Abstract: The experiment was carried out on the fresh water bivalve mollusk, Lamellidens marginalis during January 2019 to December 2020 to study the behavioural changes. The behavioural changes were recorded in this bivalve when subjected to mean LC₅₀ concentrations of cadmium chloride for different exposure periods. Different physiological and morphological changes were observed in experimental animals including protective response, foot movements and its secretion, response to external stimuli and mucus secretion.

Keywords: Behaviour, Cadmium chloride, Lamellidens marginalis, Pesticides.

INTRODUCTION
The toxicology not only protects human and the environment from the deleterious effects of toxicants, but also to facilitate the development of more selective toxicants with clinical drugs and pesticides. The importance of dose is well illustrated by metals that are essential in the diet but proved toxic at higher doses. For example, iron, copper, magnesium, cobalt, manganese and zinc can be present in the diet at too low level (deficiency), at an appropriate level (maintenance) or at too higher level (toxic) (Ernest Hodgson, 2004). According to Rothstein (1959), heavy metals directly affect the tissues and may interact with cell membrane. Higher concentration of toxic metals, in aquatic environment can cause adverse effects on aquatic organisms at cellular or molecular level and ultimately leads to disorder in biochemical composition of organisms and behaviour (Holden, 1973; Kumar et al., 2019).

Pesticide use in order to enhance the crop production is now days a common practice. Its excess use is harmful not only to plants and animals but also to human beings (Prakash and Verma, 2014 & 2020; Verma and Prakash, 2018). Herbal pesticides are therefore the need of the hour as these are relatively harmless to non-target organisms and human beings (Kaur and Mishra, 2019; Tripathi, 2021). Molluscs, therefore are of interest not only to farmers and the pesticide industry but also to ecotoxicologists as monitor species for environmental pollution. They encounter toxic materials either by contact or during feeding. If they crawl over surfaces recently sprayed with pesticides, on toxic molluscicide baits or in heavily polluted substrates, the skin is
the first point of contact (Triebkorn et al., 1996; Kumar et al., 2010; Kumar et al., 2016).

The behaviour of an organism is defined as an act or conducts in particular way or exact way an organism responses to stimuli of environment especially those responses that can be observed (Websters, 2002). Some biotic as well as abiotic factors play very important role to change activities and behaviour of the animals. The toxicological nature of surrounding environment was assessed with the help of behaviour and metabolic changes in animals. The behavioural modification in animals can be taken as the most sensitive indicators of environmental stress (Eisler, 1979). Sarojini et al. (1990) studied the effect of detergents on Kasbora daniconium and correlated the behavioural changes in relation to metabolic rates. The activities like opening of operculum, extension of foot and other body parts, crawling movement were minimized when animals were exposed to folithion and lebycide (Muley and Mane, 1988). Aluminium and salicylic acid in the environment have changed the behaviour of Lymnaea stagnalis (Compbell et al., 2000). The pesticides nuvan, methyl parathion and thimet have changed the normal behaviour of Lymnaea stagnalis (Bhide, 1998).

Gokhale and Mane (1990) studied the toxicity of molluscicide in bivalves Lamellidens marginalis, where they documented that, after intoxication, bivalve closed their valves, immediately after exposure to molluscicide and showed diapedesis in all exposed animals after 12 hours with white coagulated matter of mucus. These effects can potentially give out structural and functional changes in freshwater ecosystems (Camargo, 2003). Kumar (2021) worked on plant based molluscicide, Shinde (2020) on neural aspect of Lamellidens while Yasmeen (2019) studied the cadmium induced histopathological alterations in female gonad of freshwater bivalve mollusks, Lamellidens marginalis. Present study was conducted during January 2019 to December 2020 to explore the acute toxicity of cadmium chloride on behavioural responses of freshwater bivalve mollusk, Lamellidens marginalis.

MATERIALS AND METHODS:
Several specimens of freshwater bivalve mollusk, Lamellidens marginalis (90-100 mm in shell length) were collected from Kutluq Lake, Daulatabad, near Aurangabad (M.S.), India. After collection of the animals from habitat, they were brought to the laboratory where the fouling biomass and mud on shell valves were removed without disturbing the siphonal regions. The equal sized bivalves (shell-length) were grouped and kept in sufficient quantity of water (1 animal/litre) in aquaria with aeration for 24 hours with renewal of water at an interval 12 to 13 hours. At the same time controlled group run up to 96 hours by providing natural food and aeration. For the confirmation, the whole or total experiment repeated thrice. The above procedure was followed for intoxication of pre-determined LC_{50} concentration of cadmium chloride and for the confirmation, it was repeated thrice. The differentiations of behavioural changes were recorded as per the intoxication and time of exposure by considering protective behaviour, foot movement, mucus secretion and tentacular movement.

RESULTS AND DISCUSSION
The bivalve mollusc, Lamellidens marginalis exposed to acute toxicity tests of cadmium chloride showed differential response in behavioural pattern compared to control animals and these patterns were recorded. These changes were recorded after different exposure periods at 24, 48, 72 and 96 hours. The results were shown in the table 1.

The change in the behavioural activities of animal can be taken as sensitive indicator of environmental toxicity stress. Behavioural changes due to acute toxicity of copper in freshwater snail, Bellamya bengalensis was studied by Kamble and Kamble (2014). They recorded various physiological as well as morphological changes in copper sulphate exposed snail including reduced foot movements and tentacular movements but quick response to pin touch or other stimulus was not found, mucus secretion was much more. At the end, snails became immotile and lost their movements.
Table 1: Normal behaviour of control group of freshwater bivalve *L. marginalis*.

| S. No. | Type of response | Behavior of normal group                                                                 |
|--------|-----------------|-----------------------------------------------------------------------------------------|
| 1.     | Protective response | Control group of bivalves showed quick response.                                         |
| 2.     | Visceral body     | Continuous circulation/filtration by water took place through visceral body.             |
| 3.     | Foot movements and its secretion | Foot movement was fast. It tightly attached to the surface with the help of foot. Secretion by foot was ample as per locomotory activity. |
| 4.     | Response to external stimuli | Generally they closed the shell valves at the time of immersion in water upon the valve and protruded siphons for all the time during the experimental period. |
| 5.     | Mucus secretion of gills | In control group of bivalve, mucus secretion by gill was not seen.                       |

Previous investigators like Lossanoff (1939) and Barnes (1955) studied the activity of certain mussel species. Salanki and Lukacsovics (1967) stated that partially and rhythmic valve movement in freshwater bivalves, including the quick closure and opening of the valves may cause increased water passage, partly for the time of rest period when the valves are tightly closed for hours supposedly there is water passing through the gills correspondingly filtration also stops. It is thus obvious that the effect of certain factors (temperature, pollution) influencing filtration activity cannot be interpreted as affecting only the activity of cilia but it can be an essentially important factor related to the behaviour.

Table 2: Behavioural changes in freshwater bivalve *L. marginalis* against cadmium chloride intoxication at different exposure period.

| S. No. | Types of response | Behavior of cadmium intoxication group |
|--------|-----------------|----------------------------------------|
|        |                 | 24 hours | 48 hours | 72 hours | 96 hours |
| 1.     | Protective response | Tolerate toxicity with the help of operculum | Shell-valves closed. Tightly closed | Shell-valves closed. Tightly closed | Shell-valves closed. Tightly closed |
| 2.     | Foot movements | Foot extended initially | Foot movement was slowed down | Foot retracted | Foot movement not seen |
| 3.     | Response to external stimuli | Initially quick response | Response to stimuli was reduced | Very poor response | No response |
| 4.     | Mucus secretion of gills | Mucus secretion initiated in trough | Secretion increased in trough | Mucus secretion quantitatively increased | Thick white mucus seen in trough |

In the present study, the control group animals showed immediate shell valve opening after immersion in water. The behaviour reactions of *Lamellidens marginalis* were rhythmic and periodic with extrusion of mucus and excreta is almost equal quantity throughout the experimental period. On the other hand *Lamellidens marginalis* exposed to cadmium tightly closed the shell valves for about first 12 hours and during the later period no quick closure of the shell valves occurred particularly in high concentrations of cadmium like LC_{50} in different seasons.

Authors further observed that animals became active after about 24 hours exposure to cadmium...
LC₅₀ concentrations and later could open the shell-valves and extended the body parts like those in the control. The possible cause of the permanent opening of the shell-valves and loss of the ability of the adductor muscles to contract after prolonged cadmium exposure could be due to the inhibition in the activity of a calcineurin like Ca²⁺ regulated phosphatase. The coagulated film anoxia theory in bivalve molluscs was stated by earlier workers (Westfall, 1945). The death of animals would have occurred due to interfilament space stuffed and the blood circulation in the gill got affected which might ultimately have lead to heart block and subsequent death. However, shell valves, adduction to overcome stress in ubiquitous among the bivalves (Manley, 1983).

Authors found that the failure is normal behaviour in Lamellidens marginalis in LC₅₀ test concentrations in different seasons when compared to controls probably reveals the disfunctioning in ionic regulation even when the valves are fully opened / closed. It was observed further that the bivalve exposed to different test concentrations of cadmium showed diapedesis in the form of extrusion of white coagulated matter with much mucus, particularly more in summer than monsoon. It is of interest in this view to note that this species is more sensitive to the cadmium stress in summer than monsoon season. The physico-chemical factor of the water also plays an important role in toxicity studies. It was observed that all the species of molluscs under present study were sensitive to cadmium in summer than monsoon and winter. It is not only due to test concentrations but the high temperature and low oxygen tension altered the tolerance limits. Behaviour affects the survival of aquatic organisms and reflects the integration of many biochemical and physiological processes.

It has been reported earlier that the clams close the shell valves when stimulated by a toxic compares or by any change in the environment (Simon, 1953). Authors found that similar was the case for first 12 hours period of exposure to cadmium, but later on clams opened the shell valves for respiration and food intake. As the cadmium penetrated inside the body, the clams widely opened the shell valves and could not close again till the death. This probably leads to an anoxic condition. Diapedesis in the amount of excreta increased during the early period of exposure in all test concentrations and later it decreased. On the contrary, the amount of mucus showed reverse pattern. The study revealed that the mussels are more sensitive to cadmium toxicity in summer, while they are more resonated in winter. The reason for this has been attributed to the environmental promoters like decreased water level, high temperature, low oxygen content and increased hardness and alkalinity. Besides, the lentic environment of the mussel appears to have some role in determining the summer sensitivity to the cadmium toxicity.

ACKNOWLEDGMENT

The authors are grateful to Principal, Dr. Rafiq Zakaria College for Women, Aurangabad for providing laboratory facilities and encouragement.

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