



## Sub Lethal Effects of Cadmium on Testicular and Ovarian Maturation in Fresh Water Crab *Paratelphusa hydrodromous*

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### ABSTRACT

Cadmium (Cd), one of the most toxic environmental and industrial pollutants, is known to exert gonadotoxic and spermiotoxic effects. In the present study, we examined the toxic effect of Cd on the testis and ovary of freshwater crab *Paratelphusa hydrodromous*. A batch of 3 each for both sexes were selected and exposed to sub lethal concentration of cadmium (10% LC<sub>50</sub> value of toxicity evaluation; 20 ppm) for 30 days. Sub lethal effect of cadmium chloride on testicular and ovarian activities was observed.

**Keywords:** Heavy metal, cadmium, *Paratelphusa hydrodromous*, LC<sub>50</sub>, testicular and ovarian activities.

### INTRODUCTION

Cadmium (Cd), a widely used heavy metal in modern industry, is one of the most abundant, ubiquitously distributed toxic elements in aquatic systems.<sup>1</sup> Cadmium (Cd) is a soft, ductile, silver-white metal that belongs together with zinc and mercury to group IIb in the Periodic Table. It has relatively low melting (320.9 °C) and boiling (765 °C) points and a relatively high vapour pressure. In the air cadmium is rapidly oxidized into cadmium oxide. Cadmium (Cd) is one of the most toxic heavy metals for humans; the main source of non occupational exposure to Cd includes smoking, air, and food and water contaminated by Cd.<sup>2</sup> In addition, herbal medicine is another source of Cd. The World Health Organization (WHO) estimates that 4 billion people or 80 percent of the world population presently use herbal medicine.<sup>3</sup> Several articles have reported of adverse effects of these herbal preparations due to the presence of high level of heavy metals such as Cd, lead, chromium, nickel, etc. Saeed et al. (2010) investigated twenty five herbal products. The results revealed that the concentrations of some heavy metals, including Cd, were far greater than the permissible limits proposed by the International Regulatory Authorities for herbal drugs. Acute or chronic exposure of Cd causes respiratory distress, lung, breast and endometrial cancers, cardiovascular disorders and endocrine dysfunction.<sup>4</sup> Cadmium (Cd), a toxic and nonessential element, is frequently used in electroplating, pigments, paints, welding, and batteries, which results in both biotic and abiotic environments.<sup>5</sup> Unlike organic compounds, Cd is not biodegradable and has a very long biological half-life.<sup>6</sup>

Metal contamination in aquatic systems are gradually increasing in consequences of natural and anthropogenic effects such as mining activities, agricultural applications, domestic and industrial waste discharges. Besides, coastal urban centers are important contaminant sources for aquatic environments.<sup>7</sup> Among the various heavy metal pollutants, cadmium merits special attention due to its

potential hazards to aquatic biota<sup>8</sup> as well as to human beings.<sup>9</sup> This heavy metal is a common aquatic pollutant and is known to be highly toxic to most organisms, even at small concentrations in natural waters.<sup>10</sup> Cd in waters can be absorbed by aquatic organisms via respiratory system, digestive system and body surface without significant excretion.<sup>11</sup>

### MATERIALS AND METHODS

#### Chemicals

The bioassays were carried out using analytical grade cadmium chloride (CdCl<sub>2</sub>) (E.Merck) was used.

#### Animals and Treatments

Healthy and active crabs *Paratelphusa hydrodromous* were collected from the river bed, canals, paddy fields, etc., situated in and around the rivers of Cauvery and Bhavani. Both sexes of crab at their intermoult stage having an average carapace length of 3.0 ± 0.5 cm and breadth of 4.0 ± 0.5 cm were used for this study. They were maintained in a large cement tank (size : Length – 120 cm; Breadth – 60 cm; Height – 100cm) and were acclimatized to laboratory conditions for a week before the experiment in freshwater (salinity - 0.5 ± 0.1 ‰ ; pH – 7.1 ± 0.2 ; Temperature – 28° C ± 2° C) water was changed daily and aerated continuously. To perform a study on the gonadal maturity of the animal, intermoult stage.<sup>12</sup> Weighing about 16 ± 2 gm (both male and female) were collected and acclimatized in the laboratory. A batch of 3 each for both sexes were selected and exposed to sub lethal concentration of cadmium (10% LC<sub>50</sub> value of toxicity evaluation; 20 ppm) for 30 days. A control group of crabs were also maintained. These crabs were maintained in proper aeration, regular diet of weighed soyabean and on 24hr sub lethal toxicity renewal bioassay system.



**Sample Preparation**

The gonads (ovary and testis) were dissected out after the experimental period of 30 days and fixed in 10% formalin. After 24 hours of fixation tissues mentioned were dehydrated and embedded separately in paraffin wax (M.P. 58 - 60°C). Serial sections were prepared with the help of microtome. Section cuttings were made at 5µm thick and were stained with Heidenhain’s haemotoxylin and alcoholic eosin and mounted on DPX.<sup>13</sup> For each crab two cross sections of gonads and also from the sections of the tissues of control animal.<sup>14</sup>

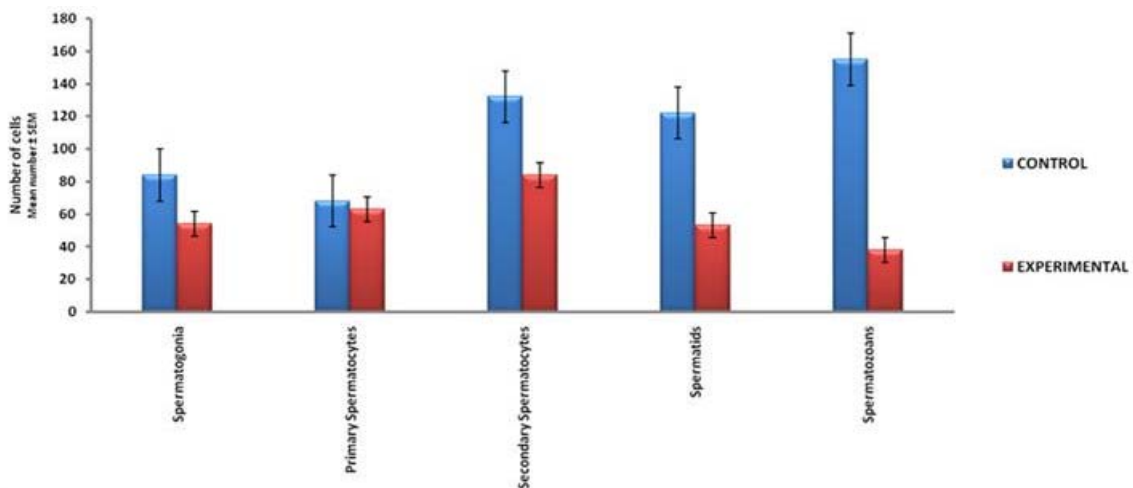
Histological observations on germ cells during oogenesis were made based on the criteria followed.<sup>15</sup> The stages of spermatogenesis were observed on the basis of the nature of chromatin as followed earlier.<sup>16</sup> The sizes of the cells were measured using the oculometer.

**RESULTS AND DISCUSSION**

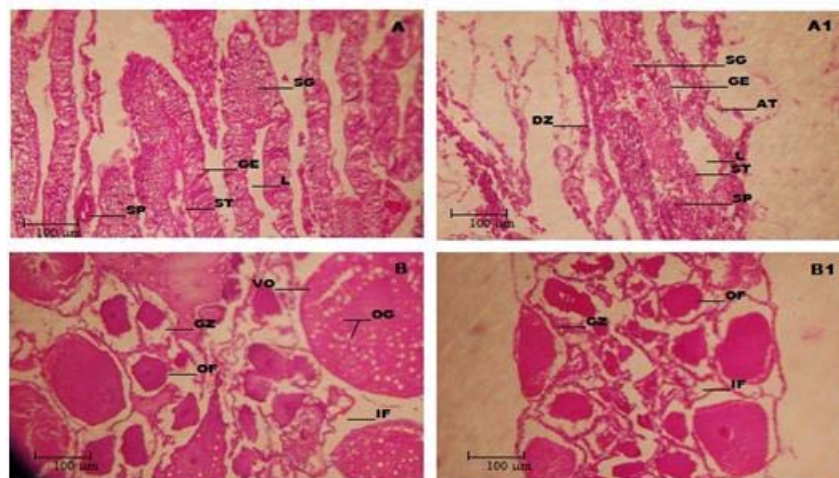
**Testis**

The testis consists of a number of seminiferous tubules with a lumen inside of varying size and shape. They were chiefly made up of linear type tubules (500 – 600 µm in length and about 150 – 250 µm in diameter). Oval shaped tubules were also present (150 – 250 µm in diameter). The germinal zone consists of spherical spermatogonia (6 – 10 µm in diameter); primary spermatocyte (5 µm in diameter) with chromatin, spermatids with chromatin grouping and spermatozoa with crescent shaped head. The untreated male crabs showed normal testicular activity. The proliferative spermatogonia comprised about 15% of the total germ cells (relative number per unit area of microscope 10 X 10x). The primary and secondary spermatocytes constituted 12% and 24% respectively. The spermatids and spermatozoa comprised about 22% and 27% respectively. Apart from the germinal zone, non – germinal zones containing nutritional and supporting cells (Fig 2). The effect of cadmium on testicular activity of *Paratelphusa hydrodromous* in Fig 1.

**Figure 1:** Effect of cadmium on testicular activity of *Paratelphusa hydrodromous*



**Figure 2:** T.S. of testis and ovary of the crab *p.hydrodromous* exposed to sublethal concentration of cadmium for 30 days



A, A1 = T.S. OF TESTIS; B, B1 = T.S. OF OVARY; A, B= CONTROL (10x 6x); A1, B1 = EXPERIMENTAL (10x 6x)

AT = Atrophy; OF = Ovarian Follicle; DZ = Degenerative Zone; OG = Oil Globules; GE = Germinal Epithelium; SG = Spermatogonium; GZ = Germinal Zone; SP = Spermatids; IF = Inter Follicular Space; ST = Seminiferous Tubules; L = Lumen; VO = Vitellogenic Oocytes

On sub lethal treatment with cadmium histological variations in testis were found to be prominent after 30 days. In the seminiferous tubules, diminishing testicular activities were well pronounced. It was indicated with the lower number of cells were noted (Fig: 2). Further the increased size of lumen in the seminiferous tubules, disorganization of tubules, lower chromatin content of the proliferating cells and on germinal epithelium were found to be the clear indications of the tendency of this tissue towards atropic conditions (Fig : 2). There were a lot of reviews of decapod reproduction in the past decades about general aspects of the reproduction of the crabs which could be compared with other crustaceans.<sup>17</sup> Recent studies have been more specific on the nature of pollutants on the crustacean physiology. The sexual maturity studies in the male decapod have been related to histological studies especially in females. A direct influence of spermatogenesis by the factors regulating the moult cycle have been proposed in the previous studies.<sup>18</sup> Materials as contaminants in waste waters would exert a direct effect on the metabolism on which further cause stress on the reproductive physiology. The results on the present study could be well augmented with the earlier studies.<sup>19</sup> The inhibition of spermatogonia process with the effect of mercury administration in rats could be correlated with the present findings.<sup>20</sup>

### Ovary

In the normal untreated female crab, the oocytes were found outside the germinal zone and developed into yolk granule stage. Oocytes during the stage, many small yolk granules in the cytoplasm along with the oil globules which were increased in number, moving to the periphery. The oocytes measured about 400 – 800 µm in diameter in average. The increased vitellogenic activity was found to be clear. The germinal zone of the ovary was found to be fully occupied by the vitellogenic oocytes (Fig 2).

The treated crabs with cadmium after 30 days showed a declining trend of maturity. A decrease in the proliferation of the oocytes and the average diameter of the oocytes were around 80 – 150 µm in diameter. The presences of oil globule and yolk platelets were found to be meager (fig 2. The degenerative zones of follicles were found to be leading to the increase of interfollicular space and the resorption of relict oocytes. In general histological observations on sub lethal toxicity showed that proliferating zone showed only pre vitellogenic oocytes with a notable vacuolization.

The anatomical characteristics of ovary and the process of oogenesis were reported for many crabs.<sup>21</sup> Several studies were conducted on the toxic effect of heavy metal on their bioaccumulation on marine crabs.<sup>22</sup> However, lack of information on the influence of metallic toxicants on fresh water crabs. Seasonal changes on the oogenesis were carried out on a fresh water crab *Geotelphusa dehaani*.<sup>23</sup>

The degeneration of oocytes and the decrease of their proliferation of the present study as previously demonstrated for a freshwater crab *Barytelphus guerini* subjected to zinc contamination.<sup>13</sup> The accumulation of cadmium was found to be depending on the ovarian maturation and other physiological status of the crab.<sup>24</sup> The inhibition of ovarian growth by cadmium which was indicated by lower oocyte diameter was also reported.<sup>25</sup> Similarly, slower maturity and reactional atresia in secondary oocytes in *Chasmagnathus granulata* where also reported with the effect of cadmium.<sup>26</sup>

### CONCLUSION

Cadmium was found to inhibit gonadal maturity. The diminishing testicular activity was indicated by the disorganization of seminiferous tubules and the lower number of spermatocytes and the atrophy of the germinal epithelium. The effect of cadmium the maturation of oocytes in ovary was found to be delayed which was indicated by the presence of degenerative zones of follicles and lower accumulation of oil globule in the previtellogenic oocytes in the proliferating zones.

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