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Discernible Behavioural Differences among the Certain Molluscan Species Exposed To Boric Acid

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Abstract

Study aimed to observe how boric acid induced alterations in behavioural pattern of certain molluscan species as land slug Semperula maculata, land snail Macrochlamys indica and freshwater snail Bellamya bengalensis. Prominent patterns including courting, self-protection, mucus secretion and tentacular movements were keenly observed from 24 to 96 hours among these selected gastropods.

Boric acid significantly impacted S. maculata's courtship activity during the initial 72-hour exposure period, while M. indica and B. bengalensis' courtship terminated after 96 hours. B. bengalensis and M. indica displayed poor defensive behavior after 96 hours, while the majority of S. maculata slugs remained on the soil's surface after 72 hours to prevent respiratory stress from boric acid. After 96 hours of exposure to boric acid, M. indica and B. bengalensis produced abundance mucus trails, however, S. maculata showed a notable increase in mucus output during an earlier exposure period of roughly 48 hours. S. maculata showed gradual tentacular movement after 48 hours of boric acid intoxication, but M. indica and B. bengalensis exhibited this movement after 72 hours.

The behavioural patterns revealed that intoxication of boric acid reacted more swiftly in S. maculata to alter a various behavioural mechanisms within a short exposure time compared to M. indica and B. bengalensis, indicating higher sensitivity of S. macualta against boric acid. The study implies concentration, and exposure dependent significant influence over behavioral patterns. The results were comparatively interpreted for pathological symptoms in experimental animals.

Keywords: *Molluscan, Boric acid, Behaviour, Toxicity, Courting, Exposure.*

Introduction

In order to pinpoint environmental contributing factors, scientists studied manifold animal behavioural conducts in both natural and artificial situations. Nowadays the physiological processes in the organisms severely disturbed by many envrionmental polluting substances (Dell'Omo, 2002). Ait *et al.*, (2011) found that toxic compounds significantly altered an animal's typical behavior, strengthening its function as a biological measure for the disturbances in the animals various life process. Potdar and Kamble, (2014) found that the basic life activities corresponds with the behavioural conduct in underwater and land invertebrates influenced by their surrounding ecological conditions.

Lethal concentrations (LC_{50}) of boric acid exposed to *Bellamya bengalensis* (4066.52 ppm), *Semperula maculata* (5459.45 ppm), and *Macrochlamys indica* (5187.78 ppm), caused variations in their different behavioural traits. Thus, ethology studies connect animal existence to its environment, influencing their habitat and distribution, and contributing to the preservation of endangered species by protecting gene pools and conserving animal biodiversity.

By considering the available literature we decided to observe induced behavioural alterations from some selected molluscan species against boric acid doses.

Materials and Methods:

After collection, land gastropods like *M. indica* and *S. maculata* were transported to laboratory, given time to adjust, and then put in a large plastic trough filled with soil by providing ample aeration. These terrestrial pulmonates daily fed by vegetable leaves of cauliflower, cabbage, dry mulberry plant leaves, tomatoes along with water. On the other hand, freshwater snails *Bellamya bengalensis* were kept in separate trough having ample amount of water with an aerator and daily fed with planktons (Gaikwad *et al.*, 2021).

All these 3 molluscan species were adapted for a five to seven days before subjected to boric acid and then arranged 10 animals each in five sets as First Set (Control group), and subsequent intoxicated groups from Second through fifth set (Potdar and Kamble, 2014 and Kamble and Kamble, 2014).

The experimental set groups (second, third, fourth and fifth) were tested for the mean lethal concentration of (LC_{50}) boric acid at different intervals of 24, 48, 72, and 96 hours. These different five sets of each molluscan species including, *Semperula maculata*, *Macrochlamys indica* and *Bellamya bengalensis* treated with boric acid with mean LC_{50} of 5459.45 ppm, 5187.78 ppm and 4066.52 ppm respectively (Gaikwad *et al.*, 2021) which were calculated by using Finney's, (1971) method. The experiment was conducted three times for confirmation.

The four behavioural features that were visually seen in the experimental molluscan animals under control and intoxication during the present study are listed below.

1. Courtship behaviour
2. Protective behaviour
3. Mucus secretion
4. Tentacular movement

Results:

Behavioural study in control group of slug *Semperula maculata:*

Throughout the experimental work, it was noted that the control group of land slugs, *Semperula maculata* were found to be active, frequently spreading their bodies with regular foot movement and do not exhibited noteworthy alterations in their mating, self protection, tentacular movements and mucus secretion. Table No. 1 display the following specific normal behaviors, along with Figure No.1 to 4 on Plate No.1

1. **Courtship Behaviour:** (Figure No.1 of Plate No.1)

A troop of *Semperula maculata* slugs were observed alluring in courting under the soil and debris with secreted large amounts of mucus, remained as a bright white line over their foot.

2. **Protective Behaviour –** (Figure No. 2 of Plate No.1)

Slugs were found in damp soil and litter beneath leaves. After being relocated from their natural home in the lab, very few of them tried to climb out of a trough, while others adapted for chewing on leaves and travelled naturally over the soil surface and trough wall.

3. **Mucus secretion –** (Figure No. 3 of Plate No.1)

Slugs normally expelled shiny mucus, leaving a slivery slime trails on the soil surface during crawling in search of food and marking their search areas. Initial excessive mucus output was eventually controlled and remained so.

4. **Tentacular movements -** (Figure No. 4 of Plate No.1)

Slugs freely extended their tentacles upto 5 or 7 mm. They frequently do this when crawling, occasionally while at rest and quickly moving during sense of an external stimulus.

Behavioural changes in *S. maculata* after exposure to Boric acid :

Semperula maculata exhibited behavioral alterations after being inebriated to a predetermined mean LC_{50} concentration (5459.45 ppm) of boric acid for 24 to 96 hours duration, affecting their ability to move tentacles, reproduce, defend themselves, and exude mucus, as shown in Figure No. 5 to 8 of Plate No. 1 and Table No.1. The following text provides an overview of the observations of slug behavior at different time intervals.

a. In comparison to control group, *Semperula maculata* exposed to boric acid for 24 hours showed signs of regular mating but few were scattered, some were fleeing or hiding under the soil and displaying increased mucus discharge during crawling, while also responding quickly to stimuli with slightly faster tentacular movement.
b. *Semperula maculata* slugs were found deeply burried into the soil after 48 hours to reduce toxicity load, some scattered with exception of few mate regularly and secrete mucus, resulting in a shiny mantle and slow down their tentacular motions, stretching upto 4 to 6 mm.
c. Most slugs remained on the surface with very few dug holes in the ground and some slugs strewn quiet in the trough with a lack of courting in them after 72 hours exposure. They showed very slow tentacular motion (with 3 to 5 mm stretching), increased mucus discharge, pale to dark yellow ventral side of the body and stopped moving feet. Additionally some slugs suffered toxicity-stress related damage to their outer mantle layer.

Slugs treated with boric acid after 96 hours, halted mating while others remaining survived with their feeble bodies and attempting to escape. They were covered in a thick layer of mucus, difficult to retrieve even after being mechanically stimulated and exhibited subpar responses to external stimuli with no any tentacular movements but emerged on the soil surface due to lack of oxygen, so their bodies then expanded and stationary.

Behavioural study in control group of snail *Macrochlamys indica* :

During lab acclimatization, the control group of *Macrochlamys indica*, like *Semperula maculata*, displayed typical behavioral attributes, including tentacular motions, mucus secretion, courting, and defensive mechanisms. Distinguishable behavioral traits in *Macrochlamys indica*, with notable observations shown in Figure No. 1 to 4 of Plate No. 2 and Table No. 2

Courtship Behaviour – (Figure No. 1 of Plate No. 2).

The control group of *Macrochlamys indica* travelled near the soil surface for face-to-face contact during natural mating, forming two or three groups, each participating in a standard courtship ritual and occasionally on the upper trough edges, where they displayed courtship dance before separating after some time. The study observed that the act of piercing the opposing partner's body with a white dart during mating significantly alters snails' courtship behavior, as revealed through behavioral pattern analysis.

Table No. 1

The Behaviour of control group of *Semperula maculata* and experimental group of *Semperula maculata* exposed to Boric acid.

Sr. No.	Experimental Group of Molluscan Animal - ***Semperula maculata***				
	Behavioural responses	**Courtship Behaviour**	**Protective Behaviour**	**Mucus secretion**	**Tentacular Movement**
I.	**Control group of *Semperula maculata***	Troops of slugs and in usual contact with each other	Buried in soil	Normal discharge of shiny mucus	Free tentacular movement, extended upto 5 – 7 mm
II.	**Intoxicated group of *S. maculata***	**Behavioural responses in *Semperula maculata* exposed to Boric acid at different time intervals from 24 hours to 96 hours.**			
1.	24 hrs.	Few engaged in mating and others few dispersed.	Some buried in the soil, while others attempt to surface.	Slightly high mucus discharge	Some with slight fast tentacular movement, extended upto 5 – 7 mm
2.	48 hrs.	Few mate regularly with someone scattered	A small number burried in the soil and the rest strive to the surface.	Increased mucus secretion	Slightly slow tentacular movement, extended upto 4 – 6 mm
3.	72 hrs.	No courtship conduct and the last few slugs were scattered	Majority of the slugs located on the soil surface	Increased mucus secretion	Very Slow tentacular motions with stretching upto 3 – 5 mm
4.	96 hrs.	No mating occurs and the remaining slugs were dispersed	The remaining live slugs were found strewn about, and attempting to escape and weak	Excessive mucus secretion	Tentacular movements stopped

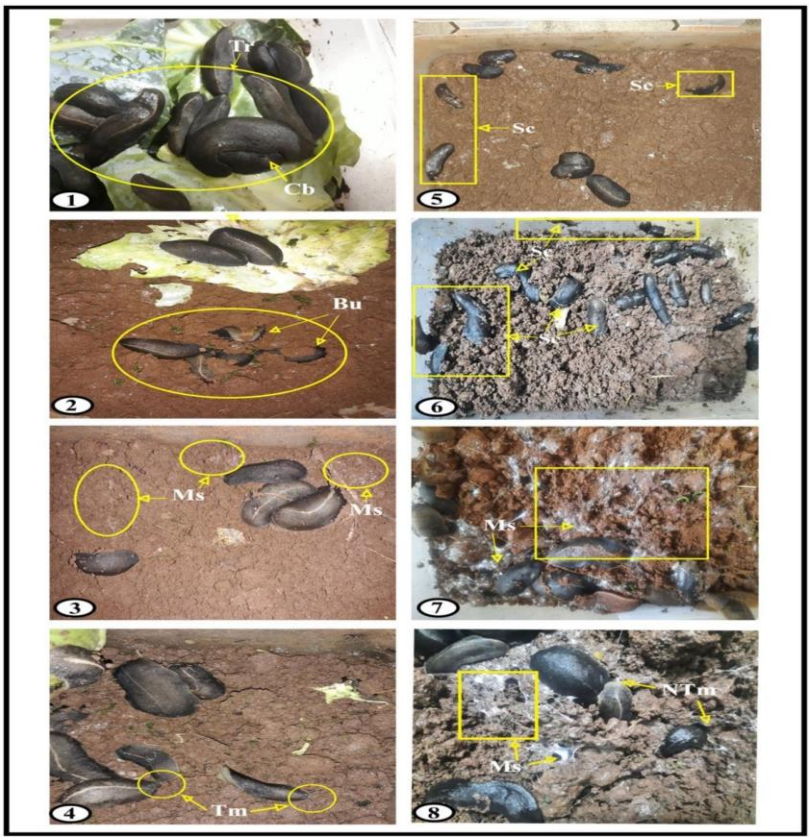

Plate No. 1 - Behavioural observations in terrestrial Slug – *Semperula maculata.*

Figure 1, 2, 3 and 4 - Normal behaviour observed in *S. maculata*, Fig. 1 – Normal courtship behaviour, Fig. 2 – Most of the slugs burried in the soil, Fig. 3 - Normal Mucus secretion. Fig. 4 – Free tentacular movement, Figure 5, 6, 7 and 8 - Altered behaviour in *S maculata* after exposure to Boric acid. Fig. 5 – Slugs scattered and disturbed courtship behaviour, Fig. 6 – Slugs trying to escape outside, Fig. 7 – Increased mucus secretion. Fig. 8 –

Stopped tentacular movement. Cb = Courtship behaviour, Bu = Burried in the soil, Ms = Mucus secretion, Tm = Tentacular movement, Sc = Scattered, NTm = No tentacular movement.

1. **Protective Behaviour –** (Figure No. 2 of Plate No. 2).

In the controlled environment, snails seal their operculum to completely hide inside their shell, a behavior observed in self-defense mechanism, influenced by responses to external stimuli in a certain circumstances.

2. **Mucus Secretion –** (Figure No. 3 of Plate No. 2).

The snails in the control group secreted a very slight mucus trails on the soil's surface, indicating that they were moving very slowly. While agitated snails, emited extremely viscous, sticky yellow mucus, giving the impression that they are experiencing danger.

3. **Tentacular Movements –** (Figure No. 4 of Plate No. 2).

In the normal state, snail's tentacles moved regularly, extending upto 7- 8 millimeters in length, used for testing and examining objects, responding to chemicals, and perceiving tactile cues.

1. **Behavioural changes in *M. indica* after exposure to Boric acid :**

The terrestrial snail *Macrochlamys indica* exhibited altered behavior after a 24 to 96-hours treatment with boric acid (LC_{50} = 5187.78 ppm), primarily affecting tentacle movement, courting, mucus discharge, and protective behaviour. Table No. 2 and Figure No. 5 to 8 on Plate No. 2 display the results and the following is a descripton of the observations of snail behaviour at various time intervals.

1. Even after 24 hours of exposure to boric acid, the same behavioural patterns were noted as in the control group of the *M. indica* snails. They just carried on acting in their customary usual protective way, with thin mucus streaks on the soil surface and no appreciable differences in tentacular movements.
2. Snails were found travelled slowly, partially withdrawn bodies inside the shell, some of them displayed poor mating behaviour, while others scattered over the trough with languid tentacular movements and at certain locations on the soil surface released mucus after being subjected to fatal concentration of boric acid for 48 hours.
3. The bulk of the snails were noticed to be apathetic about coition after being treated with boric acid for 72 hours and just a small percentage of them were in groups. As the snail gradually retreated into its shell, some of its tentacles were visible outside and were seen to move slowly and lethargically. Some areas of the soil surface showed signs of an excess of mucus.
4. Only a small number of the live snails that remained were seen in groups throughout the ensuing extended 96-hour boric acid intoxication, the rest were found scattered and unable for sexual conduct. The tentacles progressively withdrew along with their body, exhibiting a feeble defensive behavioural response. During the intoxication stage, an abnormal amount of mucus trails were observed to be secreted in certain areas, along with an exceptionally sluggish reaction to tentacular movement.

2. **Behavioural study in control group of snail *Bellamya bengalensis* :**

Following immersion in trough water and lab acclimatisation, the control group of freshwater snails, *Bellamya bengalensis* showed some typical, normal behavioural reactions including tentacular motions, mucus secretion, courtship and defence mechanisms are what keep them alive.

The behavioral study observations, as shown in Figure No. 1 to 4 of Plate No. 3 and represented in Table No. 3, providing valuable insights into the subject matter.

1. **Courtship Behaviour :** (Figure No.1 of Plate No. 3)

The control group of *Bellamya bengalensis* mate successfully when they they move in pairs, stick together, or stay above one another for long stretches of time, raised their heads to press against each other and eventually after a while they separated. This typical mating behaviour has been noted in Table No. 3 and is also presented in Figure No. 1 of Plate No. 3.

2. **Protective Behaviour :** (Figure No. 2 of Plate No. 3)

These freshwater snails tested water quality, opened operculum, resumed their regular movements, protruding their bodies outside the shell opening,

Table No. 2

The Behaviour of control group of *Macrochlamys indica* and experimental group of *Macrochlamys indica* exposed to boric acid.

Sr. No.	Experimental Group of Molluscan Animal – *Macrochlamys indica*				
	Behavioural responses	**Courtship Behaviour**	**Protective Behaviour**	**Mucus secretion**	**Tentacular Movement**
I.	**Control group of *M. indica***	Typical courting was seen among 2 or 3 groups of the snails	Snails closed operculum to hide their entire body wihtin shell	A faint mucus trail was seen on the soil's surface.	Tentacles move regularly, reaching a maximum of 7 – 8 mm
II.	**Intoxicated group of *M. indica***	**Behavioural responses in *Macrochlamys indica* exposed to Boric acid at different time intervals from 24 hours to 96 hours.**			
1.	24 hrs.	After a day, same observations were noted as in the control group.	Protective behavior was remained unchanged as in control group	A slight mucus trails on the soil surface	The observations were found identical with that of the control group
2.	48 hrs.	Poor mating in a couple of the snails occurs with someone dispersed across the trough	Snails moved slowly, with their bodies somewhat drawn in	Mucus found at some places.	Slow tentacular movements.
3.	72 hrs.	Most of the snails were lethargic for coition and scattered	Tentacles were partially exposed to the shell	Excessive mucus discharge at certain places	Tentacular motions quiet slow
4.	96 hrs.	The remaining live snails were found to be dispersed and do not mate	Poor defensive behavioral response, as the tentacles slowly withdrew as the body withdrew	Excessively secreted mucus tracks found at some places.	Incredibly sluggish tentacular motions

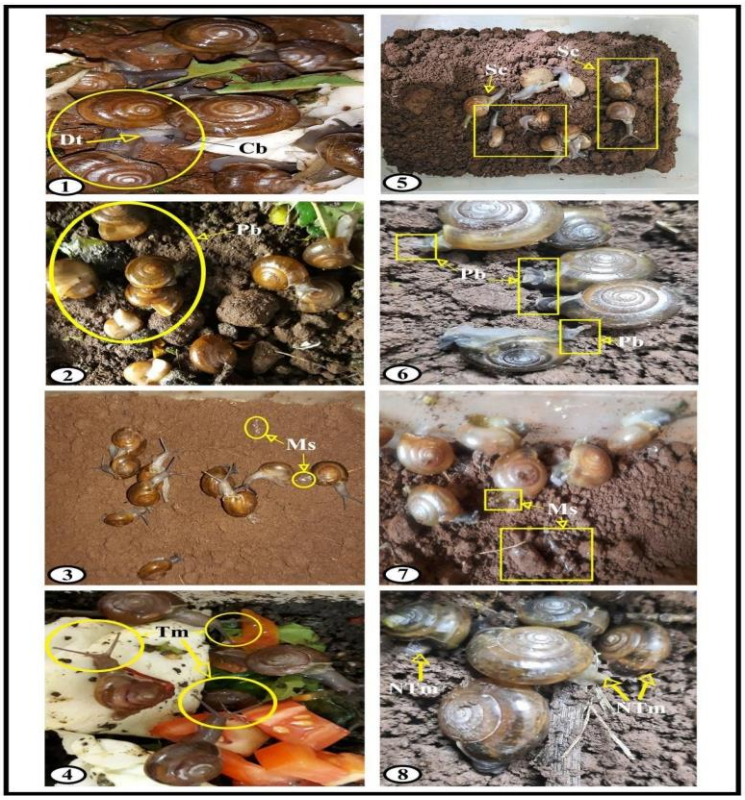

Plate No. 2 - Behavioural observations in terrestrial snail – *Macrochlamys indica.*

Figure 1, 2, 3 and 4 - Normal behaviour observed in *M.indica*, Fig. 1 - Normal courtship behaviour, Fig. 2 – Normal protective behaviour by closing operculum, Fig. 3 - Normal Mucus secretion, Fig. 4 – Regular tentacular movement, Figure 5, 6, 7 and 8 - Altered behaviour in *M. indica* after exposure to Boric acid. Fig. 5 - Snails scattered in the soil, Fig. 6 – Snails retracted body inside the shell quiet slowly, Fig. 7 - Increased mucus secretion. Fig. 8 – Sluggish tentacular movement. Cb = Courtship behaviour, Pb = Protective behaviour, Ms = Mucus secretion, Tm = Tentacular movement, Dt = Dart,
Sc = Scattered, NTm = No tentacular movement.

remained still with opened mouth, clung to trough edges, thus demonstrating quick protective reactions. Snails also demonstrate parental care by attaching their juveniles to the exterior of their shell (Figure No. 3 of Plate No. 3)

3. Mucus secretion:

In the trough, the control group of the snails discharges minimal or no mucus, as reported in Table No. 3.

4. Tentacular movements: (Figure No. 4 of Plate No. 3)

The snails in the control group moved like a whip because their tentacular motions were so fast. Snails extended their tentacles to inspect anything they came into contact with while they were moving, coiled quickly and returned to their usual tentacular movements.

The experimental study found that all snails remained active and no death was observed in the control group of snails during 24 to 96 hour intervals.

Alterations in *B.bengalensis* behvioural patterns after Boric acid intoxication:

After being exposed to the mean LC_{50} concentration of 4066. 52 ppm of boric acid, the typical behavioural traits in *B. bengalensis* mostly turned abruptly the way they mate, defend themselves, secrete mucus and move tentacles. The results depicted in Table No. 3 and also presented in Figure No. 5 to 8 on Plate No. 3. The observations made about snail behavior at different time periods from 24 to 96 hours are described below.

Snails' operculum closed due to toxic stress after 24-hour exposure to boric acid, opening later to prevent suffocation. They rarely mate, have no mucus secretion, freely move their tentacles during crawling and respond swiftly to water vibrations or sunlight. Snails have fully expanded feet on trough surfaces.

a. At 48 hours exposure, tentacular and foot movements slightly slow down. Most of them remained at the bottom, releasing some mucus occasionally, while others clung to the margins of the trough where water was at the top.

A day later, the undifferentiated outcome results of their courtship and defensive actions were noted.

b. Once exposed to boric acid for 72 hours, the majority of the snails broke off from one another and avoided mating. Most of the snails closed their operculum to protect themselves from the unfavourable environmental condition. Tentacular movements were weak and in contrast to the previous time intervals of 24 and 48 hours, mucus secretion increased.

c. The live sails that make it through up to 96 hours of exposure, stop mating or refrain having any sex at all, get lethargic and failed in defence manoeuvres.

They emit a lot of dense, white, sticky mucus bubbles and also terminated their tentacular motions.

Comparative observations on the behavioural alterations produced by exposure to boric acid in *Semperula maculat*a, *Macrochlamys indica* and *Bellamya bengalensis*:

The study comparatively examined how boric acid affects the mating habits, defensive tendencies, mucus secretion, and tentacular movements of the freshwater snail *Bellamya bengalensis*, the terrestrial slug *Semperula maculata*, and the land snail *Macrochlamys indica*.

The following is a summary of the analysis and observations made regarding the comparative effects of boric acid on different behavioural patterns in these 3 molluscan species during the experimental study.

1. The behaviour study revealed that boric acid has a greater impact on *Semperula maculata* courtship activity during an early exposure period of roughly about 72 hours, after which it get halted but in *Macrochlamys indica* and *Bellamya bengalensis* the courtship ended after 96 hours.
2. After 72 hours, most of the *Semperula maculata* slugs located on the surface of the soil to avoid respiratory stress caused by boric acid while *Bellamya bengalensis* and *Macrochlamys indica* exhibited a very poor protective behaviour after 96 hours by embedding their body inside the shell and became motionless.

Table No. 3
The Behaviour of control group of *Bellamya bengalensis* and experimental group of *Bellamya bengalensis* exposed to Boric acid.

Sr. No.	Experimental Group of Molluscan Animal – *Bellamya bengalensis*				
	Behavioural responses	**Courtship Behaviour**	**Protective Behaviour**	**Mucus secretion**	**Tentacular Movement**
I.	**Control group of *Bellamya bengalensis***	Typical mating between two snails, positioned above each other.	Snails opened their operculum by testing the water quality They also showed parental care of their young ones.	Very little or sometimes no mucus discharge was seen	Free tentacular movements
II.	**Intoxicated group of *Bellamya bengalensis***	**Behavioural responses in *Bellamya bengalensis* exposed to Boric acid at different time intervals from 24 hours to 96 hours.**			
1.	24 hrs.	Few snails shows courtship behaviour	Closed their operculum for short time till upto the testing water quality	Mucus secretion was not observed.	Free tentacular movement while crawling
2.	48 hrs.	The same results were observed a day later	The same results were observed a day later	Slightly increased mucus secretion.	Reduced the intensity of tentacular movements slightly
3.	72 hrs.	Majority of snails stay apart from one another and refrain from copulating	Most of the snails closed operculum for self-defence from the stressful environment of the water	Their mantle secretes a lot of cloudy mucus	Weak tentacular movements seen.
4.	96 hrs.	The left live sails either stop mating or refrain from having no sex	Remaining live snails becomes lethargic and failed in defence activity	A large amount of viscous whitish mucus bubbles were discharged	Snails loosely closed their operculum with no any tentacular motions

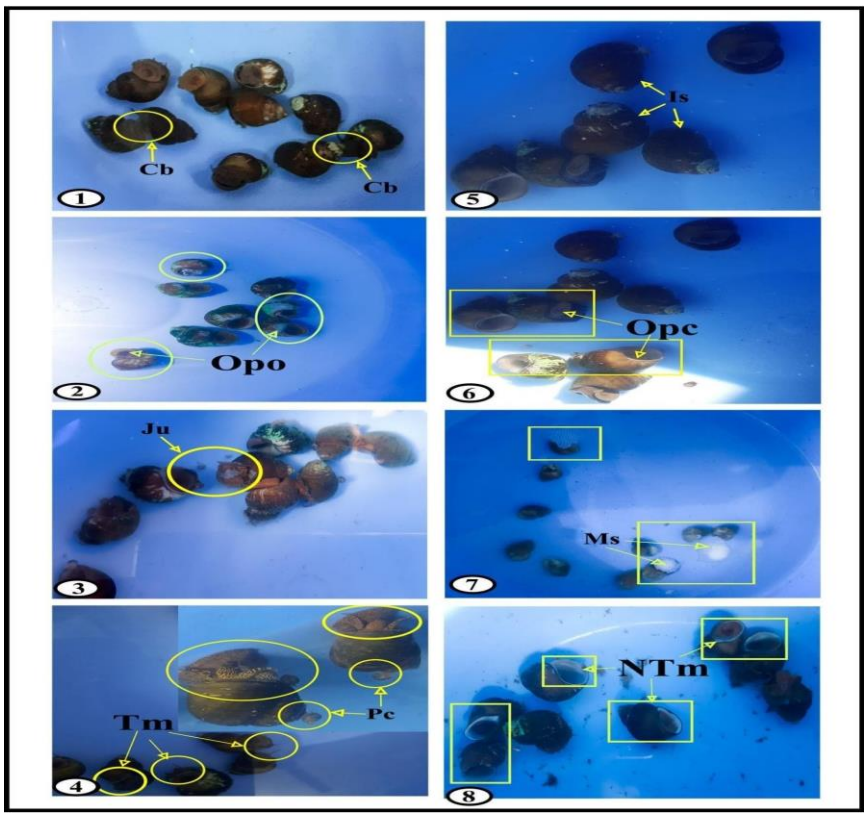

Plate No. 3 - Behavioural observations in freshwater snail – *Bellamya bengalensis.*

Figure 1, 2, 3 and 4 - Normal behaviour observed in *B. bengalensis*. 1 - Normal courtship behaviour, 2 – Snails opened operculum for the movement, 3 – No mucus secretion and parental care seen, 4 – Free tentacular movement, Figure 5, 6, 7 and 8 - Altered behaviour in *B. bengalensis* after exposure to Boric acid. 5 – Snails isolate to avoid mating, 6 – Snails retracted body inside the shell and closed operculum, 7 – Discharge of white mucus bubbles, 8 – Stopped tentacular movement. Cb = Courtship behaviour, Opo = Operculum opened, Ju = Juvenile, Pc = Parental care, Tm = Tentacular movement, Is = Isolated,
Opc = Operculum closed, Ms = Mucus secretion, NTm = No tentacular movement.

An excess amount of mucus trails by *Macrochlamys indica* and an increased secretion of viscous, whitish mucus bubbles by *Bellamya bengalensis* occurred after 96 hours exposure to boric acid, however, *Semperula maculata* showing a significant increase in mucus secretion in earlier exposure period of about 48 hours.

A quiet slow tentacular movement started to appear after 48 hours of boric acid intoxication in *Semperula maculata* whereas it was found in later exposure period of 72 hours in both *Macrochlamys indica* and *Bellamya bengalensis*.

Discussion:

Numerous researchers looked into how an animal's biology, biochemistry, physiology, and behaviour were affected by high doses of boric acid. The study of animal behavior can provide an accurate method to determine the toxicity levels of hazardous compounds in aquatic and terrestrial ecosystems.

Ranbhare *et al.*, (2020) reported that the misuse of pesticides and heavy toxic metals can negatively impact the physiology and behavior of molluscs, potentially disrupting the food web of the environment. The usual behaviour of land slug *Semperula maculata* changes after deprivation (Bhosale and Nanaware, 2002) and also after mercury chloride administration (Londhe *et al.*, 2015). Londhe *et al.*, (2015) too reported behavioural alterations including lethargy, body swelling, poor self-defense, mating avoidance, absence of movements and elevated mucus secretion. All of these results are in line with present study regarding normal behaviour and behavioural abnormalities in *Semperula maculata* following exposure to boric acid.

According to Sudhakar *et al.*, (2013) pesticides like Thiamethoxam and Diafenthiuron caused abnormal symptoms in terrestrial snail *Macrochlamys indica*, such as body enlargement with withdrawn head, highly secreted mucus and ended with mortality. These finding results are consistent with a study on the behaviour of mucus secretion in *Macrochlamys indica* that was subjected to boric acid intoxication over a period of time varying from 24 to 96 hours. These result findings by researchers in different terrestrial snail species are in contrast with the observations regarding self- defense in *Macrochlamys indica*, where the snails hid their entire body inside their shells to fend off the toxicity of boric acids, while remaining snails became motionless and displaying a weak defence strategy as exposure times increased upto 96 hours.

The causative factor of behavioural changes in aquatic animals is due to mostly their vulnerability for chemical components and environmental parameters. The present study confirms Kamble and Kamble, (2014) research findings that the typical behaviour of *Bellamya bengalensis* decreased with an increase in the intoxication period of copper sulphate and *Acacia sinuata* plant extract from 24 to 96 hours. A mucus discharge by *Bellamya bengalensis* after boric acid poisoning was compatible with Nanware and Awati, (2004) observations of increased mucus production in *Viviparous bengalensis* snails due to natural and artificial chemical molluscicides. The noticed behavioural response in the present study regarding mucus secretion in *Bellamya bengalensis* against boric acid toxicity are found to be comparable to those of Awati and Nanaware, (2004), who showed that the gills of *B. bengalensis* secreted a lot of mucus in response to molluscicides.

Animal toxicity is determined by internal cellular makeup, metabolism, and species' capacity to absorb toxicants. Gastropodan species may have reduced foot and tentacular motions due to nervous system disruption. The results of this study can be used to guide future investigations on how chemical compounds are used in limited level by human ascendancy.

Conclusion:

Overall behavioural pattern of *Semperula maculata*, *Macrochlamys indica* and *Bellamya bengalensis* showed alterations as per concentration and time of exposure. The study implies that the more hazardous chemicals were linked to significant behavioral changes within a brief exposure period. The animal's susceptibility to chemicals serves as a gauge for its likelihood of survival. The investigation revealed that *Semperula maculata* was more susceptible to boric acid than *Macrochlamys indica* and *Bellamya bengalensis*. In light of these results, it was determined that *Semperula macualata* was more lethargic and sluggish than *Macrochlamys indica* and *Bellamya bengalensis*. Behavioral research on animals could significantly enhance the realism of such investigations, crucial for an accurate ecotoxicological risk assessment.

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Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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