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## Analysis of heavy metal-induced toxicity on the successful development and hatching of grasshopper

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### Abstract

Heavy metals toxicity is a significant concern for primary consumers, like grasshoppers. In a lab setting, the effects of HgCl<sub>2</sub>, CdCl<sub>2</sub>, and ZnCl<sub>2</sub> on egg hatchability and embryonic duration of *Oxya hyla hyla* and *Oedaleus abruptus* (Acrididae) species were investigated. The same-aged healthy eggs were incubated in sand that had been exposed to various quantities of the metals. When metal concentrations in both experimental species increased relative to the control, egg hatching considerably decreased. Similar patterns were also seen when looking at the length of the embryonic duration.

**Keywords:** heavy metals, egg, hatchability, embryonic duration

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### Introduction

Heavy metals are one of the many environmental hazards that organisms can face. Heavy metal pollution comes from a variety of sources, including mining (Navarro *et al.* 2008)<sup>[9]</sup>, smelting procedures (Brumelis *et al.* 1999)<sup>[2]</sup>, and agriculture (Vaalgamaa *et al.* 2008)<sup>[11]</sup>. In addition to these, the natural activity, chemical and metallurgical industries are the most important pathways for heavy metals in the environment (Cortes *et al.* 2003)<sup>[4]</sup>. Dust fall, bulk precipitation, and gas or aerosol adsorption are the three main mechanisms by which heavy metals are transported from the atmosphere to the soil (Andersen *et al.* 1978)<sup>[1]</sup>.

Eggs are the first life stage of a new generation and the embryonic development is very important in insect life history. In grasshoppers, eggs are immobile and remain in contact with soil; they cannot surpass environmental pollution. Like adult ones their resistance to soil pollutants might be relatively weak (Devkota and Schmidt, 1999)<sup>[5]</sup>. Hatching of eggs is an important event in insect reproduction. Hatching success depends in part on the quality or health of the embryo in an egg. Because pores and tiny channels in the egg hull allow egg respiration and water uptake from the environment, heavy metals in soil may enter eggs along with water that are toxic to embryos and consequently reduce hatching success (Xu *et al.* 2009)<sup>[12]</sup>. These noxious substances in the soil influence the vitality and reproduction of grasshoppers, and eggs laid in heavy metal loaded soil exhibit a marked reduction in hatching rate (Schmidt *et al.* 1991; Devkota and Schmidt, 1999)<sup>[10, 5]</sup>.

One approach to study the effects of heavy metals on eggs is to expose healthy eggs to different concentrations of metals in soil and measure effects on hatching success. In the current study, healthy eggs of *Oxya hyla hyla* and *Oedaleus abruptus* (Orthoptera: Acrididae) were exposed to soil containing Hg, Cd, and Zn at varying concentrations in a lab setting. The hatching success and embryonic duration of the exposed eggs were then recorded.

### Materials and Methods

#### *Insects and eggs*

*Oxya hyla hyla* and *Oedaleus abruptus* were obtained from the insectaria of Entomology Research Unit of the Zoology Department in Visva-Bharati University, Santiniketan, India. Mass culture procedures suggested by Haldar *et al.* (1998)<sup>[6]</sup> were implemented with minor adjustments in order to generate a colony of the desired acridid species. Same aged individuals were separated and allowed to oviposit in sterilized sand trays and collected with special care within an hour after oviposition.

#### *Preparation of sterilized sand*

Sand (approximately 0.05 mm diameter) was collected from the bank of Mayurakshi river, near the Department of Zoology, Suri Vidyasagar College. To eliminate any soil or metal particles connected to the samples, they were carefully cleaned with filtered water and ran under running tap water. After washing the sand was oven dried and kept for the experiment.

#### *Experimental set up*

The experimental set up was followed by the method of Malakar *et al.* (2009)<sup>[8]</sup> with slight modifications. Grasshoppers lay eggs in moist soil. To recreate such an environment for the experiment, sand was provided as

the incubation medium. Sterilized dried sand was taken in plastic cups (10cm deep and 6 cm diameter) with 100 gm of oven dried sand for each species. For every concentration of metal chlorides and controls, five replications were taken keeping single egg pod per cup for either grasshopper species. Thus a total of 180 plastic cups were used for the experiment. After that egg pods laid on the same day were put in separate cups for both the species. Sand was contaminated by adding solutions of HgCl<sub>2</sub>, CdCl<sub>2</sub> and ZnCl<sub>2</sub>. Solutions were made by dissolving salts into double distilled water and further diluting them. The diluted solutions were added into plastic cups containing same amount of sand to obtain a range of metal concentrations of 0 mg/kg (control), 5 mg/kg, 10 mg/kg, 20 mg/kg, 40 mg/kg and 80 mg/kg dry sand. 10 ml solutions of different concentrations of these heavy metals were given in each plastic cup for moistening the substrate (sand) to achieve 70-80 % humidity. Any loss of moisture due to evaporation was compensated by adding proper amount of de-mineralized water.

All the plastic cups were placed in an environmental chamber at 35±2°C for 7 weeks. When the nymphs started hatching, their numbers were recorded twice daily. The period between start of treatment and hatching was regarded as the embryonic duration. Developmental periods of each nymph from each set were considered to calculate the mean duration of development. After completion of the experiment (7 weeks) the number of undeveloped eggs was recorded from each set. This could provide information about the effects the heavy metals have on egg mortality.

### Analysis of Data

All the data for successful hatching and embryonic duration for each metal treatment were subjected to one way analysis of variance (ANOVA) and Tukey's posthoc test were carried out to compare the values between different doses using PAST version 3.02.

### Results and Discussion:

#### Effects of heavy metals on hatching

Experiment shows that number of hatchlings reduces with increased doses of metal concentration. In *Oxya hyla hyla* Hg, and Zn shows decrease in hatching success significantly (F=65.85778, P<0.01 for Hg; F=49.92821, P<0.01 for Zn), when metal concentrations reached 20 mg/kg, 40 mg/kg and 80 mg/kg dry sand. But for Cd significant decrease (F=47.96735, P<0.001) was observed only in the two highest treatments. Zn exerted less effect on *Oedaleus abruptus* where in the highest dose significant decrease (39.67273, P<0.001) was found but in Hg and Cd treatments hatching success decreased significantly from second dose onwards (F=65.31282, P<0.001 for Hg; F=73.54667, P<0.001 for Cd) (Table 1).

Results of the present study are in concert with several other workers. While working on *Aiolopus thalassinus* Devkota and Schmidt (1999) [5] observed that egg hatching rate decreases with the increase of Hg, Cd and Pb concentrations. In *Oncopeltus fasciatus* reduction in the percentage of hatchlings throughout the oviposition period was observed with increasing Cd concentrations in drinking water (Cervera *et al.* 2004) [3]. Xu *et al.* (2009) [12] observed that increased Zn concentration results in the decrease of egg hatching in *Folsomia candida*. Some authors have also attributed the reduction in hatchability to a possible harmful effect Hg and Cd have on embryonic development (Schmidt *et al.* 1991 and Cervera *et al.* 2004) [10, 3]. The present study supports these facts.

**Table 1:** Effects of Hg, Cd, and Zn on number of hatched eggs per egg pod in *Oxya hyla hyla* and *Oedaleus abruptus* after 7 weeks exposure in soil.

Eggs hatched per pod						
<i>Oxya hyla hyla</i>			<i>Oedaleus abruptus</i>			
Metal concentration						
(mg /kg dry sand)	Hg	Cd	Zn	Hg	Cd	Zn
Cont	10.60±0.89a	10.60±1.14a	10.80±1.0a	9.20±0.45a	9.80±0.45a	9.40±0.89a
5	9.20±0.84a	9.80±0.84a	10.20±1.48a	8.80±0.84a	9.20±0.45a	9.60±0.55a
10	7.60±0.89a, b	8.80±0.84a	9.20±0.45a	7.40±0.55b	8.00±0.70b	8.60±0.55a
20	6.20±0.45b	7.20±0.84a	8.20±0.45b	6.60±1.14b	7.80±0.84b	7.80±0.84a
40	3.80±0.84c	4.80±0.84b	5.40±0.55c	3.80±0.84c	4.80±0.84c	6.80±0.84a, b
80	2.40±1.14c	3.40±0.89b	4.60±0.55c	1.80±0.84d	2.80±0.84d	4.00±0.70b

**Notes:** Means within a column bearing the same letter were not significantly different (P<0.05)

#### Effects of heavy metals on embryonic duration

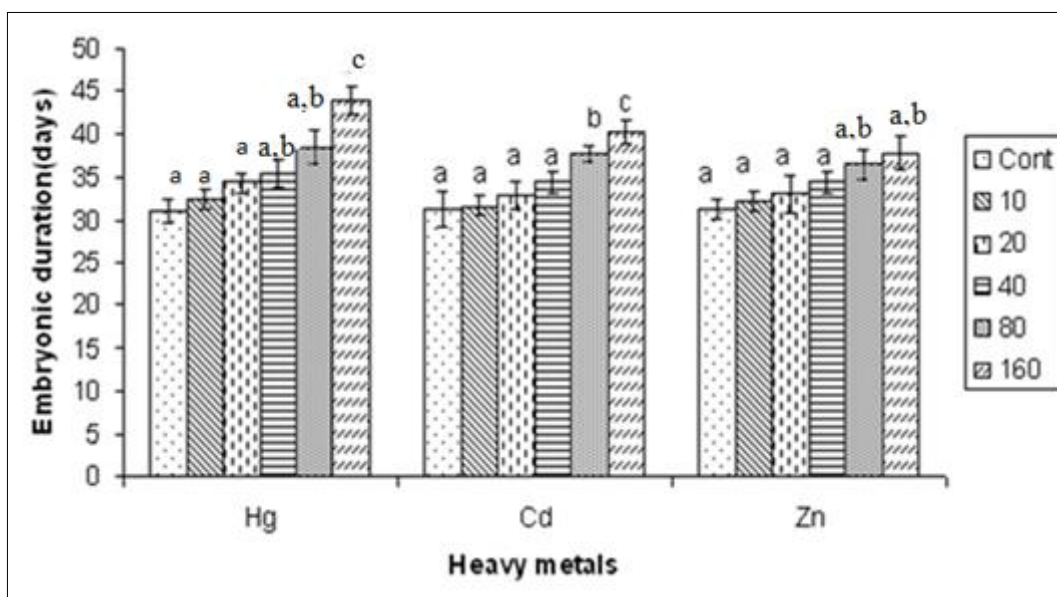
In case of both *Oxya hyla hyla* and *Oedaleus abruptus* a trend of prolonged embryonic duration was observed along with increase of doses of all the three metals. But significant increase was observed in the two higher doses of Cd and in the highest dose of Hg in case of *Oxya hyla hyla*. However, Zn showed insignificant results in those mentioned doses (Fig-1). On the other hand in *Oedaleus abruptus* significant increase was observed only in the highest dose of Zn but in case of Hg and Cd insignificant results were observed (Fig-2).

Devkota and Schmidt (1999) [5] observed that differences in the concentrations of Hg and Cd were found to have no influence on the mean duration of *A. thalassinus*. But in the present experiment the case was somewhat

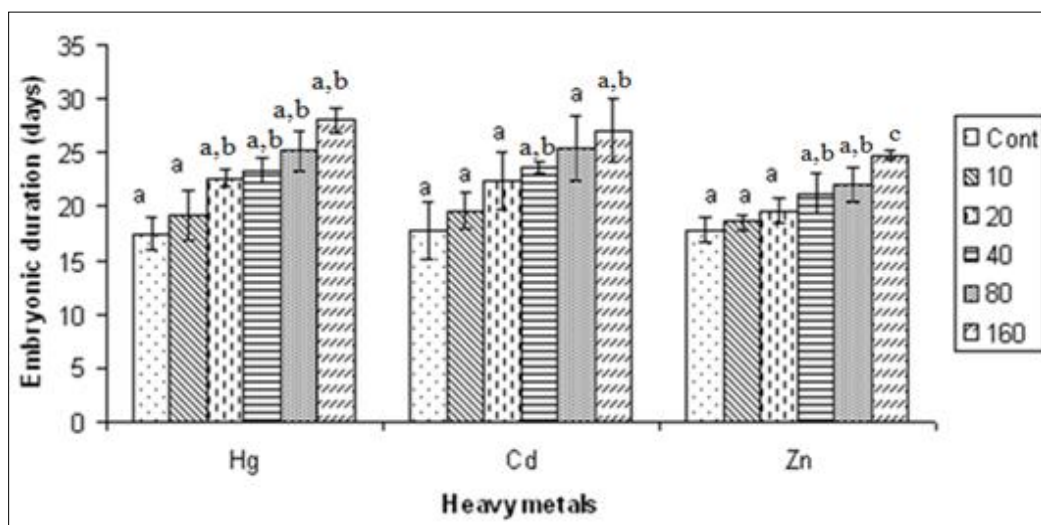
different. I conclude that heavy metals reduce the hatching rate and increases the embryonic duration in acridid eggs.

### Conclusion

In the present study I have determined the toxicity of selected heavy metals on hatching abilities and embryonic duration of two short horned Indian grasshopper, *Oxya hyla hyla* and *Oedaleus abruptus*. The results revealed that in *Oxya hyla hyla* the range of severity of Hg and Zn is more than Cd. On the other hand in case of *Oedaleus abruptus* Zn exerts less effect than Hg and Cd. This reveals that selective permeation of various metal ions is in part responsible for determining the embryonic development in non shelled eggs. In nature gradual increase of heavy metal lode both from anthropogenic and geogenic sources may pose a serious problem among the insects who lays their eggs in soil. The present experiment also revealed that heavy metals produces prolonged embryonic duration particularly in Hg and Cd treatment groups of all the experimental Acridid species indicating both these metals delay the embryonic pathways during development. Whether effects (low hatching rate or prolonged embryonic duration) are temporary or permanent is particularly important in terms of overall impacts on fitness and survival grasshoppers in the wild. Nymphal mortality in grasshoppers is normally very high, but further decrease in hatching and increase in embryonic duration can result in major decreases in populations. In addition heavy metals could biomagnify in these insects due to their phytophagous nature and the food (mainly the grass) they are taking grown on metal polluted soil which could join hand with the present findings and may cause mass mortality and may lead these species on the verge of extinction.



**Fig 1:** Effects of Hg, Cd, and Zn on embryonic duration of *Oxya hyla hyla*. Each bar represents mean  $\pm$  SD. Different superscripts denote significant differences between the mean values ( $P < 0.01$ )



**Fig 2:** Effects of Hg, Cd, and Zn on embryonic duration of *Oedaleus abruptus*. Each bar represents mean  $\pm$  SD. Different superscripts denote significant differences between the mean values ( $P < 0.01$ )

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