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Research Article

LC_{50} Dependent Safe Concentration of Sodium Arsenate against Crustacean Freshwater Crab, *Barytelphusa cunicularis* (Westwood in Sykes, 1836)

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ABSTRACT

Aim/Background: The study investigates the toxic impact of inorganic arsenic compound (sodium arsenate) on the freshwater crab *Barytelphusa cunicularis*. Sodium arsenate is a widely used chemical in various applications including insecticides, rodenticides, herbicides, antibacterial agents, and dye manufacturing, which can contaminate aquatic and terrestrial environments. **Materials and Methods:** The research conducted lethal concentration (LC_{50}) tests using sodium arsenate on *Barytelphusa cunicularis*, analyzing the toxic effects at different exposure durations (24, 48, 72, and 96 hr). Statistical analysis was performed using SPSS with a 95% confidence level to determine the lethal and safe concentrations. **Results:** The LC_{50} values were found to be: 24 hr: 675.60 (645.96-38825) ppm, 48 hr: 657.32 (633.35-4062.70) ppm, 72 hr: 621.85 (605.32-645.02) ppm, 96 hr: 605.41 (589.92-615.39) ppm. The safe concentration for sodium arsenate was determined to be 186.68 ppm. **Conclusion:** The differentiation between mean LC_{50} values and safe concentration provides insights for developing conservative strategies to protect economically important crustacean species like *Barytelphusa cunicularis* from arsenic toxicity.

Keywords: *Barytelphusa cunicularis*, LC_{50}, Safe concentration, Sodium arsenate, SPSS.

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INTRODUCTION

Worldwide, aquatic pollution is a serious problem. Various pollutants get entered in the aquatic bodies and affect the quality and quantity of the aquatic flora and fauna.[1] In the developing country like India, modernization, industrialization and agricultural revolution has severely contaminated natural resources including aquatic and terrestrial. Aquatic organisms showed prominent biochemical, physiological, histopathological alterations.[2,3] Industrial activities, domestic wastefulness, husbandry wastes, agricultural wastes and many other activities have liberated and released heavy metals, pesticides, herbicides, aromatic compounds and many other organic and inorganic discharge in different habitats. These contaminants lead to undesirable changes in the environment and thereby disturbed the ecological harmony of the surrounding.[2-4]

Among the chemical contaminants, Arsenic is a naturally occurring compound that is widely distributed in earth's atmosphere through weathering and volcanism and through arsenic-bearing minerals which undergo oxidation and release content of arsenic in water. It is also released by anthropogenic activities like mining, smelting, and agricultural activities.[5,6] Arsenic reported as a potent toxicant which exists in several oxidative states among number of inorganic and organic forms. Biochemically, Arsenic if deposited can cause number of adverse health effects like neurological, cardiovascular, respiratory, metabolic diseases and known as carcinogenic component.[7] Scientists reported that, trace amount of Arsenic is essential for the metabolic processes of organisms including humans and is ingested over time.[8,9] However, it becomes toxic to living organisms if its concentration level get elevated than the permissible level.[10] Biologically arsenic get accumulate in different cells of animal body and do not degrade from the environment and thereby enters in to the food web. Chronic exposure of inorganic arsenic to humans through drinking water resulted in pathetic reproductive issues including excess miscarriages, stillbirths, preterm births and infants with low birth weights etc. Arsenic may induce change in reproductive organs of both sexes with decreased weight causing prominent inflammation of reproductive organs.[11]

Nowadays, Arsenic compounds are used in agriculture and forestry as pesticides, herbicides and silvicides. Also used in the glass and ceramics industries.[12] Both surface water and ground water get contaminated with arsenic. The permissible level of

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arsenic in drinking water is 0.01 mg/L (10 µg/L).[13] But this level found elevated in both surface and ground water. Groundwater contamination by arsenic has been reported worldwide, of which majority of countries are from South Asia and South American region. The South and Southeast Asian belt reported as severely arsenic polluted areas including India, Bangladesh, Nepal, Vietnam and China.[14] In India, Arsenic contamination in ground water was first reported in West Bengal in 1978 and it includes 79 blocks in 8 districts among 26 districts where arsenic concentration in ground water exceeded 50 µg/L.[15,16] Vegetables and crops were found grown on arsenic contaminated ground water can cause daily intake of arsenic through food rather than drinking water. In West Bengal, tube well water showed 0.042 to 0.251 ppm arsenic which was found more than permissible limit as per World Health Organization, due to which, livestock population was severely affected by Arsenic contamination. Pathological data reported about arsenic deposition in excretory organs and has severe impact on urinary system.[17,18] Number of heavy metals also accumulates in body of aquatic organisms in higher concentration than concentration in water and biomagnified in food chain that causes physiological damage at higher trophic levels and in human.[9] Diet intake was main source of arsenic exposure where, rice and sea food products were key sources of arsenic exposure.[7,19]

Pertaining to toxicity study in crustaceans, heavy metals including arsenic get accumulated at different levels in different parts of the body and thereby affect normal physiology of crustaceans.[10] Freshwater crab, *Barytelphusa cunicularis* is a commonly occurring black coloured crab which is strong enough to live without water in moist and muddy burrows and can air breath and remain alive without food for 3-4 days.[20,21]

Considering available literature, we found that freshwater crabs are often exposed to number of toxic chemicals through agricultural runoff in their natural aquatic habitat, but scanty or no report is available regarding acute toxicities of Sodium arsenate on freshwater crab *Barytelphusa cunicularis*. Taking account of toxic features for arsenic, present study aimed for the determination of lethal effect of arsenic against crustacean species *Barytelphusa cunicularis*.

MATERIALS AND METHODS

Animal under study and its acclimatization

Healthy adult specimens of freshwater crab, *Barytelphusa cunicularis* were collected along the bank of Dudhganga River (16°25.23122′*N*; 74°5.46582′*E*) Radhanagari tehasil, Kolhapur district, Maharashtra, India (Figures 1a and 1b). Crabs were collected at night with minimal disturbance to the habitat by putting bamboo traps with flesh bait (Figures 1c and 1d). The crab species was identified by Zoological Survey of India, Akurdi, Pune. For the present investigation, animals were used as per the approval of Maharashtra State Biodiversity Board (MSBB/ Desk-5/ Research/ 657/2023).

Adult crabs with optimum similar growth size and uniform Carapace Width (CW) 6.5- 7.0 cm and weight 90 to 105 g were brought to the laboratory. Animals were kept in a plastic trough (110 L) filled with sufficient tap water, so that crabs were submerged. Animals were fed with fresh flesh, dried shrimps and prawns etc. The temperature and photoperiod conditions were maintained to 25±2°C and 12 L: 12D respectively. Water was replenished daily. All animals were acclimatized in laboratory condition for 7 days prior to experimentation.[22]

Water samples from study areas were assessed for different physicochemical parameters, similarly experimental water (tap water) was assessed for temperature, pH, dissolved oxygen, and total hardness before the experiment.[23] Animals were under observation for their every activity. Naturally died animals were removed from trough immediately. Finally healthy, active animals Were selected for toxicity experiments.

Experimental procedure for acute toxicity test

For the present investigation, toxicant used in static bioassays was 98.50% Sodium arsenate which is usually handled as a Sodium arsenate dibasic heptahydrate ($Na_2HAsO_4.7H_2O$) and is water miscible purchased from HiMedia (Figure 1e). Chemically it is an odourless, white crystalline chemical compound (Figure 1f).

To determine the LC_{50} value of Sodium arsenate, the four-day static renewal acute toxicity test was carried out in the laboratory applying probit analysis method.[24,25] The animals were starved a day before experimentation to avoid metabolic differences, if any, due to differential feeding. Adult crabs (n=10) having approximately same carapace width and weight were kept in separate plastic trough (40 L capacity) containing 10 L water. Initially animals were exposed to different concentrations of Sodium arsenate (590, 600, 610, 620, 640 and 650 ppm) for 24, 48, 72 and 96 hr. A control group, containing water without any concentration of Sodium arsenate was also maintained throughout the experimental procedure. The water from control and experimental trough was changed after every 24 hr. Dead animals were removed immediately. To determine accurate lethal concentration and confirm results, all experiments were repeated thrice.

After completion of all intoxications and exposure periods, observed data for mortality and survivability were subjected to calculate LC_{50} by Probit analysis.[25] To get a safe permissible level of Sodium arsenate, the safe concentration was determined by applying protocol of Hart *et al.*[26] Confidential limits (upper and lower) of the regression line with chi-square test were also calculated by applying SPSS software 26 version.[9,27]

RESULTS

Experimental water parameters

The physicochemical parameters of water used in experiments and from where animals were collected are shown in Table 1. In general, the experimental water parameters were suitable for the freshwater crab, *Barytelphusa cunicularis*. But, as the present investigation was carried out in laboratory conditions and by mixing of dose dependent sodium arsenate in the experimental troughs, we found negligible silent change in the aquatic media. Overall, the altered physicochemical parameters also contributed their role in the mortality of experimental animals. To confirm the data, total procedure was repeated thrice and finally tabulated the results.

Toxicity test

It is well known that toxicity of any chemical component depends upon its chemical form, the presence of other pollutants, the physiological status of the organisms and the environmental, physico-chemical parameters like temperature, dissolved oxygen, pH, and hardness of water.[9] Arsenic occurs in two major chemical groups; Organic and inorganic. Organic forms of Arsenic are present in plant and animal cells, which are less toxic, whereas inorganic forms of arsenic are highly toxic and cause significant health hazards. The prevalent forms of inorganic arsenic species are arsenite [As (III)] and arsenate [As (V)], both are differed in their mobility, bioavailability and toxicity.[28] As (Ill) i.e. arsenite found five times more toxic than as (V). Although reported, modes of toxicity were different for both arsenite and arsenate against living cells.[29,30]

The cumulative mortality of the freshwater crab *B. cunicularis* after exposure to 590 ppm, 600 ppm, 610 ppm, 620 ppm, 640 ppm and 650 ppm concentrations of Sodium arsenate for 24, 48, 72 and 96 hr respectively have been shown in Table 2. No mortality was found in control group and up to 500 ppm Sodium arsenate concentration.

The percent mortality observed for each concentration was calculated and converted to probit by means of SPSS software. The LC_{50} values and the 95% confidence limits of Sodium arsenate for freshwater crab, *Barytelphusa cunicularis* for 24, 48, 72 and 96 hr are presented in Table 3.

The LC_{50} values with 95% confidence level of Sodium arsenate for freshwater crab, *Barytelphusa cunicularis* were found to be; for 24 hr 675.60 (645.96-38825) ppm, for 48 hr 657.32 (633.35-4062.70) ppm, for 72 hr 621.85 (605.32-645.02) ppm and for 96 hr 605.41 (589.92-615.39) ppm. However, the crabs in control group observed to be healthy and normal. The mean LC_{50} value of Sodium arsenate is 640.04 ppm and the safe concentration was determined following the formula,[25] C=0.3×48 hr TLM×(24 hr TLM/48 hr TLM)^2 (C=Safe concentration). Therefore, C=0.3×65 7.32×(675.60÷657.32)2=186.68 ppm.

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

Sl. No.	Parameter	Unit	River water	Experimental water (Tap water)
1	Temperature	°C	21±1	19±1
2	pH	-	7.22	6.98
3	Electric conductivity	µmhos/cm	70±2	80±2
4	Total hardness	mg/L	19±1	20±2
5	Free chlorine	mg/L	<0.1	<0.1
6	Dissolved oxygen	mg/L	8.30	8.10
7	Arsenic	mg/L	<0.001	<0.001

Table 2: Mortality of the freshwater crab *Barytelphusa cunicularis* after 24, 48, 72 and 96 hr exposure to Sodium arsenate.

Concentration in ppm	Number of animals	Number of dead animals			
		24 hr	48 hr	72 hr	96 hr
Control	10	0	0	0	0
590	10	0	1	2	3
600	10	1	1	3	4
610	10	1	3	4	5
620	10	2	3	5	8
640	10	2	4	7	9
650	10	3	4	7	9

Figure 1: a) Geographical site of Dudhganga river in Radhanagari Tehasil, Kolhapur district, Maharashtra, India. b) Actual site of selected crab *Barytelphusa cunicularis* in a muddy habitat. c) Morphological feature of selected adult freshwater crab *Barytelphusa cunicularis*. d) Handmade bamboo trap used for collection of experimental crab *Barytelphusa cunicularis*. e) Sodium arsenate procured from Himedia used for intoxication to determine mortality. f) Actual crystals.

In the present study the result revealed that, the mortality rate increased with increasing dose concentration. The plot of Finney's probit against Log concentration for calculating LC_{50} value of Sodium arsenate for 24, 48, 72 and 96 hr were presented in Figures 2-5 as follows:

The crabs treated with Sodium arsenate exhibited some behavioural changes like excited and erratic body movements, irregular locomotion, excessive forth and bubbles are seen. Loss of balance was also noticed and ultimately, they turn over ventrally and finally slowed movements, sensation, and died.

DISCUSSION

Although sodium arsenate is widely used as a pesticide, herbicide, and fungicide in agriculture, as well as a wood preservative and in production of dyes and pigments, also used in the food industry as a preservative and to prevent spoilage, LD_{50}/LC_{50} information of Sodium arsenate is not available pertaining to its toxicological aspect. Its lethal and sub lethal effects on various animals are as follows: Intraperitoneal injection of 0.2 mg/kg BW of sodium arsenate to domestic goat, *Capra hircus*, at age 2 months were reported deafness,[31] In Hamster, *Cricetus cricetus*, 8 mg/kg dose intravenously caused increased incidence of malformation and resorption and 16 mg/kg dose killed all embryos.[32] In mouse, *Mus* spp. (FVB/NJ mice), 20 mg/kg BW dose through intraperitoneal route to the 18 days pregnant mice caused abortion or maternal death over 24 hr.[33] In rat, *Rattus norvegicus*, Single intraperitoneal injection of 5 to 12 mg/kg on days 7 to 12 of gestation produced eye defects, exencephaly, and faulty development of kidney and gonads.[34]

In the present investigation, as compared to available literature, we found the growth and development of the animals with normal behavioural pattern is associated with the physicochemical parameters. All the animals were survived in the natural conditions, but when induced with significantly increased dose of sodium arsenate from 590 to 650 ppm, mortality percent in the animals were increased up to 96 hr of exposure. Similar data have been reported by number of scientists in relation to toxicity features of different chemicals against invertebrates and vertebrates. We applied obtained data to the SPSS software and calculated 95% confidential limit values and find out the mean LC_{50} value as 640.04 ppm which can strengthen our toxicity study as a calculated value. Compered to this our data showed 186.68 ppm as a safe concentration. The observed and calculated values were

Table 3: LC_{50} values with 95% confidence limit for *Barytelphusa cunicularis* exposed to Sodium arsenate.

Exposure period (hr)	LC_{50} values of Sodium arsenate (ppm)	95% confidence limit (ppm)		Regression Equation	Chi-Square Value	Coefficient of determination (R^2 Linear)	Mean LC_{50} (ppm)	Safe Concentration (ppm)
		Lower limit	Upper limit					
24	675.60	645.96	38825	Y=59.36+20.9 X	1.093	0.854	640.04	186.68
48	657.32	633.35	4062.70	Y=74.95+26.61 X	1.000	0.820		
72	621.85	605.32	645.02	Y=94.63+33.87 X	0.193	0.978		
96	605.41	589.92	615.39	Y=1.32+47.58 X	1.004	0.936		

Figure 2: Relationship between Probit mortality at 24 hrs and Log concentration of Sodium arsenate for freshwater crab, *B. cunicularis*.

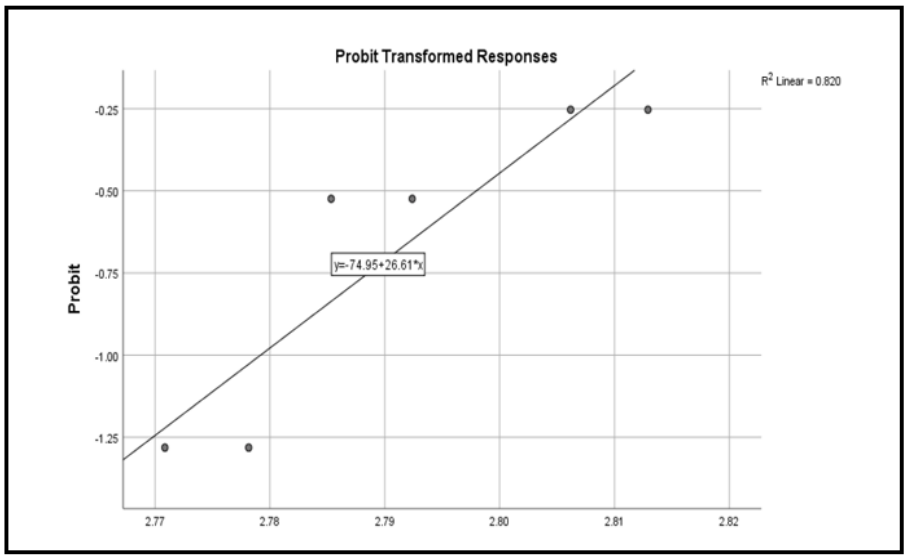

Figure 3: Relationship between Probit mortality at 48 hrs and Log concentration of Sodium arsenate for freshwater crab, *B. cunicularis*

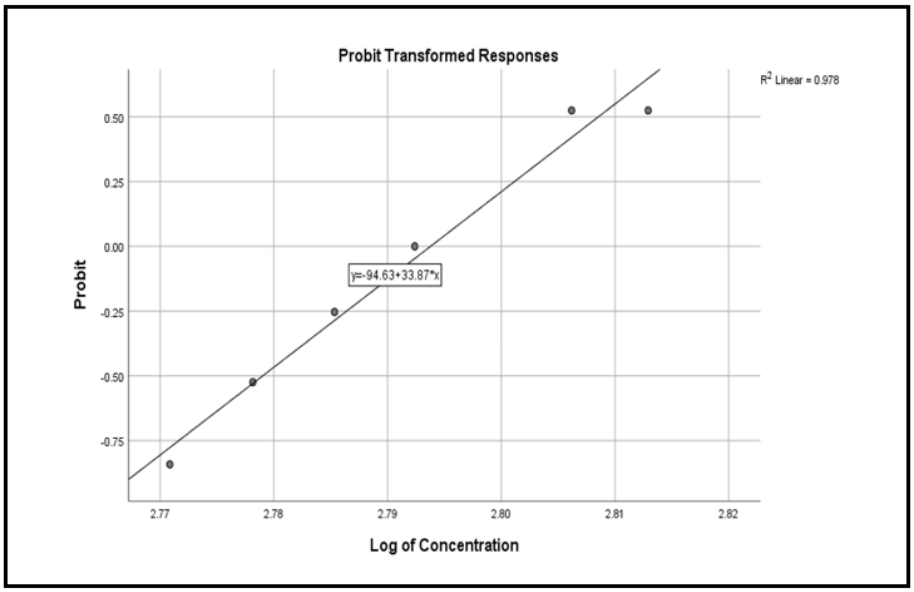

Figure 4: Relationship between Probit mortality at 72 hrs and Log concentration of Sodium arsenate for freshwater crab, *B. cunicularis*

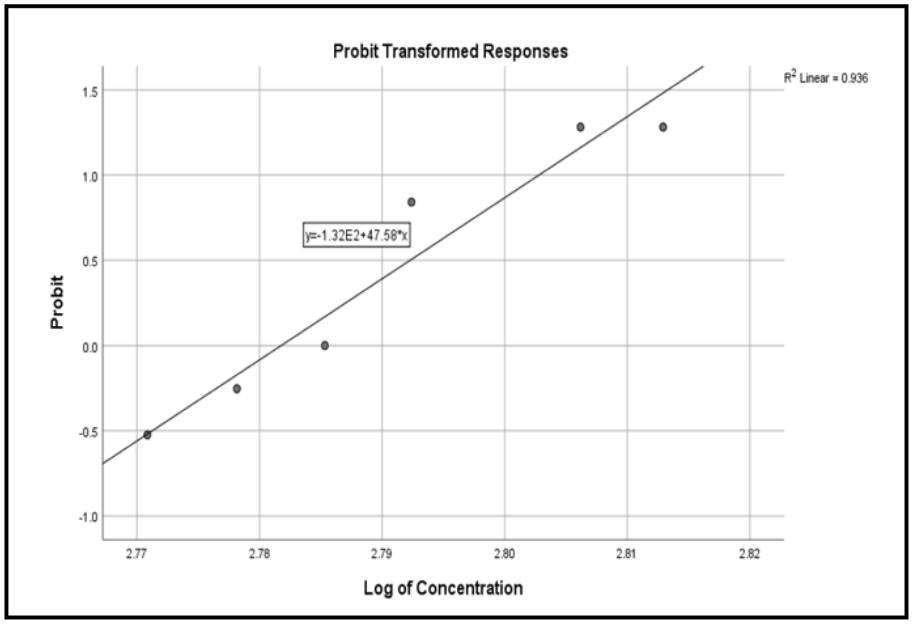

Figure 5: Relationship between Probit mortality at 96 hrs and Log concentration of Sodium arsenate for freshwater crab, *B. cunicularis*.

well supported by previous investigators in the field of toxicology. The graphical presentation of study and its comparative account relates with the data documented by many scientists.[1,2,10,17] Overall, our results in relation with physicochemical parameters in toxication of arsenic and mortality percent of crustacean *Barytelphusa cunicularis* strengthen the capability of sodium arsenate for its bioaccumulation, bioconcentration and biomagnification features.

Arsenic reported as toxic metalloid of environment which has potentiality to affect the normal physiology of crustaceans.[3] Inorganic arsenic results in acute, subacute and chronic toxic effects like abdominal cramping, hyperesthesia in extremities, peripheral vascular disturbances resulting in gangrene and a disease Black foots disease.[35] The level of heavy metal arsenic and chromium in some tissues (muscle, gills, hepatopancreas) of crab, *Barytelphusa cunicularis* is higher (Arsenic- 1.28 ppm and Chromium- 23.33 ppm) than the recommended maximum allowable standards in food, which shows Godavari river is contaminated with heavy metals and the consumption of crabs of the river can cause health hazards to man.[36] Treatment of 3 ppm of sodium arsenite for 30 days to mud crab, *Scylla serrata* resulted in alteration in morphology of hemocyte nuclei.[6] The

concentration of As and Cu in mud crabs, *Scylla serrata* is varies according to environmental factors, physiological characteristics and species of the organism. The level of both As and Cu are exceeding the permissible limit set by WHO (2012), FEPA (2001) and JEFCA (2002).[10] Marine organisms living in coastal waters and estuaries that are heavily contaminated with organic arsenic may accumulate inorganic arsenic to high concentration in some tissues like gills and digestive glands (liver).[37]

According to Chourpagar *et al*., (2016), *Barytelphusa cunicularis* reported as sensitive animal for heavy metal both at acute toxicity and at accumulation level, indicating the possible use of this species in monitoring pollution.[38]

CONCLUSION

Due to modernization, hazardous chemicals were released in to the aquatic and terrestrial media and finally get accumulated in the animals. Present study enlightens prominent feature of sodium arsenate when artificially induced against *Barytelphusa cunicularis*. With respect to the physicochemical parameters, induced study showed heavy intoxication of sodium arsenate in the vital tissues of experimental animal *Barytelphusa cunicularis* with the significant exposure up to 96 hr. The mean LC_{50} of Sodium arsenate is 640.04 ppm whereas it's experimentally calculated safe concentration was 186.68 ppm against freshwater crab, *Barytelphusa cunicularis*. The present investigation in support with available literature concludes that, at increased dose and exposure period, sodium arsenate is responsible for mortality in the crab, *Barytelphusa cunicularis*.

The death of an animal may be because of irreversible damage to the vital organs and so pathophysiology and biochemical studies are necessary for to investigate and focus on toxicological features of sodium arsenate. The work is continued in the toxicological lab to strengthen the present investigation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

SPSS: Statistical Package for Social Sciences; **LC_{50}:** Lethal concentration 50%; **LD_{50}:** Lethal dose 50%; **mg/L:** Milligrams per liter; **µg/L:** Micrograms per liter; **mg/kg:** Milligrams per kilogram; **ppm:** Parts per million; **TLM:** Median tolerance limit; **BW:** Body weight; **CW:** Carapace width; **L:** Light; **D:** dark; **hr:** Hours; **µmhos/cm:** Micromhos per centimeter.

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ETHICAL APPROVAL

Animals under study were used as per the approval of Maharashtra State Biodiversity Board (MSBB/Desk-5/Research/657/2023).

SUMMARY

Under the toxicological investigation, present study aimed for experimental evaluation of toxic LC_{50} dose of sodium arsenate and its permissible safe concentration against crustacean experimental crab model, *Barytelphusa cunicularis*. Adult specimens were collected and acclimatized in the laboratory conditions. Selected intoxicant was used for preparation of preliminary doses and animals were first time exposed up to 96 hr with 24 hr of interval. After completion of exposure period and thrice repetitions, obtained data was subjected for calculation of LC_{50} values and 95% confidential limit for the toxicant. All the animals were critically assessed and observed for survivability, so that LC_{50} dependent safe concentration was calculated and confirmed for livability of animals. The obtained results were meticulously discussed with previous work, available literature and references. We found that, to the LC_{50} concentration, the animal was more sensitive and unable to survive where as calculated safe concentration permitted the animal to carry out normal biological metabolism under the laboratory conditions. The obtained data provides information about possible and permissible limit for to use the sodium arsenate for to avoid mortality of the experimental animal in the natural condition. If we restrict the indiscriminate use of selected toxicant in the agricultural field, it will be helpful for to maintain the natural balance in the ecosystem.

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