Toxicity of herbicide Atrazine against Freshwater Crab, *Barytelphusa cunicularis* (Westwood in Sykes, 1836): Analysis of Lethal and Safe Concentration with Behavioral Study.

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Abstract

Atrazine ($C_8H_{14}ClN_5$) reported as one of the most widely used herbicide, primarily for controlling weeds in crops specifically for corn and sugarcane. However, it causes a significant toxic threat to aquatic organisms. Present investigation aimed for to evaluate the lethal concentration (LC_{50}) and safe concentration of atrazine against freshwater crab, *Barytelphusa cunicularis*. During experiment toxicity tests were conducted and the LC_{50} values were determined using SPSS software with a 95% confidence level. The calculated LC_{50} values for atrazine exposure were: 3694.44 ppm (3454.25 - 9431.34) for 24 hours, 3639.57 ppm (3374.86 - 44409) for 48 hours, 3396.97 ppm (3218.99 - 5295.51) for 72 hours, and 3037.62 ppm (2754.46 - 3162.47) for 96 hours. The safe concentration for *Barytelphusa cunicularis* was ranges from 151.88 to 1215.04 ppm. Exposure of herbicide atrazine also induced some abnormal and stress related behavioral changes in experimental animals. These findings were highlighted specific potential ecological risks of atrazine contamination in aquatic environments, emphasizing the need for careful monitoring and regulation of use of atrazine in agricultural field.

Keywords- *Barytelphusa cunicularis*, Atrazine toxicity, LC_{50}, Safe concentration, SPSS.

1. Introduction-

Agricultural pesticide use has been increasing worldwide, especially in countries with large-scale agricultural production, such as the United States, China, India, and Brazil (James *et al.* 2012 and Reddy *et al.* 2024).

In the agroindustry, pesticide pollution reported as considerable environmental issue which has affected ecosystems and human health. Aquatic contamination occurred due to such agricultural chemicals (like pesticides, herbicides, rodenticides, insecticides etc.) was found significant environment concern, as these chemicals can easily make their way into water systems

through runoff, leaching, or direct application. The widespread use of chemical pesticides in agriculture to control pests, weeds, and diseases has led to various forms of pollution, with negative impacts on soil, water, air, and biodiversity. Agricultural Pesticides, Herbicides, Insecticides etc. were reported toxic not only to the targeted pests but also to non-target organisms, including insects, birds, and other wildlife and may affect their endocrine, nervous, excretory, reproductive and immune systems (Tulgar, 2018; Yadav, 2010; Relyea, 2005 and Simpson and Roger, 1995). Generally Pesticides from agricultural runoff are recognized as significant stressors, adversely affecting invertebrate populations and, consequently, the entire aquatic ecosystem (Gull *et al*., 2019). They often run off into rivers, lakes, and groundwater through rainfall or irrigation, causing contamination. This runoff can lead to toxic effects on aquatic life, disrupt ecosystems, and make water unsafe for human consumption and seriously affects Aquatic biota. These effects vary depending on the specific herbicide used, its concentration, and the types of organisms present (Miyamoto *et al.*, 2008).

Aquatic invertebrates (such as insects, crustaceans, and molluscs) were crucial for the health of aquatic ecosystems. Herbicides can reduce the abundance and diversity of aquatic invertebrates, which were crucial for nutrient cycling and energy transfer in food webs they can directly affect invertebrates, reducing their populations and disrupting their role in the food web (Chaumot *et al*., 2024, Rabo, 2024, Abdallah, 2017).

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) is one of the most extensively used herbicides worldwide, particularly in the agricultural sector. It was primarily applied for pre- and post-emergence control of broadleaf and grassy weeds in major crops such as corn, sorghum, and sugarcane (Silvestre *et al*., 2002; Alvarez *et al*., 2014 and Prasad *et al*., 1994). Atrazine belongs to the triazine class of herbicidal compounds, which were characterized by a symmetrical triazine ring and various substituted side chains that contribute to their selective herbicidal activity (Breckenridge *et al*., 2010). It's a member of the triazine class of chemicals and works by inhibiting photosynthesis in plants, effectively preventing them from producing energy, which leads to their death (Silveyra *et al*., 2017)

Despite its effectiveness and widespread use, atrazine has become a subject of environmental and health concerns due to its persistence in soil and potential to contaminate groundwater and surface waters. It is relatively stable under natural conditions, with a half-life in soil ranging from several

weeks to months, depending on temperature, moisture, and microbial activity (Gammon et al., 2005 and Arabi *et al*., 2024). Atrazine was detected in drinking water sources in some agricultural regions, prompting regulatory scrutiny in several countries. In the United States, it remains registered for use under the Environmental Protection Agency (EPA) guidelines, whereas the European Union banned its use in 2004 due to groundwater contamination risks (Bethsass and Colangelo 2006). Atrazine has raised concerns due to its potential environmental and health impacts. It is known to contaminate groundwater, and there are concerns about its effects on aquatic life and wildlife. In addition, studies have suggested that atrazine may have hormone-disrupting effects, potentially influencing reproductive health in animals and possibly humans (Pathak and Dikshit, 2011).

Atrazine has raised serious environmental concerns due to its high toxicity and persistence in the environment. It adversely affects a wide range of non-target organisms, including aquatic life and terrestrial animals (Mada et al., 2013). For instance, Fernández-Naveira et al. (2016) reported that atrazine significantly inhibited the growth and nitrate reductase activity of the freshwater algae, *Chlamydomonas reinhardtii*. One of the key indicators of environmental stress caused by atrazine was dissolved oxygen. Furthermore, Almberg *et al*. (2018) found a correlation between atrazine-contaminated drinking water and reduced birth weights in infants and highlighted potential risk to human health. Several studies have also documented the detrimental effects of atrazine on fish, showed its interference with normal physiological functions (Waring and Moore, 2004; Westmoreland, 2018 and Ahmad *et al*., 2021).

In addition, the toxic impact of atrazine on marine crabs has been widely reported (Silveyra *et al*., 2017; Pillai *et al*., 1979; Silvestre *et al*., 2002; Prasad *et al*., 1995 and Alvarez *et al*., 2014), further emphasizing the widespread ecological risks associated with this herbicide. Freshwater crabs were continuously exposed to various harmful chemical herbicides through agricultural runoff in their natural aquatic habitats; however, they have received comparatively little scientific attention. Several studies have explored the impact of different pesticides on different freshwater crab species; for instance, Deyashi *et al*. (2016) investigated the acute toxicity of the biopesticide Nimbicidine Plus on the freshwater crab *Varuna litterata*. Similarly, Deshai *et al.* (2012), Desai, (2021), Barde and Jagtap, (2019), and Mali and Afsar, (2011) reported the toxic effects of Endosulfan, Dimethoate, Acephate, and Zinc Sulphate, respectively, on *Barytelphusa guerini*.

Singh *et al*. (2021) examined the sublethal effects of the insecticide Malathion on *Poppiana dentata* (Randall, 1840), while Taher and Muneeb, (2019) described histological alterations in *Barytelphusa cunicularis* caused by the herbicide Glyphosate. In addition, Vartak and Bondre, (2013) studied the growth and survival of *Barytelphusa cunicularis* under different feeding conditions, and Padghane and Chavan, (2018) explored the habitat preferences and maintenance of species under study.

As per available literature, limited research has been found related to effects of Atrazine, a widely used herbicide on freshwater crabs, particularly in the Kolhapur district. Therefore, the present study was carried out to assess the impact of Atrazine on the freshwater crab *Barytelphusa cunicularis*, focusing on determining the lethal concentration (LC_{50}), safe concentration levels and behavioral responses.

2. Materials and Methods

2.1 Animal under study

Live freshwater crabs (*Barytelphusa cunicularis*) were collected from the banks of the Dudhganga River (16º25.23122′ N; 74º5.46582′ E) in Radhanagari Tehsil, Kolhapur District, Maharashtra, India. Collection was carried out at night using bamboo traps baited with flesh, with minimal disturbance to their natural habitat. The crab species was identified by the Zoological Survey of India, Akurdi, Pune and were used as per the approval of Maharashtra State Biodiversity Board (MSBB/Desk-5/Research/657/2023).

Adult crabs of uniform size, weighing between 105 and 115 grams and with a carapace width of 6.5 to 7.0 cm, were transported to the laboratory in the same bamboo traps. In the lab, experimental animals housed in plastic troughs (110 L capacity) filled with sufficient tap water to keep them submerged. The crabs were fed with a fresh flesh, dried shrimps, and prawns. Ambient temperature and natural photoperiod (12 L: 12D) were maintained, and the water in the troughs was replaced after every 24 hours. All animals were acclimatized in laboratory condition for seven days before experimentation.

2.2 Preparation of test chemicals

Technical grade atrazine (purity >99%) was obtained from Ram Shree Chemicals, Mulund, Mumbai. Stock solutions were prepared using dimethyl sulfoxide (DMSO), purchased from Hi-Media, as the solvent. DMSO was commonly used, cost-effective aprotic polar solvent known for its low toxicity at concentrations below 10% (Kumar et al., 2025 and Kuroda *et al*., 2020). Stock solutions were freshly prepared just prior to each experiment. In all cases, atrazine was first dissolved in DMSO and then diluted with phosphate-buffered saline (PBS, pH 7.2). A typical working solution contained approximately 0.1 mg/mL atrazine in a 1:10 mixture of DMSO and PBS. Different concentrations of atrazine were prepared for initial pilot studies.

2.3 Water quality assessment

Prior to the experimentation, a comprehensive analysis of the physicochemical properties of two water sources; experimental tap water and the water sample collected from the designated study area was conducted. The parameters measured included temperature, pH, electrical conductivity, dissolved oxygen (DO), total hardness, and the concentration of the herbicide atrazine (Deyashi *et al*., 2019). These parameters were selected due to their relevance in assessing water quality and potential impact on experimental outcomes. All these parameters were measured using appropriate apparatus and recorded. All measurements were performed in triplicate to ensure accuracy and reliability of the data.

2.4 Experimental protocol

A preliminary pilot study was conducted to determine the appropriate range of atrazine concentrations for acute toxicity testing. In this study, varying doses of atrazine ranging from 5 µg to 4000 µg were administered to separate sets of crabs, with each set consisting of 10 individuals (n=10). Based on the mortality data obtained from the pilot study, a four-day static renewal acute toxicity test was subsequently carried out (APHA, AWWA, WEF, 2017). The data from this toxicity test were analyzed using probit analysis as described by Finney, (1971) to determine the median lethal concentration (LC_{50}) of atrazine.

In the acute toxicity experiment, adult male and female freshwater crabs of *Barytelphusa cunicularis* **(Figure 1)** were randomly divided into treatment groups, each comprising 10 individuals. Each group was placed in a separate plastic trough (40 L capacity) containing 10 liters

of tap water. The crabs were exposed to six different concentrations of atrazine: 2900 ppm, 3000 ppm, 3100 ppm, 3200 ppm, 3300 ppm, and 3500 ppm. In addition, a control group was maintained under identical conditions, except that the water contained a 1:10 mixture of dimethyl sulfoxide (DMSO) and aqueous phosphate-buffered saline (PBS) without any atrazine.

Throughout the 96-hour exposure period, the water in each trough, including the control, was renewed every 24 hours to maintain water quality and consistent exposure levels. Mortality was recorded at regular intervals, and any dead individuals were promptly removed to prevent water quality deterioration and potential secondary effects and mortality rate was recorded for all the doses. The entire experiment was conducted in triplicate to ensure the reproducibility and reliability of the results. This approach allowed for accurate determination of the LC_{50} value of atrazine for *Barytelphusa cunicularis* under controlled laboratory conditions. Behaviors of all groups of test crabs were observed and recorded for interpretation.

2.5 Safe concentration determination

The safe concentration of atrazine for *B. cunicularis* was calculated by multiplying the 96 h LC_{50} with different application factors (AF) based on Edwards and Brown, (1966), Burdick, (1967), Sprague, (1971), International Joint Commission, (1977), European Inland Fisheries Advisory Commission, (1983) and Canadian Council of Resources and Environmental Ministry, (1971) and also by Hart et al., (1945).

2.6 Data collection and Analysis

After completion of the toxicity experiment and exposure periods, observed data for mortality and survivability were collected, tabulated and subjected to Probit analysis for statistical analysis which was performed using SPSS software 26 version (Chandra and Verma, 2021 and Tendulkar and Kamble, 2023).

Figure 1: Freshwater crab, *Barytelphusa cunicularis* - a) Male dorsal view b) Male ventral view c)Female dorsal view d) Female ventral view

3. Results

As per the standard literature, toxicology is the scientific study of the harmful effects of chemical, biological, or physical agents on living organisms and the environment. It plays a crucial role in identifying and understanding substances that can cause adverse health effects, and it informs regulatory decisions on safety levels and permissible exposure limits. Toxicological research provides evidence about the potential hazards of substances, while mortality studies reveal whether those hazards lead to increased death rates in real-world populations (Gordon, 2024).

Present investigation aimed for toxicological profile of commonly used herbicide, Atrazine against freshwater crab, *Barytelphusa cunicularis* which often resides near agricultural fields, in shallow aquatic bodies.

3.1 Water analysis

The physicochemical properties of experimental tap water and water from where crabs were collected were presented in **Table 1**. The dissolved oxygen (DO) levels in both river water (8.30 ± 0.15 mg/l) and tap water (8.10 ± 0.12 mg/l) were high, indicating good water quality and the potential to support aquatic life or aerobic biological activity. The temperature of the river water (23.4 ± 0.67°C) was found higher than that of tap water (20.5 ± 0.84°C), which was expected as river water was more directly exposed to different environmental conditions. When examining electrical conductivity (EC), which reflects the concentration of dissolved ions and salts, tap water exhibited a slightly higher value (80 ± 2.05 µmhos/cm) as compared to river water (70 ± 2.01 µmhos/cm). While both values were well within acceptable limits.

The pH values revealed that river water was slightly alkaline (7.22 ± 0.03) compared to the mildly acidic tap water (6.98 ± 0.05). Both pH leveled were within the safe range for drinking water (6.5-8.5). Free chlorine level was found below 0.1 mg/l in both samples, which was a positive indicator from a health standpoint, suggested minimal chemical disinfection residues.

Table 1- Base level analysis of experimental (Tap water) and river water

Sr. No.	Parameter	Unit	Water from study area (River water)	Experimental water (Tap water)
1	Dissolved oxygen	mg/l	8.30 ± 0.15	8.10 ± 0.12
2	Temperature	^{O} C	23.4 ± 0.67	20.5 ± 0.84
3	Electric conductivity	µmhos/cm	70 ± 2.01	80 ± 2.05
4	pH	-	7.22 ± 0.03	6.98 ± 0.05
5	Free chlorine	mg/l	< 0.1	< 0.1
6	Total hardness	mg/l	19.2 ± 1.01	20. 0 ± 1.05
7	Atrazine	ppm	< 0.002	< 0.002

The total hardness values, which were nearly identical; 19.2 ± 1.01 mg/l for river water and 20.0 ± 1.05 mg/l for tap water; classify both as soft water. It indicated low concentrations of calcium and magnesium salts, which was generally favorable for normal aquatic life. Lastly, the Atrazine levels were below 0.002 ppm in both water types, indicating no detectable contamination from this herbicide.

Overall, both river and tap water samples demonstrate good quality, with minor differences attributable to environmental exposure i.e. for the freshwater crab, *Barytelphusa cunicularis.* But, as the present investigation was carried out in laboratory conditions by mixing of dose dependent herbicide atrazine in the experimental plastic troughs, we found negligible silent change in the aquatic media.

3.2 Assessment of toxicity test

Exposure period (hrs)	**LC_{50} values of Atrazine (ppm)**	**95% Confidence limits (ppm)**		**Regression Equation**	**Chi-Square value**	**Coefficient of determination (R^2 Linear)**	**Mean LC50 (ppm)**
		Lower limit	**Upper limit**				
24	3694.44	3454.25	9431.341	Y = 52.62 + 14.66 X	1.173	0.756	3442.15
48	3639.57	3374.86	44409.09	Y = 51.19 + 14.37 X	0.391	0.893	
72	3396.97	3218.99	5295.517	Y = 51.22 + 14.5 X	0.628	0.882	
96	3037.62	2754.46	3162.479	Y = 65.08 + 18.69 X	1.834	0.811	

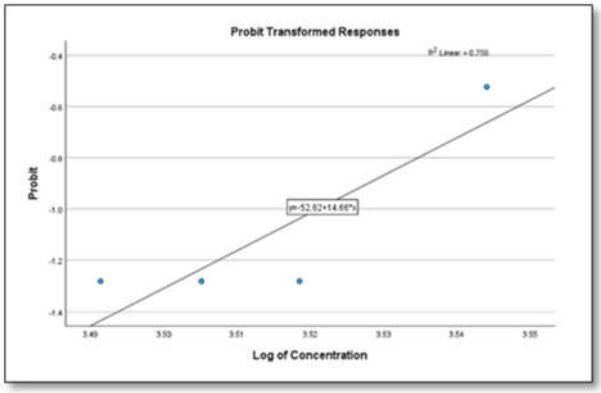
Table 2 - LC50 values with 95% confidence limits for *Barytelphusa cunicularis* exposed to Atrazine.

The LC_{50} values and 95% confidence limits of herbicide Atrazine for freshwater crab, *B. cunicularis* at 24 hr, 48 hr, 72 hr and 96 hr were presented in **Table 2**. (For 24 hrs - 3694.44 [3454.25 - 9431.34] ppm, for 48 hrs - 3639.57 [3374.86 - 44409.09] ppm, for 72 hrs - 3396.97 [3218.99 – 5295.51] and for 96 hrs – 3037.62 [2754.46 – 3162.47]). The mean LC_{50} value of herbicide atrazine was 3442.15 ppm. In our study it was observed that, LC_{50} values decrease with increasing exposure time; from 3694.44 ppm at 24 hours to 3037.62 ppm at 96 hours. Results indicated that the compound became more toxic over time, a common outcome due to cumulative exposure effects. The confidence intervals provide additional insight into the reliability of these estimates.

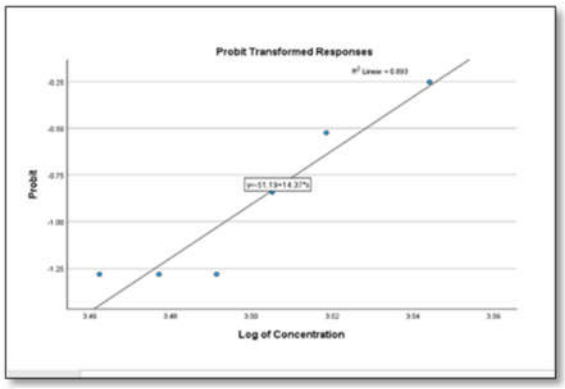
Regression equations for each time point, such as Y = 52.62 + 14.66X for the 24-hour exposure, suggest a linear dose-response relationship. The slope of the regression line increases

over time, with the steepest at 96 hours (18.69), indicated heightened sensitivity to changes in concentration as exposure duration increased. The Chi-square values, all relatively low (ranging from 0.391 to 1.834), demonstrated that the regression models fit the data well, with no significant divergence between observed and expected mortality. The coefficient of determination (R^2) values were ranged from 0.756 to 0.893, further support the strength and reliability of these observations. The plot of Finney's probits against Log 10 concentration of for calculating LC_{50} values of atrazine against freshwater crab, *B. cunicularis* for 24, 48, 72 and 96 hrs has been presented and calculated for confirmation of obtained results **(Graph 1 to 4)**.

The cumulative mortality of the *B. cunicularis* after exposed to 2900 ppm, 3000 ppm, 3100 ppm, 3200 ppm, 3300 ppm and 3500 ppm concentrations of herbicide atrazine up to 96 hrs have been shown in **Table 3.** All the observed data of mortality was subjected to SPSS to confirm the expected mortality response, observed mortality response, residual values and probability values.



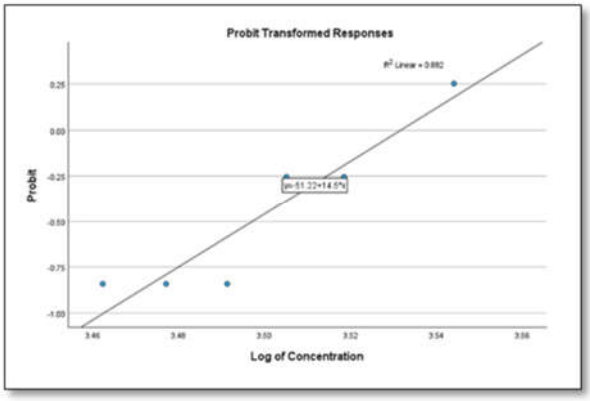
Graph 1: Relationship between Probit mortality at 24 hrs and Log concentration of Atrazine for freshwater crab, *B. Cunicularis*



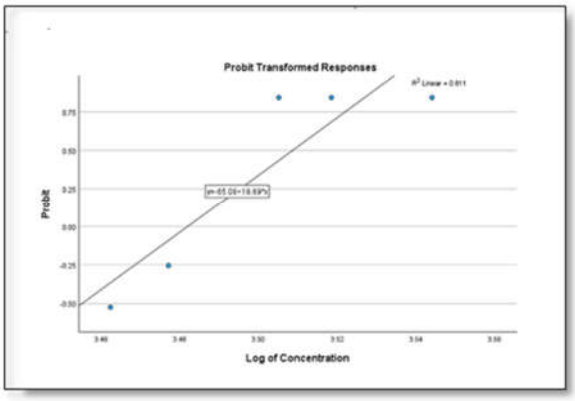
Graph 2: Relationship between Probit mortality at 48 hrs and Log concentration of Atrazine for freshwater crab, *B. Cunicularis*

Table 3 illustrated the acute toxicity of the herbicide atrazine, which was evaluated across a range of dose concentrations from 2900 to 3500 ppm using a total of 240 animals, with 40 animals exposed at each concentration level. A dose-dependent increase in mortality was observed, with the number of dead animals rising from 6 at 2900 ppm to 21 at 3500 ppm. The expected mortality responses, derived from a statistical model, closely approximated the observed responses across the tested doses. Residuals, representing the differences between observed and expected deaths. Additionally, the calculated probabilities of mortality increased with dose concentration, ranging from 0.586 at the lowest dose to 2.162 at the highest, indicating the increased lethality

with higher exposure. Obtained pattern indicated that, the biological response was not only dose-dependent but also significantly time-dependent, with prolonged exposure amplifying the effects even at relatively moderate doses.



Graph 3: Relationship between Probit mortality at 72 hrs and Log concentration of Atrazine for freshwater crab, *B. Cunicularis*



Graph 4: Relationship between Probit mortality at 96 hrs and Log concentration of Atrazine for freshwater crab, *B. Cunicularis*

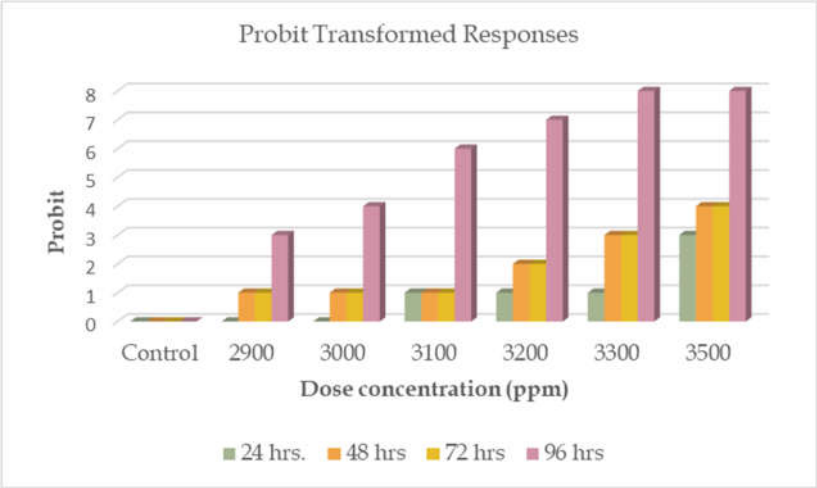
Overall, the **Graph 5** highlights a synergistic relationship between concentration and exposure time in influencing the magnitude of the biological response (Mortality).

Sr. No.	Dose concentration (ppm)	Total No. of animals	Observed Responses (No. of dead animals)	Expected Mortality Responses	Residual	Probability
1	2900	40	06	5.864	-0.746	0.586
2	3000	40	07	8.018	-1.018	0.801
3	3100	40	10	10.449	-0.449	1.045
4	3200	40	15	13.091	1.909	1.308
5	3300	40	16	15.879	0.121	1.588
6	3500	40	21	21.622	0.622	2.162

Table 3 - Probit analysis for induced dose of Atrazine against *Barytelphusa cunicularis* to find out LC_{50} up to 96 hrs.

3.3 Safe concentration

The safe permissible level or safe concentration of herbicide Atrazine for freshwater crab, *Barytelphusa cunicularis* were calculated using different methods and regulatory sources were showed in **Table 4.** These safe concentrations were calculated by multiplying 96 hr.



Graph 5: The graph represents Probit analysis for induced dose of herbicide Atrazine to Freshwater crab, *B. cunicularis* to find out LC_{50} for 24, 48, 72 and 96 hrs.

96 hr LC_{50} value of Atrazine	**Method used**	**Safe concentration (ppm)**
3037.62 ppm	Hart et al., (1945)	1059.77
	Edwards & Brown (1966)	1215.04
	Burdick (1967), Sprague (1971) & EIFAC (1983)	303.76
	IJC (1977)	151.88
	CCREM (1991)	151.88

Table 4 – Safe concentrations of herbicide Atrazine for freshwater crab, *B. cunicularis*

LC_{50} with respective application factors and the formula given by Hart et al., (1945) : C = 48 hr $LC_{50} \times 0.3 / S^2$ where, C was the presumable harmless concentration and S = (24h LC_{50} / 48h LC_{50})^2. Based on these methods, **safe concentration values**; i.e. presumable harmless concentration levels considered safe for *B. cunicularis* ranged from **151.88 ppm to 1215.04 ppm.**

3.4 Behavioral responses

The behavioral responses of both control and Atrazine-exposed crabs were carefully observed and documented over a 96 hour period. In the control group, the crabs displayed normal, healthy behavior throughout the duration of the observation. They exhibited regular locomotion and the typical whirling motion of water currents associated with normal respiration. Air bubbles were rarely seen during respiration, indicating efficient gas exchange. These crabs remained calm

and fully submerged in water, consistently maintaining a stable posture with their bodies aligned parallel to the bottom surface of the container **(Figure 2: a),** which was characteristic of unstressed, healthy individuals. In contrast, the crabs were exposed to the herbicide Atrazine exhibited a range of abnormal and distress-related behaviors. Shortly after exposure, treated crabs showed signs of agitation and discomfort. They persistently tried to escape from the contaminated water, frequently climbing the walls of the container in an effort to remove themselves from the environment **(Figure 2: b)**.

One of the most prominent and concerning observations was the appearance of excessive white frothy bubbles emerging from their mouths **(Figure 2: c)**, which had indicated severe respiratory stress or a physiological reaction to chemical toxicity.

As the exposure progressed, both male and female crabs began to lose their walking appendages; a symptom suggested neuromuscular impairment or systemic toxicity induced by Atrazine. The affected crabs subsequently lost their ability to maintain balance and orientation, eventually collapsing into a vertical downward position **(Figure 2: d and e)**. The state of immobility was typically followed by death, confirming the lethal effect of prolonged Atrazine exposure at the tested concentration.

4. Discussion

Study was aimed to evaluate the acute toxicological effects of the herbicide Atrazine on the freshwater crab *Barytelphusa cunicularis*, with a specific focus on determining the median lethal concentration (LC_{50}) values at different exposure durations using probit analysis. Experimental animals were exposed to varying concentrations of Atrazine for 24, 48, 72, and 96 hours under controlled laboratory conditions. Each experimental set included a corresponding control group, which received no herbicide treatment. Notably, no mortality was recorded in any of the control groups, confirming that all observed lethal effects were attributable solely to Atrazine exposure rather than to environmental stress or handling. Mortality data collected at each time interval were subjected to probit analysis using the SPSS statistical software package. The LC_{50} values determined for Atrazine exposure were 3694.44 ppm at 24 hours, 3639.57 ppm at 48

hours, 3396.97 ppm at 72 hours, and 3037.62 ppm at 96 hours and mean LC_{50} value we have calculated as 3442.15 ppm.

Figure 2: Behavioural responses of freshwater crab, B. cunicularis - a) normal behaviour of control crabs b) atrazine induced escaping behaviour c) froth production d) loss of walking legs in male crab e) loss of walking legs in female crab.

These values indicated a progressive decrease in LC_{50} over time, reflecting the time-dependent nature of Atrazine toxicity and suggesting that prolonged exposure enhances the herbicide's lethality to the test organism. The trend of decreasing LC_{50} values with increasing exposure duration supports the hypothesis of cumulative toxic effects and underscores the persistent nature of Atrazine in aquatic environments.

The consistency and reproducibility of the results across multiple time points enhance the credibility of the findings. Moreover, the absence of mortality in control groups strengthens the conclusion that Atrazine was directly responsible for the observed adverse effects. A probit plot was also generated by plotting the probit transformed mortality rates against the log of Atrazine concentrations, integrating data across all exposure durations. This graphical representation further confirmed the dose-response relationship and provided a robust statistical basis for estimating the LC_{50} values (Chandra and Verma, 2021and Sharikmaslat and Kamble, 2024).

Among the species tested via oral administration, rats appear to be the most sensitive, with an LD_{50} of 672 mg/kg, followed by rabbits (750 mg/kg) and mice (850 mg/kg). The route of exposure significantly influences the level of toxicity. Intraperitoneal injection in rats results in a much lower LD_{50} (235 mg/kg), indicating higher toxicity through this method compared to oral ingestion. Similarly, mice show greater sensitivity via intraperitoneal injection (626 mg/kg) than oral exposure. On the other hand, dermal exposure in rabbits has a considerably higher LD_{50} value (7500 mg/kg), suggested that atrazine has low toxicity through skin contact and was poorly absorbed through the skin. Inhalation toxicity in rats was moderate, with an LD_{50} of 5200 mg/m³ over four hours (Ghosh and Philip, 2006). Under experimentation, we calculated the safe concentration of atrazine against freshwater crab *Barytelphusa cunicularis*, ranging from 151.88 ppm to 1215.04 ppm. These values were derived using various established methods and regulatory sources, including those proposed by Hart *et al*. (1945), Edwards and Brown (1966), Burdick (1967), Sprague (1971), EIFAC (1983), IJC (1977) and CCREM (1971). While these estimates offer a broad perspective on permissible exposure levels in aquatic invertebrates, they highlight a substantial disparity when compared to regulatory thresholds established for drinking water and other aquatic organisms.

Singh *et al*., (2018) reported the maximum acceptable concentration (MAC) of atrazine in drinking water to be 5 µg/L. Similarly, U.S. Environmental Protection Agency (EPA, 2003) has recognized that concentrations above this threshold may induce reproductive effects in fish. Main concern was findings from studies on amphibians, which indicated that adverse effects such as hermaphroditism and gonadal dysgenesis in leopard frogs (*Rana pipiens*) can occur at atrazine concentrations as low as 0.1 ppb (Hayes *et al*., 2002; Erickson, 2016). These results suggested that certain aquatic taxa, particularly amphibians, exhibit heightened sensitivity to atrazine, raising

concerns about its ecological safety at even trace levels. Ezemonye and Tongo, (2009), studied the lethal and sub-lethal effect of atrazine to amphibian larvae, *Ptychadena bibroni* and estimated 96 hours LC_{50} ranges from 230.06 – 431.32 µg/L. According to Almberg, *et al*., (2018), atrazine exposure through drinking water is associated with reduced birth weight among infants in early and mid-pregnancy. Kwairanga *et al*., (2022), reported that, farm raised fish, *Clarias garipienus* showed 40% mortality after 96 hours of Atrazine exposure and the highest mortality was at 4 mg/L.

Moreover, a recent meta-analysis conducted by Oliveira *et al*. (2024) further corroborates the potential toxicity of atrazine by demonstrating increased mortality rates in various fish species exposed to environmentally relevant concentrations. This growing body of evidence underscores the need for species-specific risk assessments and reinforces the importance of considering sub-lethal and chronic effects, especially in ecologically vulnerable organisms.

We have estimated the safe concentration level of atrazine for freshwater crab, *B. cunicularis* which was ranged from **151.88 ppm to 1215.04 ppm** by using different methods and regulatory sources (Hart *et al*., 1945; Edwards and Brown, (1966); Burdick, (1967); Sprague, (1971); EIFAC (1983); IJC (1977) and CCREM (1971). According to Singh *et al*., (2018); maximum acceptable concentration (MAC) for atrazine in drinking water is 5 µg/L but, The U.S. Environmental Protection Agency, suggests that atrazine concentrations above 5 µg/L can lead to reproductive effects in fish, while some studies indicate that effects on amphibians may occur at levels as low as 0.1 ppb, atrazine at 0.1 ppb can cause hermaphroditism and gonadal dysgenesis in leopard frogs (Erickson, 2016) and Hayes *et al*., 2002). However, a meta-analysis indicates increased mortality rates in various fish species exposed to environmental concentrations (Oliveira *et al*., 2024).

These findings align with those reported by Westmoreland (2018), who observed that *Orconectes virilis* crayfish exposed to Atrazine similarly lost their claws within 24 hours. Such consistency across species underscores the potential for Atrazine to cause acute toxicity and impair critical motor functions in aquatic arthropods. Additionally, Deyashi et al. (2019) reported comparable behavioral disturbances in the freshwater grapsid crab *Varuna litterata* exposed to mahua oil cake (MOC) extract, including improper mouthpart movement, froth release,

aggregation, escape responses, and a progressive loss of balance and coordination leading to mortality.

Conclusion

The present study provided compelling evidence of the acute toxicological effects of Atrazine on the freshwater crab *Barytelphusa cunicularis*. The time-dependent decrease in LC_{50} values, coupled with the observed physiological and behavioral changes, highlights Atrazine's cumulative and persistent nature in aquatic environments. Study revealed that exposure to the herbicide Atrazine induced a range of abnormal and stress-related behaviors in the test crabs. Notably, individuals exposed to Atrazine demonstrated an immediate escape response, attempting to leave the contaminated environment. This behavior was accompanied by the excessive production of white frothy bubbles, which may indicate respiratory stress or disturbance of normal physiological processes. Over time, both male and female crabs exhibited the loss of walking appendages, suggesting that Atrazine may interfere with neuromuscular coordination or contribute to tissue degradation.

The estimated safe concentration range for *B. cunicularis* (151.88–1215.04 ppm) was significantly higher than thresholds reported for fish, amphibians, and human health, emphasizing the need for species-specific risk assessments. Given the ecological importance of freshwater crabs in benthic food webs and nutrient cycling, Atrazine contamination poses a potential threat to aquatic ecosystem stability. These findings reinforce the urgent need for stricter regulatory monitoring and ecotoxicological evaluation of commonly used herbicides like Atrazine to safeguard aquatic biodiversity.

Ethical Approval

All experimental animals were used in compliance with the guidelines and approval of the Maharashtra State Biodiversity Board. (MSBB/ Desk-5/ Research/ 657/2023).

Acknowledgement

The authors express their sincere gratitude to the Head, Department of Zoology, Shivaji University, Kolhapur, for providing the necessary laboratory facilities to conduct the present study.

We also acknowledge with thanks the financial support provided by SARTHI – the Chhatrapati Shahu Maharaj Institute of Research, Training, and Human Development, Pune, which greatly facilitated the successful completion of this research.

Conflict of Interest

The authors declare that there is no conflict of interest.

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