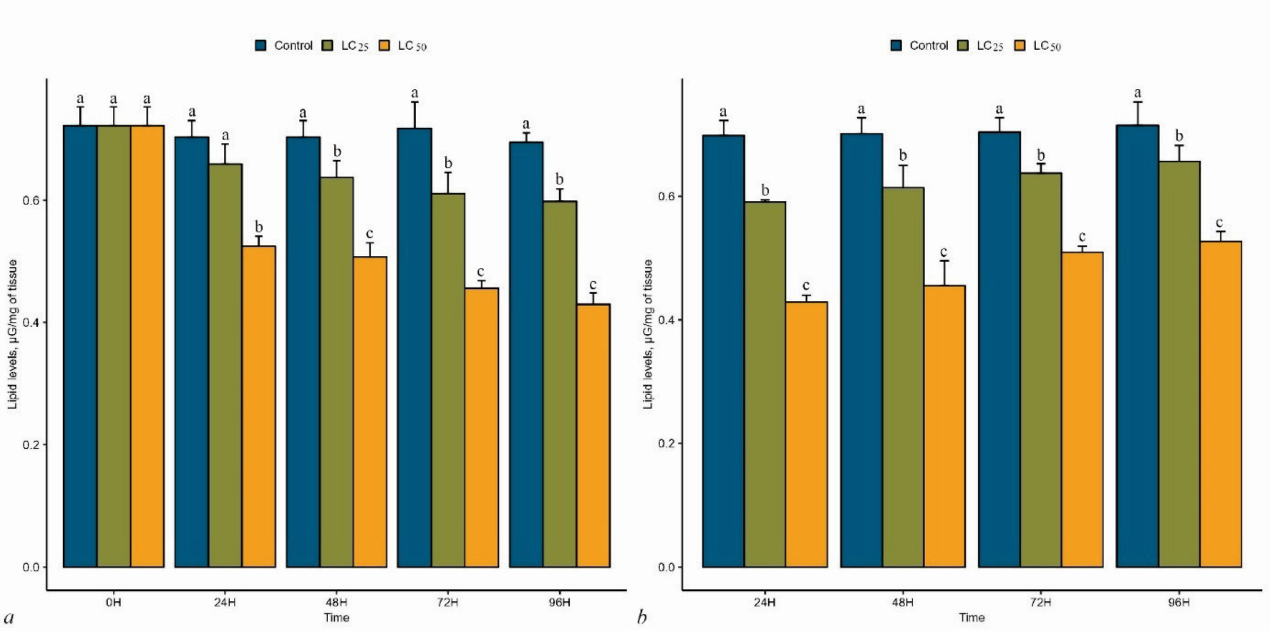

Fig. 6. Effect of glyphosate on lipids in *P. adspersus* during the treatment (*a*) and recovery (*b*) periods ($LC_{25} = 1.06$ mg/L and $LC_{50} = 1.14$ mg/L): different letters indicate differences between dose within one time (hours) of observation (Tukey's test, $P < 0.05$); x ± SD, n = 5

Discussion

Decapod crustaceans are a large and diverse group of organisms. Ecologically, they are key species in the food chain, play an essential role in ecosystem function, and are economically important species in the fisheries sector. They are also excellent biological models for determining the impact of xenobiotics such as *Gammarus* spp. (J.C. Fabricius, 1775), (Amphipoda, Gammaridae) (Consolandi et al., 2019), *Gammarus fossarum* (Koch, 1836) (Amphipoda, Gammaridae) (Lebrun & Gismondi, 2020), *Palaemon serratus* (Pennat, 1777) (Decapoda, Palaemonidae) (González-Ortegón et al., 2014), *Macrobrachium rosenbergii* (De Man, 1879) (Decapoda, Palaemonidae) (Mostafiz et al., 2020). Owing to their worldwide distribution and sensitivity to most pesticides, shrimps have been proposed as indicators of estuarine health. This justifies their use as a model for assessing the impact of pesticides. In addition, the extensive application of pesticides can accidentally lead to their introduction into fresh and marine surface water. These contaminants pose a high ecotoxicological risk to aquatic organisms, particularly in their early life stages. Although, there is growing interest in the presence of herbicides, such as glyphosate, in aquatic ecosystems, information on the effects of this compound on non-target marine species, particularly invertebrates, is limited. Therefore, it is crucial to investigate the effects of this compound on non-target aquatic species. The objectives of this study were to examine the acute toxicity of Rondo® (a commercial formulation of glyphosate) against the adult shrimp *P. adspersus*, by determining the various lethal and sublethal concentrations. Next, enzymatic activities were measured in the head and flesh fragments of the shrimp to quantify acetylcholinesterase, glutathione S-transferase, and malondialdehyde activity, followed by the quantification of lipids in the treatment and depuration phases.

Indeed, the 24 and 96-hours sublethal and lethal LC_{50} and LC_{25} concentrations are estimated at around 1.35 and 1.25 mg/L at 24 hours and 1.14 and 1.06 mg/L at 96-hours, respectively. So, shrimp are sensitive to glyphosate, and their lethal concentrations are lower than those of other species, such as *Macrobrachium nipponense* (De Haan, 1849) (Decapoda, Palaemonidae) (Hong et al., 2018), *Eriochier sinensis* (H. Milne Edwards, 1853) (Decapoda, Varunidae) (Hong et al., 2017), and *Caridina nilotica* (Roux, 1833) (Decapoda, Atyidae) (Filho et al., 2014). To date, several studies have demonstrated that glyphosate and its commercial formulation have significant toxic effects on freshwater organisms, particularly crustaceans and fish (Bastos Gonçalves et al., 2020). Hong et al. (2019) demonstrated considerable variability in the 96-hours LC_{50} value of glyphosate, depending on factors such as species, life stage, and environmental conditions. For larvae and adults of the freshwater shrimp *C. nilotica*, the respective values were 2.45 and 27.76 mg/L (Mensah et al., 2015). Neotropical fish *Prochilodus lineatus* (Valenciennes, 1837) (Characiformes, Prochilodontidae) exhibited values around 13.69 mg/L (Langiano & Martinez, 2008), while the cold-water species *Salmo salar* (Linnaeus, 1758) (Salmoniformes, Salmonidae) recorded a value of 42 mg/L (Servizi et al., 1987).

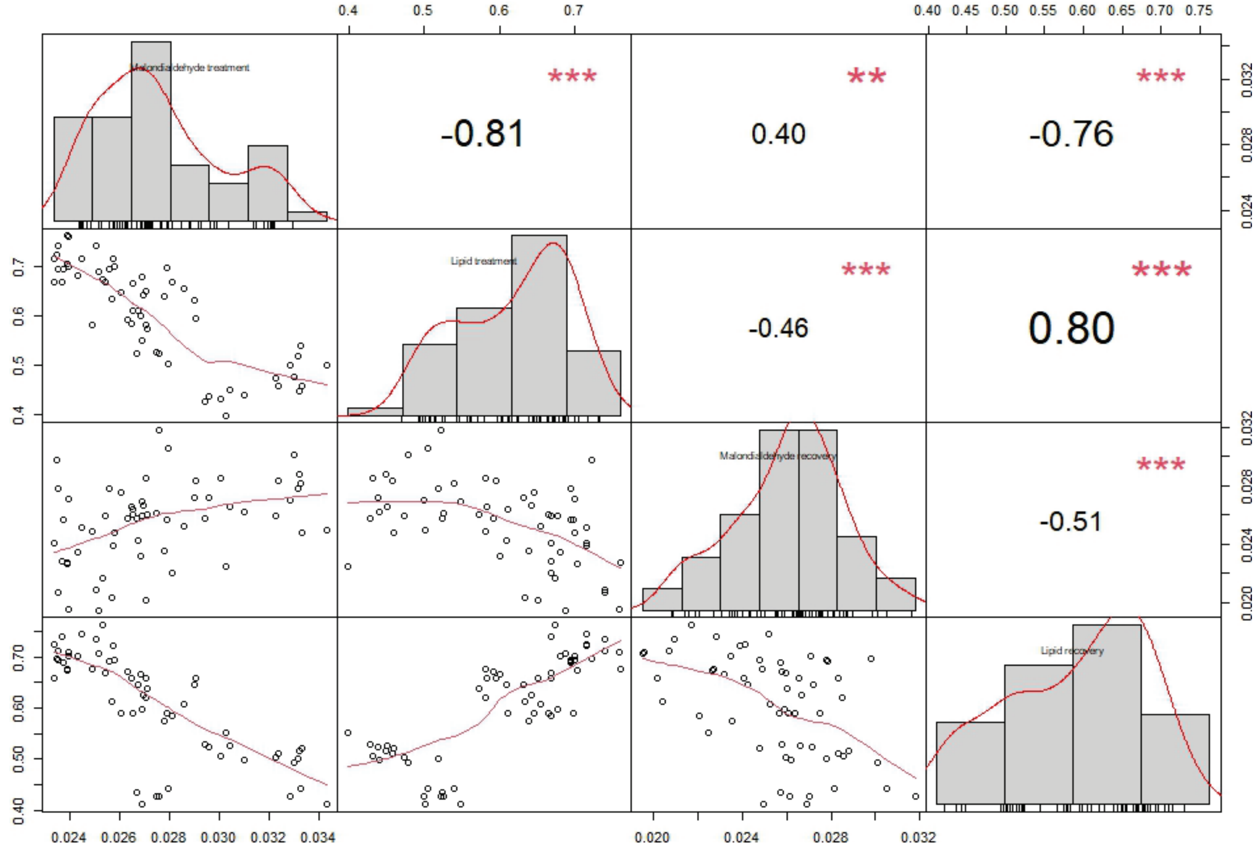

Fig. 7. Pearson correlation test between malondialdehyde and lipid levels (n = 60)

Furthermore, research conducted on the freshwater shrimp *M. nipponense* has estimated the LC_{50} value of glyphosate after 96 hours of exposure to be approximately 57.68 mg/L. In addition, according to (Hong et al., 2017), the lethal concentration (LC_{50}) of glyphosate in the crab *E. sinensis* was significantly high, reaching 97.89 mg/L, whereas that in the shrimp *Gammarus pulex* (Linnaeus, 1758) (Amphipoda, Gammaridae) did not exceed 403 µg/L (Pala, 2019). Moreover, Osterberg et al. (2012) showed that the 24-hours LC_{50} value of Roundup (the glyphosate-based commercial formulation) in the juvenile blue crab *Callinectes sapidus* (Rathbun, 1896) (Decapoda, Portunidae) reached 316 mg/L. Furthermore, in our study, shrimp generally exhibited the first negative effect after 48 hours. When the first effects of glyphosate appeared, the mortality increased at very short concentration intervals. Indeed, $LC_{10,}$ $LC_{25,}$ LC_{50} and LC_{90} range from 0.99 to 1.31 mg/L. With depuration, shrimp were able to return to their initial state after 48 hours. These results suggest a difference in shrimp sensitivity to glyphosate-based herbicides. However, various authors have documented that pure glyphosate may be relatively less toxic to aquatic organisms (Bridi et al., 2017). However, its formulations are often more toxic to aquatic organisms because of the addition of surfactants which are used to improve its penetration into plants (Fiorino et al., 2018). This confirmed that shrimp were highly sensitive to glyphosate-based herbicide formulations. This is due to a variety of behaviours and habitats.

Biochemical biomarkers are often assessed when an organism is exposed to pollutants, leading to a cascade of biological responses triggered by stress. To analyse the probable adverse effects of glyphosate on *P. adspersus*, a set of neurotoxicity and oxidative stress biomarkers was determined. The tissue acetylcholinesterase level is commonly used as a sensitive parameter in the assessment of pesticide neurotoxicity in non-target organisms. As modulators of neurotransmission, changes in acetylcholinesterase activity have also been associated with changes in behavioural patterns (Bonifacio et al., 2020). Glyphosate is categorised as a non-acetylcholinesterase inhibitor in animals (Sandrini et al., 2013). However, the results of the present study indicate that acetylcholinesterase activity in *P. adspersus* was inhibited in a dose-dependent manner after 24 to 96-hours and when individuals were transferred to clean water, they showed rapid recovery patterns. Numerous research endeavors have reported a decrease in acetylcholinesterase activity in aquatic organisms exposed to pure glyphosate or glyphosate formulations (Menéndez-Helman et al., 2012). As well, Pala (2019) revealed inhibition of acetylcholinesterase activity in the shrimp *G. pulex* treated with glyphosate at sublethal concentrations (10, 20, and 40 µg/L) for 24 and 96 hours. Similarly, pure glyphosate inhibited acetylcholinesterase activity *in vitro* in a concentration-dependent manner in fish, such as *Danio rerio* (Hamilton, 1822), (Cypriniformes, Cyprinidae) and *Jenynsia multidenta* (Jenyns, 1842), (Cyprinodontiformes, Anablepidae) (Sandrini et al., 2013). Previous studies have shown that acetylcholinesterase activity was inhibited after exposure to concentrations of 0.2 and 0.4 mg/L of Roundup® (glyphosate) for 96 hours in the fish *Rhamdia quelen* (Quoy & Gaimard, 1824) (Siluriformes, Heptapteridae) (Glusczak et al., 2007). Similarly, a significant 1.5-fold reduction in acetylcholinesterase activity in Polychaeta *Laeonereis acuta* (Treadwell, 1923) (Phyllodocida, Nereididae) was observed after exposure to Roundup at concentrations of 3.25 and 5.35 mg/L for 96 hours (De Melo Tarouco et al., 2017). In addition, pure concentrations of glyphosate, ranging from 0.075 to 15 mM, inhibited acetylcholinesterase activity in a dose-dependent manner in the gills and muscles of the marine brown mussel, *Perna perna* (Linnaeus, 1758) (Mytiloida, Mytilidae). This enzymatic activity was suppressed by more than 50%, even at the lowest concentrations tested (0.75 to 1 mM) in *P. perna* than in *D. rerio* and *J. multidenta* (Matozzo et al., 2020). Inhibition of acetylcholinesterase activity has also been observed in crabs *Carcinus maenas* (Linnaeus, 1758), (Decapoda, Carcinidae) exposed to fluoranthene (Rodrigues & Pardal, 2014), in the shrimp *Macrobrachium borelli* (Nobili, 1896) (Decapoda, Palaemonidae) contaminated by organophosphates (Lavarías et al., 2013), and in the freshwater shrimp *Palaemonetes argentinus* (Nobili, 1901) (Decapoda, Palaemonidae) exposed to organophosphorus fenitrothion (Lavarías & García, 2015). Moreover, glyphosate has also been shown to affect haemocyte parameters in bivalve species (Matozzo et al., 2019), and to disrupt genes involved in apoptosis, immune response, energy metabolism, Ca^{2+} homeostasis, cell signalling, and endoplasmic reticulum stress response.

Glutathione S-transferases constitute an enzyme family pivotal in sustaining a significant detoxification mechanism. They inactivate secondary metabolites such as unsaturated aldehydes, epoxides, and hydroperoxides. Glutathione S-transferases also play an important role in the detoxification of electrophilic toxicants such as some groups of pesticides (Piner Benli & Çelik, 2021). In addition, they metabolise and eliminate a range of environmental pollutants, heavy metals, and carcinogens (Mazari et al., 2023). In the current study, glutathione S-transferase activity in the flesh of glyphosate-treated series at both concentrations (LC_{25} and LC_{50}) increased progressively and significantly at 48, 72, and 96 hours. However, a latency period was observed in the shrimp before the first induction. During the depuration phase, glutathione S-transferase activity decreased in a dose-dependent manner at 72 and 96-hours. Diverse research using various compounds against shrimp species have shown a significant increase in glutathione S-transferase activity, including *P. argentinus* exposed to fenitrothion (Lavarías & García, 2015), *M. borelli* treated with organophosphorus insecticides, and *P. serratus* exposed to benzopyrene (BaP) (Silva et al., 2013). This increase has already been observed in previous studies of *P. adspersus* exposed to thiamethoxam, a neonicotinoid with the commercial product actara (Berghiche et al., 2018), and diflubenzuron, a chitin synthesis inhibitor (Lechekhab & Soltani, 2018), at lethal and sublethal concentrations. According to Velisek et al. (2020), glutathione S-transferase levels increased in marbled crayfish (decapod) after exposure to metazachlor and its primary metabolite, metazachlor OA, at both concentrations (0.0115 and 0.0790 µmol/L) and metazachlor OA (0.0117 and 0.0805 µmol/L). Roundup induces oxidative stress in aquatic organisms, such as goldfish, *Carassius auratus* (Linnaeus, 1758) (Cypriniformes, Cyprinidae) (Lushchak et al., 2009), *P. lineatus* (Modesto & Martinez, 2010), and *Anabas testudineus* (Bloch, 1792) (Perciformes, Anabantidae) (Samanta et al., 2014). Glutathione S-transferase activity in zebrafish increased significantly in a time-dependent manner after exposure to three sulfonamides (sulfamethoxazole, sulfadiazine and sulfadimidine) for three days (Lin et al., 2014). Previous authors reported similar effects on *Donax trunculus* (Linnaeus, 1758) (Cardiida, Donacidae), Amira et al. (2018) highlighted the implication of metal accumulation in sediments, Lechekhab (2018) observed effects by applying spiromesifen on *P. adspersus*, and finally, through the application of thiamethoxan Cheghib et al. (2020) also demonstrated a significant effect in *Gambusia affinis* (Baird & Girard, 1853) (Cyprinodontiformes, Poeciliidae) at various concentrations.

Lipid peroxidation is considered an important biomarker of cell damage because of the interaction between radicals and membrane lipids. Antioxidant and detoxification systems are unable to neutralise the active intermediates produced by xenobiotics and their metabolites, resulting in the production of malondialdehyde, which is a primary indicator of lipid peroxidation. Pesticides, metals, and other xenobiotics cause lipid peroxidation, which is the first stage of cellular membrane damage and is accepted as an important indicator of oxidative damage in cellular components (Sule et al., 2022). The present experiment showed that the glyphosate-exposed group had higher levels of malondialdehyde in a dose-dependent manner than the controls. Additionally, when shrimp were removed from the glyphosate medium and replaced with untreated seawater, malondialdehyde levels in the shrimp flesh rapidly returned to normal levels, i.e. after 24 hours of depuration. Furthermore, the negative correlation between malondialdehyde and lipid levels reflected lipid peroxidation. Multiple studies have also reported similar changes in the malondialdehyde levels in certain aquatic organisms exposed to glyphosate. The results of a freshwater amphipod study, *G. pulex* (crustacean), by Pala, 2019, indicate a significant increase in malondialdehyde levels in groups treated with glyphosate herbicide for all exposure durations. Previous research has shown that glyphosate induced inflammation contributes to lipid metabolism in fish (Liu et al., 2021). Samanta et al. (2014) noted a significant increase in lipid peroxidation levels in various tissues of fish *A. testudineus* exposed to glyphosate (formulation of glyphosate-based-hebicide, Excel Mera-71) for 30 days. Kaya et al. (2012) reported elevated malondialdehyde levels in the plasma of fish *Capoeta capoeta* (Güldenstädt, 1773) (Cyprinodontiformes, Cyprinidae), after a 10 days exposure to glyphosate at a concentration of 0.02 mg/L. Several studies have been carried out on aquatic organisms and have shown that malondialdehyde levels increase as a function of exposure time and pesticide concentration. For example, Khebbeb et al. (2010) demonstrated a significant induction of malondialdehyde in several tissues of the clam *Ruditapes decussatus* (Linnaeus, 1758) (Veneroida, Veneridae) exposed to cadmium. Another study conducted in *D. trunculus* collected from the Sidi Salem site due of domestic and industrial contamination in the Gulf of Annaba in eastern Algeria noted an induction of malondialdehyde (Amamra et al., 2019). In addition, high levels of lipid peroxidation, linked to metallic contamination, were observed in the crab *C. maenas* collected in the Bizerte Lagoon compared with a reference site (Ben-Khedher et al., 2013). By contrast, the results showed a highly significant negative correlation between the two variables during treatment and recovery phases.

Our results are complementary to those reported by other authors for glyphosate and glyphosate-based formulations and suggest a deleterious effect on animals. In crustaceans, exposure to Roundup can disrupt the production and elimination of reactive oxygen species, leading to a decrease in the efficiency of antioxidant defences or even oxidative damage (Husak et al., 2022). However, the results showed a dose-dependent effect on neurotoxicity, induction of oxidative damage, and antioxidant activity during exposure to sublethal and lethal concentrations of glyphosate.

Conclusion

Although information on glyphosate levels in aquatic environments is limited, the results of this study clearly indicate that glyphosate has a dose-dependent effect on neurotoxicity, induction of oxidative damage and antioxidant activity during exposure to sublethal and lethal concentrations of glyphosate. Furthermore, the shrimp are highly sensitive to glyphosate-based herbicide formulations and are also able to recover from the depressive effects of glyphosate. Therefore, glyphosate is a major threat to the environment and should only be used to a limited extent.

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