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Acute toxicity and antioxidant enzyme responses of *Gammarus* sp. exposed to malathion in Caspian Coastal Waters

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ABSTRACT
Malathion is a widely used organophosphate insecticide in Northern Iran, where agricultural runoff may introduce it into the Caspian coastal ecosystems and threaten non-target aquatic invertebrates. This study evaluated the acute toxicity of a commercial malathion formulation (57% emulsifiable concentrate) and the sublethal antioxidant responses of the Caspian amphipod *Gammarus* sp. collected from the western coast of Bandar Anzali, Iran. Under laboratory conditions, acute toxicity was assessed at six nominal malathion concentrations (0.2–2.2 µg L^{-1}). Probit analysis estimated the 96-h median lethal concentration (LC_{50}) at 0.97 µg L^{-1}, from which a maximum allowable concentration of 0.097 µg L^{-1} was derived. For the sublethal exposure experiment, amphipods were exposed to 0.1, 0.2, 0.3, and 0.5 µg L^{-1} malathion for up to 96 h. Superoxide dismutase (SOD) and catalase (CAT) activities were measured as biomarkers of oxidative stress. Both enzymes showed significant time- and concentration-dependent changes relative to the control. Superoxide dismutase activity increased progressively and reached its highest level after 72 h of exposure. In contrast, CAT activity showed an early transient increase, peaking between 24 and 48 h depending on malathion concentration, followed by a decline towards control levels at 96 h. The contrasting temporal patterns of SOD and CAT indicate that prolonged exposure to sublethal malathion concentrations may disrupt antioxidant defence in *Gammarus* sp. These findings indicate the high sensitivity of this amphipod to malathion and support the use of SOD and CAT activities as biomarkers of organophosphate-induced oxidative stress in the Caspian coastal environments.

Keywords: Acute toxicity, Antioxidant enzymes, Caspian Sea. Catalase, *Gammarus* sp., Malathion.

INTRODUCTION

Amphipods of the genus *Gammarus* play important ecological roles in aquatic ecosystems as detritivores and as key trophic links between organic matter and higher-level consumers, including fish and benthic predators (Grabner & Sures 2019; Giari *et al.* 2020). Owing to their broad distribution, ecological relevance, and sensitivity to environmental contamination, *Gammarus* species are widely used as bioindicators in freshwater and coastal ecotoxicological studies (Zaghloul *et al.* 2020). In the Southern Caspian region, aquatic ecosystems are increasingly affected by agricultural runoff and urban discharges, with organophosphate insecticides representing an important group of chemical stressors (Bashkin 2006; Ghaemi *et al.* 2024).

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Malathion is a widely used organophosphate insecticide applied in agricultural and vector-control programs (Easa & El-Wafa 1995; Yedjou & Moore 2015; Madzorera 2017). Its extensive use in agricultural areas surrounding the Caspian watersheds may increase the likelihood of its transport to surface waters through runoff and other diffuse pathways (Ghaemi *et al.* 2024). Although malathion is generally considered less persistent than several other organophosphate insecticides (Katagi 2009), it may still pose risks to aquatic organisms, particularly through sublethal effects at environmentally relevant concentrations. In aquatic environments and exposed organisms, malathion can be transformed into malaoxon, a more toxic oxon metabolite with greater acetylcholinesterase-inhibiting potential, thereby increasing the hazard to non-target organisms (Samocha *et al.* 2002; Aker *et al.* 2008). In addition to its established neurotoxic effects, malathion exposure may disrupt cellular redox balance by enhancing reactive oxygen species production and altering antioxidant defences (Yang *et al.* 2021; Abdelfattah & El-Bassiony 2022). Superoxide dismutase (SOD) and catalase (CAT) are essential components of the antioxidant defence system. Superoxide dismutase catalyses the conversion of superoxide radicals to hydrogen peroxide and molecular oxygen, whereas CAT decomposes hydrogen peroxide into water and oxygen. Accordingly, changes in SOD and CAT activities are commonly used as biochemical indicators of oxidative stress in aquatic organisms (Falfushynska *et al.* 2012; Huang *et al.* 2022).

Despite the ecological importance of the Southern Caspian coastal ecosystems, information on the acute toxicity of malathion and associated antioxidant responses in local *Gammarus* populations remains limited. Available ecotoxicological evidence has been derived largely from amphipod species and populations outside the Caspian region (Maltby *et al.* 2002; Ashauer *et al.* 2011). However, toxicity estimates and biomarker responses may vary among species and populations because of physiological, genetic, and environmental differences. In particular, local water-quality characteristics, including salinity and ionic composition, may influence pesticide bioavailability and subsequent biological responses (Katagi 2009; Ghaemi *et al.* 2024). Therefore, toxicity data generated using locally collected amphipods are needed to improve the ecological relevance of pesticide-risk assessments for the Caspian coastal waters.

The present study aimed to: (i) determine the 96-h median lethal concentration (LC_{50}) of a commercial malathion formulation for *Gammarus* sp. collected from the western coast of Bandar Anzali, Iran; and (ii) evaluate changes in SOD and CAT activities during a 96-h sublethal exposure period. By combining acute-toxicity testing with antioxidant-biomarker analysis, this study provides preliminary data on the sensitivity of a Caspian coastal *Gammarus* population to malathion and may support ecological risk assessment of organophosphate contamination in the region.

2. MATERIALS AND METHODS

2.1. Collection and acclimation of specimens

Adult amphipods, *Gammarus* sp. (Crustacea: Amphipoda), were collected seasonally between June 2023 and May 2024 from the shallow sandy coastal zone of the western coast of Bandar Anzali, the Southern Caspian Sea, Iran (sampling site coordinate details are shown in Fig. 1).

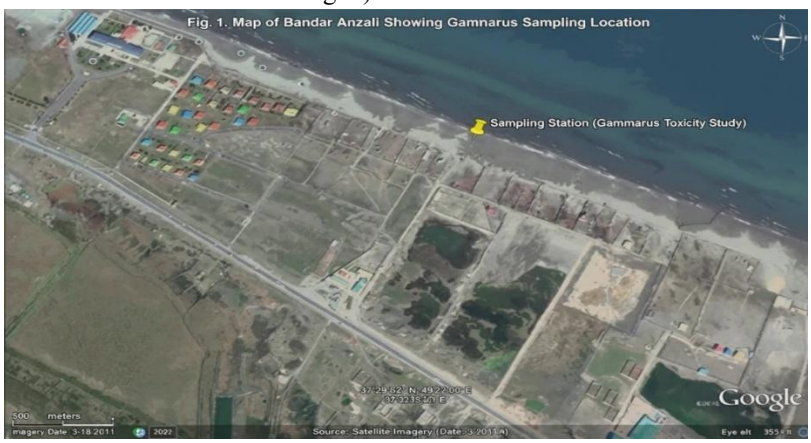

Fig. 1. Sampling location in Bandar Anzali, southern Caspian Sea.

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Sampling was conducted at water depths of 0.5–1.0 m using hand nets (mesh size: 500 µm). The collected specimens were immediately transferred to the laboratory in 10-L insulated containers filled with the aerated Caspian Sea water from the collection site.

Prior to the bioassays, the amphipods were acclimated for 14 days in 50-L glass aquaria containing filtered, aerated Caspian Sea water under controlled laboratory conditions. During acclimation, water parameters were maintained as follows: temperature 18 ± 1°C, dissolved oxygen > 7.5mg L^{-1}, pH 7.8 ± 0.2, and salinity 12 ± 0.5 ppt. The aquaria were kept under a 12 h light:12 h dark photoperiod. The organisms were fed daily with pre-decayed alder leaf litter. Feeding was discontinued 24 h before the initiation of the exposure trials. Only active, healthy adult individuals of uniform size (total length: 12 ± 2 mm; wet weight: 20 ± 5 mg) were selected for the experiments.

2.2. Acute toxicity bioassay

A commercial formulation of malathion (57% emulsifiable concentrate, EC; Aria Shimi, Tehran, Iran) was used. A primary stock solution of 100 mg L^{-1} was prepared daily in Milli-Q water. All nominal test concentrations were calculated based on the active ingredient (malathion content). Since the stock was prepared directly in Milli-Q water without organic solvents, no solvent control was required.

The acute toxicity of malathion was evaluated using a 96-h static-renewal bioassay. Based on preliminary range-finding tests, six nominal concentrations of malathion were established: 0.2, 0.4, 0.7, 1.1, 1.6, and 2.2 µg L^{-1}. A control group containing only the filtered Caspian Sea water was run in parallel. The bioassays were conducted in 400-mL glass beakers containing 300 mL of exposure solution. Ten healthy amphipods were randomly allocated to each beaker, with three independent replicate beakers per concentration, yielding a total of 30 individuals per treatment ($N = 30$). Exposure solutions in all beakers were renewed completely (100% renewal) every 24 h to maintain stable nominal concentrations and prevent the accumulation of nitrogenous waste. Continuous gentle aeration was supplied to maintain dissolved oxygen levels above 5.5 mg L^{-1}. Mortality was monitored and recorded at 24, 48, 72, and 96 h. Dead individuals, identified by the complete absence of appendage movement and lack of response to gentle mechanical stimulation with a glass probe, were recorded and immediately removed. The median lethal concentrations (LC_{10}, LC_{50}, and LC_{90}) with their respective 95% confidence intervals were calculated using probit analysis (USEPA 2002).

2.3. Sublethal Exposure and Sample Preparation

To evaluate sublethal antioxidant enzyme responses, four concentrations of malathion were selected: 0.1, 0.2, 0.3, and 0.5 µg L^{-1}, representing fractions below the 96-h LC_{50}. A control group (0 µg L^{-1}) was maintained under identical conditions. Each treatment and the control were carried out in five independent replicate 2-L glass aquaria, each containing 1.5 L of exposure solution and 20 amphipods, resulting in 100 individuals per treatment ($N = 100$). The physicochemical water conditions (temperature, pH, dissolved oxygen, salinity, and photoperiod) were identical to those maintained during the acclimation period. No food was provided during the 96-h exposure. At the sampling intervals of 24, 48, 72, and 96 h, four surviving amphipods were randomly sampled from each replicate aquarium (yielding 20 amphipods per treatment per time point). The sampled organisms were gently rinsed with cold distilled water, blotted dry on filter paper, pooled by replicate ($n = 5$ pooled samples per treatment per time point), and stored at -80 °C for biochemical analysis. For tissue preparation, the pooled whole-body samples were homogenized on ice in nine volumes (w/v) of cold 0.1 M phosphate buffer (pH 7.4) using a glass-to-glass homogenizer. The homogenates were centrifuged at $10{,}000 \times g$ for 15 min at 4 °C. The resulting supernatant (post-mitochondrial fraction) was collected, aliquoted, and stored at -80 °C until enzymatic assays.

2.4. Biochemical assays

All biochemical assays were performed spectrophotometrically (UV-Vis Spectrophotometer, Shimadzu, Kyoto, Japan) at 25 °C using methods optimized for aquatic invertebrates (Kirici 2022) and validated by Erdogan *et al.* (2023).

2.4.1. Superoxide dismutase (SOD) activity

Superoxide dismutase activity was determined by measuring its ability to inhibit the autoxidation of pyrogallol according to the method of Marklund & Marklund (1974). The reaction mixture contained 50 mM Tris-HCl buffer (pH 8.2), 1 mM ethylenediaminetetraacetic acid (EDTA), and 0.2 mM pyrogallol. The autoxidation rate of

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pyrogallol was monitored at 550 nm. One unit of SOD activity was defined as the amount of enzyme that caused 50% inhibition of the pyrogallol autoxidation rate. The specific activity of SOD was expressed as units per milligram of protein (U mg^{-1} protein).

2.4.2. Catalase (CAT) activity

Catalase activity was determined by measuring the decrease in absorbance of hydrogen peroxide (H_2O_2) at 240 nm according to Aebi (1984). The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7.0) and 10 mM H_2O_2. The reaction was initiated by adding the supernatant, and the decrease in absorbance was monitored for 2 min. Catalase activity was calculated using the molar extinction coefficient of H_2O_2 ($\epsilon = 43.6\ M^{-1}\ cm^{-1}$) and expressed as units per milligram of protein (U mg^{-1} protein), where one unit represents the decomposition of 1 µmol of H_2O_2 per minute.

2.4.3. Protein quantification

Total protein concentration in the supernatant was determined according to the Bradford (1976) method, using bovine serum albumin (BSA) as the standard. All specific enzyme activities were normalized to the total protein content of the sample.

2.5. Statistical analysis

Statistical analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Data normality and homogeneity of variances were assessed using the Shapiro–Wilk and Levene's tests, respectively. Since the biochemical datasets violated the assumptions of normality and homoscedasticity, non-parametric statistics were applied. Differences in enzyme activities among concentrations at each sampling time, and among sampling times within each concentration, were evaluated using the Kruskal–Wallis test. When significant differences were detected ($p < 0.05$), pairwise comparisons between the control and individual treatments were performed using the Mann–Whitney U test with Bonferroni correction for multiple comparisons. The significant level was set at $p < 0.05$. Results are presented as mean $\pm$ standard deviation (SD). Relationships between SOD and CAT activities were evaluated using Spearman's rank correlation coefficient (ρ).

3. RESULTS

3.1. Acute toxicity of malathion

No mortality was recorded in the control group throughout the 96-h exposure period, confirming the validity of the bioassay conditions. Exposure to malathion resulted in concentration- and time-dependent mortality in *Gammarus* sp. (Table 1). The 24-h median lethal concentration (LC_{50}) of malathion was estimated at 8.78µg L^{-1} (95% confidence interval [CI]: 6.12–12.45 µg L^{-1}). Prolonging the exposure period led to a substantial increase in toxicity; the LC_{50} decreased to 3.80 µg L^{-1} (95% CI: 2.70–5.28 µg L^{-1}) at 48 h, 1.23 µg L^{-1} (95% CI: 0.89–1.68 µg L^{-1}) at 72 h, and reached 0.97 µg L^{-1} (95% CI: 0.71–1.32 µg L^{-1}) at 96 h.

The estimated sublethal threshold (LC_{10}) at 96 h was 0.31 µg L^{-1} (95% CI: 0.18–0.44 µg L^{-1}), whereas the LC_{90} value reached 3.05 µg L^{-1} (95% CI: 2.15–4.56 µg L^{-1}). Based on the 96-h LC_{50} and applying an assessment factor of 10, the estimated safe concentration or maximum allowable concentration (MAC) of malathion for this amphipod population was determined to be 0.097 µg L^{-1}:

$$\text{MAC} = \frac{\text{96-h LC}_{50}}{10} = \frac{0.97\ \mu\text{g L}^{-1}}{10} = 0.097\ \mu\text{g L}^{-1}$$

Table 1. Lethal concentrations (LC_{10}, LC_{50}, and LC_{90}) of malathion for *Gammarus* sp. at different exposure durations (95% confidence intervals in parentheses).

Exposure time (h)	LC_{10}(µg L^{-1})	LC_{50}(µg L^{-1})	LC_{90}(µg L^{-1})
24	0.09	8.78	92.18
48	0.34	3.80	42.25
72	0.31	1.23	4.89
96	0.31	0.97	3.05

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3.2. Superoxide dismutase (SOD) activity

Sublethal exposure to malathion induced significant alterations in superoxide dismutase (SOD) activity in *Gammarus* sp., with responses varying according to concentration and exposure time (Table 2).

In the control group, SOD activity remained stable throughout the 96-h exposure period, ranging from 3.9 ± 0.2 to 4.1 ± 0.3 U mg^{-1} protein. Exposure to 0.1 µg L^{-1} malathion caused a progressive increase in SOD activity, from 4.2 ± 0.3 U mg^{-1} protein at 24 h to 5.1 ± 0.4 U mg^{-1} protein at 48 h, reaching a maximum of 6.9 ± 0.5 U mg^{-1} protein at 72 h. This value was 68.3% higher than that of the time-matched control ($p < 0.05$). At 96 h, SOD activity declined slightly to 6.2 ± 0.5 U mg^{-1} protein but remained significantly higher than the control ($p < 0.05$). A similar temporal pattern was observed at 0.2 µg L^{-1} malathion. SOD activity was 5.0 ± 0.3 U mg^{-1} protein at 24 h, increased to 6.3 ± 0.4 U mg^{-1} protein at 48 h, and reached 7.8 ± 0.6 U mg^{-1} protein at 72 h ($p < 0.05$). Activity then declined slightly to 7.1 ± 0.5 U mg^{-1} protein at 96 h.

Table 2 .Superoxide dismutase (SOD) activity (U mg^{-1} protein) in *Gammarus* sp. exposed to sublethal malathion concentrations.

Time (h)	Control	0.1 µg L^{-1}	0.2 µg L^{-1}	0.3 µg L^{-1}	0.5 µg L^{-1}
24	3.9 ± 0.2^{a}	$4.2 \pm 0.3^{a,b}$	$5.0 \pm 0.3^{b,c}$	6.1 ± 0.4^{d}	4.8 ± 0.3^{b}
48	4.0 ± 0.3^{a}	5.1 ± 0.4^{b}	6.3 ± 0.4^{c}	7.6 ± 0.5^{d}	5.8 ± 0.4^{c}
72	4.1 ± 0.3^{a}	6.9 ± 0.5^{b}	7.8 ± 0.6^{c}	8.5 ± 0.6^{d}	$6.3 \pm 0.5^{b,c}$
96	4.0 ± 0.2^{a}	6.2 ± 0.5^{b}	7.1 ± 0.5^{c}	7.4 ± 0.5^{c}	5.9 ± 0.4^{b}

Values are mean ± SD ($n = 5$ pooled samples; 4 individuals per pool). Different superscript letters within a row indicate significant differences among treatments at the same exposure time ($p < 0.05$; Kruskal–Wallis test followed by pairwise Mann–Whitney U tests with Bonferroni correction).

The highest SOD activity among all treatments was recorded after 72 h exposure to 0.3 µg L^{-1} malathion (8.5 ± 0.6 U mg^{-1} protein), representing a 2.07-fold increase relative to the time-matched control ($p < 0.05$). At this concentration, SOD activities at 24, 48, and 96 h were 6.1 ± 0.4, 7.6 ± 0.5, and 7.4 ± 0.5 U mg^{-1} protein, respectively.

Conversely, at the highest sublethal concentration (0.5 µg L^{-1}), SOD induction was less pronounced than in the 0.3 µg L^{-1} group. SOD activities were 4.8 ± 0.3, 5.8 ± 0.4, 6.3 ± 0.5, and 5.9 ± 0.4 U mg^{-1} protein at 24, 48, 72, and 96 h, respectively. Nevertheless, SOD activity remained significantly higher than in the corresponding control group at all sampling times ($p < 0.05$).

3.3. Catalase (CAT) activity

Catalase (CAT) activity in the control group remained stable throughout the bioassay, with a mean value of 5.0 ± 0.3 U mg^{-1} protein. Sublethal exposure to malathion induced an early increase in CAT activity, followed by a decline towards control values at later sampling times (Table 3).

Table 3. Catalase (CAT) activity (U mg^{-1} protein) in *Gammarus* sp. exposed to sublethal malathion concentrations.

Time (h)	Control	0.1 µg L^{-1}	0.2 µg L^{-1}	0.3 µg L^{-1}	0.5 µg L^{-1}
24	5.0 ± 0.3^{a}	5.8 ± 0.4^{b}	6.3 ± 0.4^{c}	6.8 ± 0.5^{c}	5.9 ± 0.4^{b}
48	5.0 ± 0.3^{a}	6.1 ± 0.5^{b}	6.7 ± 0.5^{c}	$6.4 \pm 0.4^{b,c}$	6.0 ± 0.4^{b}
72	5.0 ± 0.3^{a}	5.2 ± 0.4^{a}	5.6 ± 0.4^{b}	5.7 ± 0.4^{b}	5.1 ± 0.3^{a}
96	5.0 ± 0.3^{a}	4.9 ± 0.4^{a}	5.1 ± 0.4^{a}	5.2 ± 0.4^{a}	4.8 ± 0.3^{a}

Values are mean ± SD ($n = 5$ pooled samples; 4 individuals per pool). Different superscript letters within a row indicate significant differences among treatments at the same exposure time ($P < 0.05$; Kruskal–Wallis test followed by pairwise Mann–Whitney U tests with Bonferroni correction).

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In the 0.1 µg L^{-1} treatment group, CAT activity increased to 5.8 ± 0.4 U mg^{-1} protein at 24 h and reached a maximum of 6.1 ± 0.5 U mg^{-1} protein at 48 h ($p < 0.05$). Activity subsequently declined to 5.2 ± 0.4 and 4.9 ± 0.4 U mg^{-1} protein at 72 and 96 h, respectively, and did not differ significantly from the corresponding controls at these times ($p > 0.05$).

Similarly, at 0.2 µg L^{-1} malathion, CAT activity increased significantly to 6.3 ± 0.4 U mg^{-1} protein at 24 h and peaked at 6.7 ± 0.5 U mg^{-1} protein at 48 h ($p < 0.05$). It then declined to 5.6 ± 0.4 U mg^{-1} protein at 72 h and returned to the control level (5.1 ± 0.4 U mg^{-1} protein) by 96 h ($p > 0.05$).

In the 0.3 µg L^{-1} group, the maximum CAT activity occurred earlier, at 24 h (6.8 ± 0.5 U mg^{-1} protein), representing a 36.0% increase relative to the time-matched control ($p < 0.05$). Thereafter, CAT activity declined progressively to 6.4 ± 0.4 U mg^{-1} protein at 48 h and 5.7 ± 0.4 U mg^{-1} protein at 72 h, approaching the control level (5.2 ± 0.4 U mg^{-1} protein) by 96 h ($p > 0.05$).

At the highest exposure concentration (0.5 µg L^{-1}), CAT activity showed a less pronounced response, reaching 5.9 ± 0.4 U mg^{-1} protein at 24 h and 6.0 ± 0.4 U mg^{-1} protein at 48 h ($p < 0.05$). Following this early increase, CAT activity declined to 5.1 ± 0.3 U mg^{-1} protein at 72 h and 4.8 ± 0.3 U mg^{-1} protein at 96 h; these values did not differ significantly from the corresponding controls ($p > 0.05$).

3.4. Comparative temporal profiles of SOD and CAT activity

The temporal profiles of SOD and CAT activities in *Gammarus* sp. showed distinct patterns during the 96-h exposure period. SOD activity was induced progressively in all treatments, reaching maximum levels at 72 h, and remained elevated above the controls at 96 h. In contrast, CAT activity exhibited an early induction phase (peaking at 24 h or 48 h depending on the concentration) followed by a decline, returning to baseline control values by 96 h.

A Spearman rank correlation analysis was not performed, since individual paired replicate data were not available, and enzymatic activities were measured on distinct pooled biological samples. Consequently, no direct correlation between SOD and CAT activities is reported.

4. DISCUSSION

The present study demonstrates that malathion is highly toxic to the tested Caspian population of *Gammarus* sp. The 96-h LC_{50} was 0.97 µg L^{-1}, and the progressive decline in LC_{50} from 8.78 µg L^{-1} at 24 h to 0.97 µg L^{-1} at 96 h clearly indicates that toxicity increased with exposure duration. This time-dependent increase in mortality suggests that the toxic effects of malathion were not limited to an immediate response but accumulated or intensified during prolonged exposure. Such a pattern is consistent with the delayed physiological consequences of organophosphate exposure, including disruption of metabolic processes, impairment of detoxification systems, and oxidative imbalance.

The relatively low 96-h LC_{50} obtained in this study indicates that *Gammarus* sp. may be particularly vulnerable to malathion contamination in the Caspian coastal waters. Although direct comparisons among studies should be made cautiously, since toxicity values are affected by test conditions, species identity, life stage, water chemistry, and formulation type, the present findings support the view that amphipods can be sensitive indicators of pesticide contamination. Differences in susceptibility among *Gammarus* populations may result from interspecific or population-level variation in body size, physiological condition, metabolic rate, detoxification capacity, and previous exposure history (Maltby *et al.* 2002; Ashauer *et al.* 2011; Osterberg *et al.* 2012; Nyman *et al.* 2013).

The observed toxicity is ecologically relevant, since amphipods are important components of coastal food webs. They contribute to decomposition and nutrient cycling and serve as prey for fish and other aquatic organisms. Therefore, reductions in amphipod survival following pesticide exposure may affect not only the exposed population, but also ecological processes and trophic interactions in coastal ecosystems. In semi-enclosed environments such as the Caspian Sea, pesticide inputs from agricultural drainage, river discharge, and runoff may generate short-term concentration pulses. Such episodic exposures can be particularly important, since they may reach biologically harmful concentrations even when average concentrations measured during routine monitoring appear low.

The maximum allowable concentration (MAC) estimated in the present study was 0.097 µg L^{-1}, calculated as one-tenth of the 96-h LC_{50}. This value should be regarded as a laboratory-derived precautionary threshold rather than

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a regulatory water-quality criterion. Nevertheless, it provides an initial ecotoxicological reference point for evaluating potential malathion risks to sensitive amphipod populations in the Caspian coastal waters. Since this estimate is based on acute mortality in one test organism under controlled laboratory conditions, further studies incorporating chronic exposure, reproductive endpoints, behavioral responses, multiple life stages, and other representative taxa are required before establishing a formal regional guideline value.

4.1. Antioxidant enzyme responses to sublethal malathion exposure

The sublethal exposure experiment showed that malathion altered antioxidant enzyme activity in *Gammarus* sp., supporting the occurrence of a physiological stress response consistent with oxidative stress. Both SOD and CAT activities changed in a concentration- and time-dependent manner; however, their temporal patterns were different. This difference is important, since SOD and CAT act sequentially within the antioxidant defense system. SOD converts superoxide radicals to hydrogen peroxide, whereas CAT subsequently decomposes hydrogen peroxide into water and oxygen. Therefore, changes in the activity of one enzyme can influence the oxidative burden handled by the other.

SOD activity increased in exposed organisms and generally reached its maximum at 72 h. The highest SOD activity was observed at 0.3 µg L^{-1} after 72 h (8.5 ± 0.6 U mg^{-1} protein), which was substantially higher than that of the corresponding control. This response indicates that malathion exposure stimulated the enzymatic capacity to neutralize superoxide radicals. The elevation of SOD activity during the first 72 h can be interpreted as a compensatory defense mechanism against increased reactive oxygen species production. Similar increases in SOD activity have been reported in aquatic invertebrates exposed to pesticides and other chemical stressors, indicating activation of antioxidant defenses under adverse conditions (Branković *et al.* 2018; Carlsson & Örn 2024; Czarny Krzymińska *et al.* 2024; Le *et al.* 2024).

However, the decline in SOD activity at 96 h, despite values generally remaining above the control level, suggests that the antioxidant response may not be sustained indefinitely. This reduction may reflect partial enzyme inhibition, depletion of cellular resources required for enzyme synthesis or repair, or a reduced capacity to compensate for continued oxidative challenge. Thus, the SOD pattern suggests an initial induction of antioxidant defenses followed by a possible weakening of the compensatory response during longer exposure.

CAT activity showed a faster and more transient response than SOD. CAT increased during the early phase of exposure, reaching its maximum at 24 h in the 0.3 µg L^{-1} treatment (6.8 ± 0.5 U mg^{-1} protein), while peak activity in the 0.1 and 0.2 µg L^{-1} treatments occurred at 48 h. This early increase indicates rapid activation of CAT in response to hydrogen peroxide generated during malathion-induced oxidative processes. The return of CAT activity toward control values by 96 h may indicate that CAT activity was no longer sufficiently induced under prolonged exposure or that the enzyme was affected by sustained oxidative conditions.

The earlier CAT peak and later SOD peak indicate temporal decoupling between these two antioxidant enzymes. This pattern may have important physiological implications. Increased SOD activity generates hydrogen peroxide as a product of superoxide detoxification; therefore, if CAT activity declines while SOD remains elevated, hydrogen peroxide removal may become less efficient. Such an imbalance could increase the probability of intracellular hydrogen peroxide accumulation and oxidative damage during extended exposure. Although oxidative damage was not directly measured in the present study, the observed enzyme-response pattern is consistent with a disruption of antioxidant homeostasis.

The strongest enzymatic responses were not always observed at the highest malathion concentration. For example, the highest SOD and CAT activities occurred at 0.3 µg L^{-1} rather than at 0.5 µg L^{-1}. This non-linear response may indicate that moderate concentrations induced an active and measurable compensatory response, whereas the higher concentration may have imposed greater physiological stress and partially constrained enzyme activity. Such non-monotonic biomarker responses are common in toxicological studies, since enzyme activity reflects the balance between induction of defense mechanisms and toxicant-related impairment of cellular function.

Overall, the biochemical findings complement the acute toxicity results. The low 96-h LC_{50} confirms a high mortality risk at elevated exposure concentrations, while the alterations in SOD and CAT at sublethal concentrations demonstrate that malathion can disturb the antioxidant defense system before lethal effects become evident. Therefore, SOD and CAT may be useful complementary biomarkers for detecting early physiological effects of malathion exposure in *Gammarus* sp. from the Caspian coastal waters.

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5. CONCLUSION

In conclusion, the Caspian amphipod *Gammarus* sp. showed high sensitivity to malathion, as indicated by a 96-h LC_{50} of 0.97 µg L^{-1}. Sublethal malathion exposure altered the antioxidant defense system, characterized by an increase in SOD activity that peaked at 72 h and an early, transient increase in CAT activity that declined after 24–48 h. The different temporal responses of these enzymes suggest that prolonged malathion exposure may disrupt antioxidant homeostasis and reduce the organism's capacity to cope with oxidative stress.

These findings indicate that toxicity data derived from non-local or non-representative test species may not adequately protect sensitive taxa in the Caspian coastal waters. Therefore, the development of region-specific ecotoxicological benchmark values for organophosphate pesticides is important for improving environmental risk assessment and supporting the protection of aquatic communities in the Caspian Sea.

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