Cadmium Chloride Induced Behavioural alterations In Slug *Semperula Maculata*

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Abstract- *Terrestrial slugs, Semperula maculata were exposed to cadmium chloride ($CdCl_2.H_2O$) mean LC_{50} concentration 383.5 ppm which was calculated with the help of probit analysis. As the exposure period was increased from 24 hrs to 96 hrs animals were more sluggish with increased mucus secretion and percent mortality.*

Keywords- Behavioural alterations, $CdCl_2$, Percent mortality, *Semperula maculata*

I. INTRODUCTION

During last decades, heavy metal contamination has increased by its extensive use in different agricultural, chemical and industrial processes. Though, many metals play a vital role in various physiological activities of plants and animals, their excess bioaccumulation leads pathological conditions at the cellular and molecular level. Heavy metals are hazardous to all living organisms, specifically invertebrates showed widespread sensitivity to it. Generally molluscs are used in different biomonitoring projects due to its tendency to accumulate trace metals from environment. Besides responding to heavy metals, molluscan species were found affected by number of industrial effluents [1], acidified drainage from mines or factories [2], excess use of pesticides and herbicides in agricultural field [3]. Molluscan animals have 100 to 1000 time's greater bioaccumulation capacity than that of other animals.

Direct mortality of the animal has primary means of assessing the chemical contamination in ecosystems. Surwase (2009) [4] was documented the uses of behavioural measures because such methods were relatively easy to evaluate and proved more sensitive than any other endpoints. He was also showed that impact of environmental pollution leads to significant change in the behaviour pattern of non targeted organisms. Behavioural responses were considered to be rapid and the first line of defence to environmental stimuli [5, 6]. The toxicant causes stress on the organisms; the behavioural changes were immediate responses to the toxicant and proved to be indicators of possible stress [7].

Altered behaviour affects the survival of aquatic as well as terrestrial invertebrate which reflects the integration of many biochemical and physiological processes. From the literature survey it clears that, the behaviour of the targeted species provide response against toxicants as, pesticides, fertilizers, toxic chemicals, phenolic compounds, oils and heavy metals. Also provides information related to changes in environmental factors such as, pH, temperature, dissolved oxygen, hardness, salinity and season etc. from their surrounding environment. So present investigation aimed to observe changes in behavioural pattern as, protective behaviour, tentacular movements, foot movements, mucus secretion from mantle of terrestrial slug *S. maculata* in control and experimental groups when exposed to LC_{50} concentrations of $CdCl_2$ at 24, 48, 72 and 96 hrs of exposure.

II. MATERIALS AND METHODS

A) Materials:

i) Selection of animals:

Molluscan gastropod species namely, terrestrial slug Semperula maculata was selected for the present work.

ii) Collection sites: Betel leaf farms (Pan-mala) of Village Bedag from district Sangli:

Village Bedag is 10-12 Km away from Miraj city, well known for betel leaf farms. These farms have black soil, usually rich in iron with high moisture holding capacity. Betel leaf crop require more water as compared to other, therefore farmers water the farm after duration of every 8 days. Terrestrial slug *Semperula maculata* was collected from site, as these are available throughout the year.

Slugs were carried to the laboratory in plastic boxes covered with perforated lid for the ventilation and provided with moist soil along with fresh leaves of cabbage (*Brassica oleracea*), pumpkin (*Cucurbita pepo*) and mulberry (*Morus alba*), etc as a feed. Overcrowding was avoided by keeping 50 animals in each trough. Prior to the experiment, animals were acclimatized for one week, healthy and adult animals

having same size and weight (slug with length 4-6 cm and weight 3-4gm) were selected for experiment **(Plate No.-1)**.

iii) Selection of toxicant:

For the present study heavy metal cadmium chloride (Himedia, RM 469-100G) was selected against slug Semperula maculata.

Plate No. 1

Figure -A- Betel leaf (*Piper betle*) farm from village Bedag

Figure -B- Natural habitat of slug *S. maculata*

Figure -C- Expanded view of slug *S. maculata*
a) Dorsal view of slug *S. maculata*
b) Ventral view of slug *S. maculata*

B) Methods:

After the acclimatization, animals (terrestrial slug S. maculata), were divided into two sets, first as control group and other as experimental which was exposed to predetermined mean LC50 values of CdCl2. Animals were exposed to 24, 48, 72 and 96 hrs of period and behavioural alterations were observed in control and experimental group of animals. The experimental setup was as follows:

Set I - Control set, consist of 10 slugs and moistened soil with distilled water.

Set II - Experimental set, containing 10 slugs exposed up to 96 hrs mean LC50 value 383.5 ppm of Cadmium chloride.

During the whole experiment following type of behavioural alterations were recorded in slugs.

i)Protective behaviour
ii)Tentacular movements
iii)Foot movements and its mucus secretion
iv)Mucus secretion of mantle
v)Response to external stimuli (pin touch)

III. RESULTS

Behavioural changes in control group of slug S. maculata

Acclimatized slugs, when introduced in trough containing moistened soil with distilled water, initially they tried to escapes from soil, may be due to drastic change in habitat, but after few moments animals were actively moved on soil surface and along the walls of trough. Tentacles showed quick response to external stimuli like, gentle touch with needle, glass rod etc. Wiper like right and left tentacular movement, with forward and backwardly directed tentacular movement was observed. Throughout the experiment animals were active with fully expanded body and regular foot movements. Initially increased mucus secretion was regulated after some time and remained as it. Major behavioural changes was not found in protective behaviour, tentacular movements, foot movements and mucus secretion from mantle throughout the experiment (Plate No.-2).

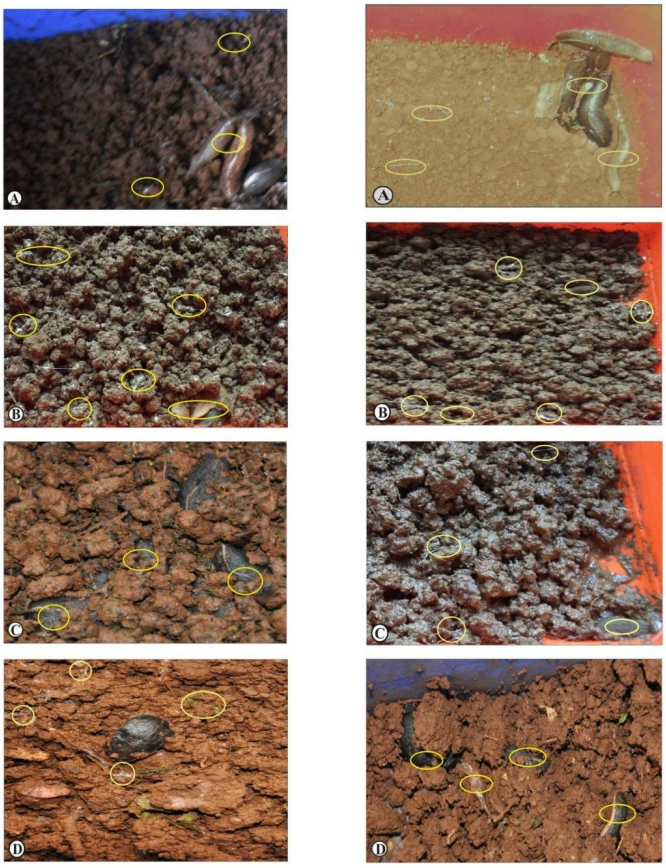
Behavioural changes in slug S. maculata exposed to $CdCl_2$:

Experimental slugs when exposed to predetermined mean LC50 concentration 383.5 ppm of CdCl2 after 24 hrs of exposure period, slugs were found attached to the walls and edges of the trough. Free tentacular movements were observed, with quick response to external stimuli. Animals were moved actively on the soil surface in trough, due to which, number of trail on soil surface was noticed. Animals were showed protective response by buried themselves deep in soil to minimise toxicity stress. Whereas, the 48 hrs. slugs were found somewhat lethargic showed reduced tentacular and

foot movements. Animals were secreting higher amount of mucus due to which mantle portion was appeared shiny.

At the 72 hrs of exposure, animals found buried in soil, to overcome the toxicity stress. Green excretory waste was found over soil surface. Due to the inactive phase (buried in soil) tentacular movements were less. Foot movements were absent; ventral side became pale yellow to dark yellow in colour. In some animals outer layer of mantle found damaged at some places due to toxicity stress. At the 96 hrs of exposure, animals were found on soil surface to overcome respiratory stress. Foot movements were totally lost and slugs becomes immobile, whole body of the animals became puffy and yellow in colour. In some animals tentacular movements were stopped, both the primary and secondary tentacles were protruded which were unable to withdraw even after mechanical stimulation of pin or pointer. Animals were poorly responds to external stimuli. Maximum amount of mucus secretion was observed (Plate No. 2).

Plate No. 2



Behaviour in control slug *S. maculata*
A- Control group of slug *S. maculata* after 24 hrs
B- Control group of slug *S. maculata* after 48 hrs
C- Control group of slug *S. maculata* after 72 hrs
D- Control group of slug *S. maculata* after 96 hrs

Behaviour alterations in slug *S. maculata* exposed to $CdCl_2$.
A- Behavioural changes in slug *S. maculata* exposed to $CdCl_2$ after 24 hrs
B- Behavioural changes in slug *S. maculata* exposed to $CdCl_2$ after 48 hrs
C- Behavioural changes in slug *S. maculata* exposed to $CdCl_2$ after 72

IV. DISCUSSION

Behavioural responses provide early warnings as standard test methods [8]. Since behaviour serves as the link between physiological and ecological processes, it proved ideal for analysing environmental pollution effects. In normal behaviour, animal followed specific sequences, which were triggered by external stimuli acting via neural networks. Disruption of these sequences resulted in to detrimental behavioural alterations. Multigenerational effects of cadmium on survival, growth and reproduction have been observed in Daphnia magna [9]. Similarly behavioural changes in freshwater snail Bellamya bengalensis due to acute toxicity of copper sulphate and Acacia sinuata was studied by [10] and observed different physiological and morphologically changes like protective response, tentacular movements, foot movements and its secretion, response to external stimuli, mucus secretion of gills and courtship behaviour in experimental animals.

In the present study, slugs from control group behave normally as they behaved in their natural environment throughout the experiment. Whereas in the experimental slugs several changes in natural behaviour was observed during exposure period mainly increased production of excreta as increase in exposure period was noted. As gastropods can excrete metabolites together with faeces [11]. Increasing excretion measured together with faeces. Excess mucus secretion by mantle was a usual phenomenon occurred in the gastropod species. It might be to counteract the toxic chemical reactions in the cells of targeted organs. Thus, it might have involved in detoxification process of the organism. Such mucus release in bivalves and fishes against pesticide exposure has also been reported by several researchers [12, 13 & 14].

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