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

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**Abstract**

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 (LC10 and LC50) and lethal (LC50 and LC90) concentrations were estimated after 24 and 96 hours of exposure. The compound was added to rearing water at LC50 and LC90 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, glutathione S-transferase, and malondialdehyde, followed by lipid quantification. Toxicological data indicated the toxicity of glyphosate against shrimp, exhibiting a dose-response effect. Lethal concentrations LC50– for 96 hours during the exposure phase (24, 48, 72, and 96 hours). Shrimp 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 intermittent 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 Arcuate 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 C3H8NO5P, it originates 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 as 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 variati-
ons 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 (LC10, LC25) and lethal (LC50, LC90) concentrations of glyphosate, commercial formulation Rondo® (480 mg/L) 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 l/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)](image)

*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 (C3H8NO5P).

*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 (LC10 and LC25) and lethal (LC50 and LC90) 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 post-molt), as described by Robenstyn et al. (1987). The compound was used at LC10 and LC50 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 recovery 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).

*Glutathione 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 mal-
Glyceraldehyde. The rate of malondialdehyde was measured at 532 nm and expressed as µmol/min/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 concentration-dependent response. The goodness of fit of the curve model was evaluated based on the R² 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).

**Table 1**

Glycophosphate lethality parameters in adult *P. adspersus* shrimp after 24 and 96-hours: the data were expressed in terms of lethai concentration (LC, %) together with the corresponding 95% fiducial limits (FL [95%]), coefficient of determination (R²), and hill slope (n = 5 repeats, each containing 15 individuals)

<table>
<thead>
<tr>
<th>Time</th>
<th>Concentration Values, mg/L</th>
<th>Fiducial limits</th>
<th>95% R²</th>
<th>Hill slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h</td>
<td>LC₀</td>
<td>1.15</td>
<td>0.82-0.90</td>
<td>0.9999</td>
</tr>
<tr>
<td></td>
<td>LC₁₀</td>
<td>1.25</td>
<td>1.03-1.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LC₂₅</td>
<td>1.35</td>
<td>1.24-1.47</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LC₅₀</td>
<td>1.50</td>
<td>2.04-2.23</td>
<td></td>
</tr>
<tr>
<td>96 h</td>
<td>LC₀</td>
<td>0.99</td>
<td>0.88-1.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LC₁₀</td>
<td>1.06</td>
<td>0.99-1.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LC₂₅</td>
<td>1.14</td>
<td>1.09-1.18</td>
<td>0.9998</td>
</tr>
<tr>
<td></td>
<td>LC₅₀</td>
<td>1.31</td>
<td>1.22-1.44</td>
<td></td>
</tr>
</tbody>
</table>

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 LC₂₅ and LC₅₀ 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 LC₅₀ (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 (F₁₀ = 11.964; P < 0.05), treatment effect (F₀ = 1428.893; P < 0.05), and time/treatment interactions (F₁₀ = 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 (LC₂₅ and LC₅₀) and control series (Fig. 3b). A two-way ANOVA revealed a highly significant effect of time (F₁₀ = 16.797; P < 0.05), treatment effect (F₀ = 27.461; P < 0.05) and time/treatment interaction (F₁₀ = 7.337; P < 0.05).
Changes in glutathione 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 LC25 and LC50 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 LC50 (1.14 mg/L). A two-way ANOVA revealed a highly significant time effect (F3, 48 = 602.007; P < 0.05), treatment effect (F2, 48 = 1411.895; P < 0.05), and time/treatment interactions (F6, 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 (F3, 48 = 1508.926; P < 0.05), treatment effect (F2, 48 = 1304.721; P < 0.05), and time/treatment interaction (F6, 48 = 378.949; P < 0.05).

Changes in malondialdehyde activity. In the control series, malondialdehyde activity in flesh fragments increased over time to 96 h. Treatment with glyphosate at both LC25 and LC50 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 LC50. A two-way ANOVA revealed a highly significant time effect (F3, 48 = 90.835; P < 0.05), treatment effect (F2, 48 = 655.325; P < 0.05), and time/treatment interaction (F6, 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 (F3, 48 = 1.764; P > 0.05), a significant treatment effect (F2, 48 = 8.008; P < 0.05), and no significant time/treatment interaction (F6, 48 = 0.039; P > 0.05).

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 LC25 and LC50 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 LC50 at the end of the treatment. A two-way ANOVA revealed a highly significant effect of treatment (F2, 48 = 1.764; P > 0.05), a significant effect of time (F3, 48 = 1.764; P > 0.05), and no significant time/treatment interaction (F6, 48 = 0.039; P > 0.05).
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.

**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* (Pennant, 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 LC₅₀ and LC₉₀ 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 *Macrobachium nipponense* (De Haan, 1849) (Decapoda, Palaemonidae) (Hong et al., 2018), *Eriocheir 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 LC₅₀ 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 (Menéndez-Helman 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 LC₉₀ 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 (LC₅₀) of glyphosate in the crab *E. sinensis* was significantly high, reaching 97.89 mg/L, whereas that in the shrimp *Gammarsus pulex* (Linnaeus, 1758) (Amphipoda, Gammaridae) did not exceed 403 μg/L (Pala, 2019). Moreover, Osterberg et al. (2012) showed that the 24-hours LC₅₀ 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, LC₅₀, LC₉₀, LC₂₅, and LC₁₀ 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 (Brindi 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 *Anyonida multidentata* (Jenyns, 1842), (Cypriniformes, Cyprinidae).
Antioxidant and detoxification systems are unable to neutralise the active
images because of the interaction between radicals and membrane lipids.

Poeciliidae) at various concentrations.

Metal accumulation in sediments, Lechekhab (2018) observed effects by
applying spiromesifen on P. adspersus, and finally, through the applicati-

sulfadiazine and sulfadimidine) for three days (Lin et al., 2014). Previous
research using various compounds against shrimp species have shown a
highly significant negative correlation between the two variables during
treatment site (Ben-Khedher et al., 2013). By contrast, 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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