Results
3. RESULTS

3.1 Morphology of female reproductive system

The female reproductive system of *U. annulipes* was of the same type as described by the earlier crustacean workers with minor difference in the maturation ranges (Williamson, 1904; Harvey, 1929; Broekhusysen, 1936; Demeusy, 1958; Ryan, 1967; Viswanathan, 1992; Kulasekharan, 1994; Ragunathan, 1995). The ovaries were paired structures resembling approximately the shape of the english letter ‘H’. They occupy the anterolateral position below the carapace and extended posteriorly and connected transversely by a bridge. Normally the ovaries were located above the alimentary canal, mostly intermingled with the hepatopancreas. Each ovary was covered by a thin layer of connective tissue which contained many black chromatophores. Each ovarian limb contained many ovarian lobes.

The colour of the ovary varied with respect to the ovarian developmental stages. In the present investigation it was observed that there was a relationship between the colouration of the ovarian stages, with respect to the enlargement of the spermatheca. Only the female crabs that were at the advanced stage of ovarian development with the carapace length ranging from 9.5 mm to 11.6 mm and breadth ranging from 15.4 mm to 19.0 mm were selected. In addition to this, only the female crabs of intermoult stage were selected. Further, the advanced stage of the ovarian
development was selected and confirmed by the window method as reported by Viswanathan (1992).

3.1.1 Morphology and histology of the ovary

3.1.1.1 Morphology of the ovary

In the mature stage, the ovary was enlarged and was distinct with many ovarian lobes. Ovary at this stage attained dark brick red colour. Black pigmentation was scarcely found on the ovary. The hepatopancreas was pale yellow in colour and also very soft and fragile.

3.1.1.2 Histology of the ovary

The histological sections of the ovary revealed the presence of many ovarian lobes in bunches. Each ovary was covered by a thin connective tissue layer (3 μm). Below the connective tissue layer was a thin layer called muscular layer (2 μm). This was followed by germinal epithelial tissue layer (4 μm). Different developing oocytes were seen at the germinal zone. The follicular cells were abundantly seen encircling the maturing oocytes.

In the mature stage, the oocytes were comparatively larger in size than the earlier stages, measuring 45.6 μm. The ooplasmic contents were more and the nucleus was very much reduced or indistinct. The chromatic granules were abundant at this stage. The ooplasm showed more of yolk granules and globules (Fig.5).
Fig. 5. Photomicrograph showing the C.S. of ovary in *U. annulipes* - Control
Stained in haematoxylin and eosin x 100.
C - Connective tissue    Y - Yolk globule
M - Muscular tissue      G - Germinal tissue
E - Epithelial layer     O - Ooplasm

Fig. 6. Photomicrograph showing the C.S. of spermatheca - Control
Stained in haematoxylin and eosin x 100.
L - Lumen                  S - Secretory substance
T - Typhiosole
Staining reaction

The outer connective tissue layer of the ovary showed reddish pink in colour in Ehrlich’s haemotoxylin and eosin. The middle muscular layer stained pink in colour in haemotoxylin and eosin. On the other hand, the germinal epithelial layer stained bluish in colour in haematoxylin and eosin. The ooplasm showed intensive staining reaction.

3.2.1 Morphology and histology of spermatheca

3.2.1.1 Morphology of spermatheca

The spermatheca was an enlarged portion of the oviduct present one on either side of the ovary. The spermatheca were comparatively equal in size with 2 to 3 folding or pouches. Depending on the maturation of the ovarian stages, the spermatheca showed differences in shape and sizes. At the mature stage the spermatheca was enlarged and pink in colour and more distinct. The external pouches were clearly visible.

3.2.1.2 Histology of the spermatheca

In the mature state, the spermatheca showed an outer cuticular layer measuring 9 μm thickness, middle muscular layer measuring 4 μm and inner epithelial layer measuring 10 μm thickness and a lumen measuring 1500 μm. The lumen of the spermatheca was endowed with granules, free sperm, sperm-mass and spermatophores (Fig.6).
Staining reactions

Staining of spermatheca showed granular substances, free sperm-mass, spermatophores in the luminal contents of the spermatheca. The secretory substances stained pink in colour with haematoxylin and eosin. The cuticular, muscular, and epithelial layers stained pink in colour. The luminal contents stained red, blue and violet in colours.

3.3.1 Morphology and histology of the hepatopancreas

3.3.1.1 Morphology of the hepatopancreas

In *U. annulipes* the hepatopancreas showed many lobules basely connected by connective tissue to form definite bunches. Each lobule enclosed a lumen. The colour of the hepatopancreas varied with respect to different ovarian maturation stages. In the mature stage the hepatopancreas exhibited yellow colour.

3.3.1.2 Histology of the hepatopancreas

The histological study of hepatopancreas revealed the occurrence of numerous elongated tubules united together by a connective tissue to form definite bunches. Each lobule possessed an outer thin cuticle and inner epithelial lining, with a lumen of varying shape and sizes. The terminal portion of the lobule was called the embryonic zone (EZ). The cells in the epithelial wall constitute the metal zone (MZ). In between the embryonic and metal zones was the secretory zone (SZ), however, the secretory zone
could not be distinguished clearly from the embryonic zone and the metal zone. In the embryonic zone, the base of the lobules divide the lobular lumen into compartments. The metal zone occupied the major portion of the lobule consisting of two types of cells (a) copper cells and (b) iron cells. Copper cells were more in number and possessed small nucleus and indistinct nucleolus, numerous vacuoles in the cytoplasm. These were the centres for storage of both organic and inorganic reserves. The iron cells possessed comparatively larger nucleus and distinct nucleolus and highly basophilic cytoplasm (Fig.7).

Staining reactions

The different zones (embryonic zone and metal zone) of the hepatopancreas and also the outer components of the lobular cells stained well in haematoxylin and eosin in red to violet colours.

3.4 Brain

The cross section of brain of *U. annulipes* showed different types of neurosecretory cells (NSC) ranging from 11-13 μm. Each cell showed a distinct nucleus and stained less in haematoxylin and eosin (brown colour). In some sections the cells exhibited the presence of nucleolus (Fig.8).

3.5 Thoracic ganglia

The cross section of the thoracic ganglia showed different types of neurosecretory cells (NSC) ranging from 11-13 μm. Each neurosecretory
Fig. 7. Photomicrograph showing the C.S. of hepatopancreas - Control. Stained in haematoxylin and eosin x 100.
L - Lumen
E - Embryonic zone
MZ - Metal zone
MC - Metal cell
Fig. 8. Photomicrograph showing different types of neurosecretory cells in the C.S. brain of *U. annulipes* - Control (less stained). Stained in haematoxylin and eosin x 100.

Fig. 9. Photomicrograph showing different types of neurosecretory cells in the C.S. thoracic ganglia of *U. annulipes* - Control (less stained). Stained in haematoxylin and eosin x 100.
cell showed a dense nucleus. In some sections nucleolus was also noticed. The neurosecretory cells (NSC) stained less in haematoxylin and eosin (brown colour) (Fig.9).

3.6 Median Lethal concentration (LC$_{50}$) of cadmium

Median lethal concentration (LC$_{50}$) of cadmium for *U. annulipes* was observed for 96 hours. The logarithm of 50% lethal concentration was obtained by finding the value on the abscissa for straight line which assumes the probit value 5. The concentrations resulting in 50% mortality and slope of the probit line were calculated for specific period of exposure as described by Finney (1971). The per cent mortality data were subjected to probit analysis and plotted against log of dose concentrations resulting in a straight line (Fig.10). The values of LC$_{50}$, upper and lower confidence limits, slope function, correlation co-efficient square and regression results of cadmium on *U. annulipes* are given in Table 1. The LC$_{50}$ values for 24, 48, 72 and 96 hrs of exposure periods were estimated as 0.202, 0.180, 0.153 and 0.132 ppm.

3.7 Effect of sublethal concentration of cadmium

The experimental crabs subjected to cadmium to two different sublethal concentrations (0.013 ppm and 0.04 ppm) and duration for 15 and 30 days exhibited changes in the ovary, spermatheca, hepatopancreas, brain and thoracic ganglia both morphologically and histologically.
Fig. 10: Graph showing the LC$_{50}$ value of cadmium on $U.$ annulipes

![Graph showing the LC$_{50}$ value of cadmium on $U.$ annulipes](image-url)
Table 1: The LC\textsubscript{50} values and regression equation for *U. annulipes* treated with cadmium

<table>
<thead>
<tr>
<th>Exposure period in days</th>
<th>LC\textsubscript{50} (ppm)</th>
<th>Upper confidence limits (UCL) (ppm)</th>
<th>Lower confidence limits (LCL) (ppm)</th>
<th>Regression results</th>
<th>Slope function (S)</th>
<th>( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>0.202</td>
<td>0.234</td>
<td>0.173</td>
<td>( Y = -5.832x + 8.285 )</td>
<td>1.326</td>
<td>0.922</td>
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<tr>
<td>48</td>
<td>0.180</td>
<td>0.209</td>
<td>0.154</td>
<td>( Y = -4.959x + 0.966 )</td>
<td>1.334</td>
<td>0.966</td>
</tr>
<tr>
<td>72</td>
<td>0.153</td>
<td>0.181</td>
<td>0.129</td>
<td>( Y = -3.997x + 7.563 )</td>
<td>1.354</td>
<td>0.982</td>
</tr>
<tr>
<td>96</td>
<td>0.132</td>
<td>0.165</td>
<td>0.105</td>
<td>( Y = -2.167x + 6.394 )</td>
<td>1.429</td>
<td>0.988</td>
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</table>
3.7.1 Morphology of the ovary

Crabs treated with lower sublethal concentration (0.013 ppm) of cadmium for 15 and 30 days and higher sublethal concentration (0.04 ppm) for 15 and 30 days

The crabs subjected to lower sublethal concentration (0.013 ppm) of cadmium for 15 and 30 days and higher sublethal concentration (0.04 ppm) for 15 and 30 days exhibited little changes in the ovary. The ovaries were soft, shiny, lobulated and dark brick red in colour with dense chromatophores. The ovary also showed turgid and collapsible condition.

3.7.1.1 Histology of the ovary

3.7.1.1 a. Crabs treated with lower sublethal concentration (0.013 ppm) of cadmium for 15 days

The cross sections of the ovary in this group exhibited little enlargement of oocytes. Small vacuoles were seen in the cytoplasm of oocytes. In some sections the cytoplasm exhibited foamy condition. The nuclei were moderately basophilic. Moderate necrosis were noticed in the oocytes (Fig.11).

3.7.1.1 b. Crabs treated with lower sublethal concentration of cadmium for 30 days

On the other hand, the crabs treated with lower sublethal concentration (0.013 ppm) of cadmium for 30 days showed enlargement and
Fig. 11. Photomicrograph showing C.S. of ovary in *U. annulipes* treated with lower sublethal concentration (0.013 ppm) of cadmium for 15 days. Stained in haematoxylin and eosin x 100.
V - Vacuole

Fig. 12. Photomicrograph showing C.S. of ovary in *U. annulipes* treated with lower sublethal concentration (0.013 ppm) of cadmium for 30 days. Stained in haematoxylin and eosin x 100.
V - Vacuole
more distension of oocytes, vacuolization and more foaming appearance in the cytoplasm and more basophilic nucleus. Necrotic conditions were also seen (Fig.12).

3.7.1.1 c. Crabs treated with higher sublethal concentration (0.04 ppm) of cadmium for 15 days

In the crabs treated with higher sublethal concentration of cadmium for 15 days, the cross section of the ovary exhibited reduction in the size of oocytes. More vacuolization and foaming conditions of the cytoplasm were noticed. Nuclei were basophilic. Necrosis in the oocytes were more than in the crabs treated with lower sublethal (0.013 ppm) concentration of cadmium for 30 days (Fig.13).

3.7.1.1 d. Crabs treated with higher sublethal concentration (0.04 ppm) of cadmium for 30 days

In this group, the cross sections of the ovary exhibited much more pronounced condition in the reduction of the size of oocytes. Vacuolization and foaming of cytoplasm, basophilic condition of nuclei, necrotic condition of oocytes were noticed (Fig.14).

3.8 Effect of cadmium on the spermatheca

The crabs treated with lower sublethal concentration (0.013 ppm) of cadmium for 15 and 30 days and higher sublethal concentration (0.04 ppm) for 15 and 30 days also exhibited differences in the different
Fig. 13. Photomicrograph showing C.S. of ovary in *U. annulipes* treated with higher sublethal concentration (0.04 ppm) of cadmium for 15 days. Stained in haematoxylin and eosin x 100.

V - Vacuole

Fig. 14. Photomicrograph showing C.S. of ovary in *U. annulipes* treated with higher sublethal concentration (0.04 ppm) of cadmium for 30 days. Stained in haematoxylin and eosin x 200.

V - Vacuole
constituent layers such as circular, muscular and epithelial layers and in
the luminal contents of the spermatheca.

3.8.1 Morphology of the spermatheca

Crabs treated with lower sublethal concentration
(0.013 ppm) for 15 and 30 days and higher sublethal
concentration (0.04 ppm) for 15 and 30 days of cadmium

In the experimental groups of lower sublethal concentration
(0.013 ppm) of cadmium for 15 and 30 days, the spermatheca exhibited less
morphological differences when compared to the control group. Here, in the
crabs, the spermatheca were little enlarged but turgid. On the other hand,
crabs treated with higher sublethal concentration (0.04 ppm) were shrunken
and highly flaccid. Similarly at the higher sublethal concentration
(0.04 ppm) for 15 and 30 days, the spermatheca showed more shrinkage and
collapsible condition.

3.8.1.1 Histology of the spermatheca

3.8.1.1 a. Crabs treated with lower sublethal concentration
(0.013 ppm) of cadmium for 15 days

In this group, crabs treated with 0.013 ppm of cadmium for
15 days, the spermatheca showed little hardness. The histological sections
revealed less changes in the cuticular, muscular and epithelial layers. There
was a reduction in thickness of these layers. The luminal contents of the
spermatheca (granular substances, sperm-mass and also spermatophores) exhibited less staining reactions (Fig.15).

3.8.1.1 b. Crabs treated with lower sublethal concentration (0.013 ppm) of cadmium for 30 days

In the crabs treated with lower sublethal concentration (0.013 ppm) of cadmium for 30 days, the cross sections of spermatheca showed changes in the thickness of the three layers and also in the luminal contents. The thickness of the layer was also reduced more than the earlier group. The staining reaction also differed in their intensity when treated for 15 and 30 days (Fig.16).

3.8.1.1 c. Crabs treated with higher sublethal concentration (0.04 ppm) of cadmium for 15 days

In higher sublethal concentration of (0.04 ppm) cadmium for 15 days, the spermatheca of treated crabs showed intense changes in the constituent layers, luminal contents as well as in the staining reactions than the crabs treated with lower sublethal concentration of cadmium (0.013 ppm) (Fig.17).

3.8.1.1 d. Crabs treated with higher sublethal concentration (0.04 ppm) of cadmium for 30 days

In the crabs exposed to higher sublethal concentration (0.04 ppm) of cadmium for 30 days showed more pronounced conditions in the
Fig. 15. Photomicrograph showing C.S. of spermatheca in *U. annulipes* treated with lower sublethal concentration (0.013 ppm) of cadmium for 15 days. Stained in haematoxylin and eosin x 100. L - Lumen LC - Luminal contents

Fig. 16. Photomicrograph showing C.S. of spermatheca in *U. annulipes* treated with lower sublethal concentration (0.013 ppm) of cadmium for 30 days. Stained in haematoxylin and eosin x 100. L - Lumen LC - Luminal contents
Fig. 17. Photomicrograph showing C.S. of spermatheca in *U. annulipes* treated with higher sublethal concentration (0.04 ppm) of cadmium for 15 days. Stained in haematoxylin and eosin x 100.
L - Lumen
LC - Luminal contents

Fig. 18. Photomicrograph showing C.S. of spermatheca in *U. annulipes* treated with higher sublethal concentration (0.04 ppm) of cadmium for 30 days. Stained in haematoxylin and eosin x 100.
C - Cuticular layer
E - Epithelial layer
M - Muscular layer
spermathecal constituent layers and also in the luminal contents than the earlier three groups 0.013 ppm of cadmium for 15 and 30 days and 0.04 ppm for 15 days (Fig. 18).

3.9 Effects of sublethal concentration of cadmium on the hepatopancreas

3.9.1 Morphology of the hepatopancreas

Crabs treated with lower sublethal concentration of cadmium (0.013 ppm) for 15 and 30 days and higher sublethal concentration of cadmium (0.04 ppm) for 15 and 30 days

In the crabs treated with lower sublethal concentration (0.013 ppm) of cadmium for 15 and 30 days and higher sublethal concentration (0.04 ppm) for 15 and 30 days showed little changes in the morphological characters when compared to the control. In the experimental crabs, the hepatopancreas was light to pale yellow in colour, but not as yellow as in the control group. The tubules were not elongated and also not united by the connective tissue to form definite bunches as found in the control. The embryonic zone was undistinguished. The metal zone also showed some changes. Similarly the secretory zone which was quite clear in the control was not distinguishable. The tubules exhibited vacuolization. The above said degenerative characters were progressively increased when the concentration and the durations were increased.
3.9.2 Histology of the hepatopancreas

The cross sections of different regions of hepatopancreas of different groups of the crabs treated with cadmium at lower sublethal concentration (0.013 ppm) for 15 and 30 days (Fig.19, 20) and higher sublethal concentration (0.04 ppm) for 15 and 30 days (Fig.21, 22) exhibited changes in the embryonic zone and also in the metal zone. The secretory products of the tubules were also quite undistinguishable and vacuoles were also found in the cytoplasm of the cell.

3.10 Effects of sublethal concentration of cadmium on the brain
3.10.1 Crabs treated with lower sublethal concentration (0.013 ppm) of cadmium for 15 and 30 days and higher sublethal concentration (0.04 ppm) for 15 and 30 days

In the crabs, U. annulipes exposed to lower sublethal concentration (0.013 ppm) of cadmium for a period of 15 and 30 days showed histopathological changes. The cytoplasm of neurosecretory cells showed deeply stained neurosecretory materials for 15 days treated crabs (Fig.23). On the other hand, crabs treated for 30 days showed intensively stained neurosecretory materials (Fig.24). In the higher sublethal concentration (0.04 ppm) for 15 days, the neurosecretory cells of the brain showed more deeply stained neurosecretory materials (Fig.25). Whereas in the crabs treated for 30 days the neurosecretory cells of the brain exhibited more intensively stained neurosecretory materials (Fig.26).
Fig. 19. Photomicrograph showing C.S. of hepatopancreas in *U. annulipes* treated with lower sublethal concentration (0.013 ppm) of cadmium for 15 days. Stained in haematoxylin and eosin x 100.  
L - Lumen  
V - Vacuole

Fig. 20. Photomicrograph showing C.S. of hepatopancreas in *U. annulipes* treated with lower sublethal concentration (0.013 ppm) of cadmium for 30 days. Stained in haematoxylin and eosin x 100.  
L - Lumen  
V - Vacuole
Fig. 21. Photomicrograph showing C.S. of hepatopancreas in *U. annulipes* treated with higher sublethal concentration (0.04 ppm) of cadmium for 15 days. Stained in haematoxylin and eosin x 100.
L - Lumen  
V - Vacuole

Fig. 22. Photomicrograph showing C.S. of hepatopancreas in *U. annulipes* treated with higher sublethal concentration (0.04 ppm) of cadmium for 30 days. Stained in haematoxylin and eosin x 100.
L - Lumen  
V - Vacuole
Fig.23. Photomicrograph showing different types of neurosecretory cells in the C.S. brain of *U. annulipes* treated with lower sublethal concentration (0.013 ppm) of cadmium for 15 days (deeply stained). Stained in haematoxylin and eosin x 100.

Fig.24. Photomicrograph showing different types of neurosecretory cells in the C.S. brain of *U. annulipes* treated with lower sublethal concentration (0.013 ppm) of cadmium for 30 days (intensively stained). Stained in haematoxylin and eosin x 100.
Fig.25. Photomicrograph showing different types of neurosecretory cells in the C.S. brain of *U. annulipes* treated with higher sublethal concentration (0.04 ppm) of cadmium for 15 days (more deeply stained). Stained in haematoxylin and eosin x 100.

Fig.26. Photomicrograph showing different types of neurosecretory cells in the C.S. brain of *U. annulipes* treated with higher sublethal concentration (0.04 ppm) of cadmium for 30 days (more intensively stained). Stained in haematoxylin and eosin x 100.
3.11 Effects of sublethal concentration of cadmium on the thoracic ganglia

3.11.1 Crabs treated with lower sublethal concentration (0.013 ppm) of cadmium for 15 and 30 days and higher sublethal concentration (0.04 ppm) for 15 and 30 days

The histopathological changes were observed in the thoracic ganglia of *U. annulipes* exposed to lower sublethal concentration (0.013 ppm) of cadmium for 15 and 30 days. The neurosecretory cells showed deeply stained neurosecretory materials for 15 days (Fig.27). On the other hand, crabs treated for 30 days showed intensively stained neurosecretory materials in the cytoplasm (Fig.28). In the crabs exposed to higher sublethal concentration (0.04 ppm) of cadmium for 15 days showed more deeply stained neurosecretory materials in the cytoplasm (Fig.29), whereas in the crabs treated for 30 days showed more intensively stained reaction with the neurosecretory materials in the cytoplasm (Fig.30).

3.12 Effect of sublethal concentration of cadmium on quantitative biochemical parameters of different tissues (ovary, spermatheca, hepatopancreas, muscle, gill and haemolymph) in *U. annulipes*

3.12.1 Effect of sublethal concentration of cadmium on protein content in different tissues of *U. annulipes*

3.12.1.1 Ovary

The protein content in the ovary of control crab *U. annulipes* was 90.31 and 90.74 mg/g wet tissue for 15 and 30 days of exposure period
Fig. 27. Photomicrograph showing different types of neurosecretory cells in the C.S. thoracic ganglia of *U. annulipes* treated with lower sublethal concentration (0.013 ppm) of cadmium for 15 days (deeply stained). Stained in haematoxylin and eosin x 100.

Fig. 28. Photomicrograph showing different types of neurosecretory cells in the C.S. thoracic ganglia of *U. annulipes* treated with lower sublethal concentration (0.013 ppm) of cadmium for 30 days (intensively stained). Stained in haematoxylin and eosin x 100.
Fig. 29. Photomicrograph showing different types of neurosecretory cells in the C.S. thoracic ganglia of *U. annulipes* treated with higher sublethal concentration (0.04 ppm) of cadmium for 15 days (more deeply stained). Stained in haematoxylin and eosin x 100.

Fig. 30. Photomicrograph showing different types of neurosecretory cells in the C.S. thoracic ganglia of *U. annulipes* treated with higher sublethal concentration (0.04 ppm) of cadmium for 30 days (more intensively stained). Stained in haematoxylin and eosin x 100.
respectively (Table 2, Fig.31). In the crabs treated with lower sublethal concentration (0.013 ppm) of cadmium the protein content was 71.89 and 60.56 mg/g wet tissue, whereas in the higher sublethal concentration (0.04 ppm), it was 66.30 and 53.31 mg/g wet tissue for 15 and 30 days of experimental periods. The protein content of ovary progressively decreased in both sublethal concentrations at both exposure periods. It was observed that there was an overall decrease in the protein content of the treated crabs on the 30th day of exposure. The decline of protein content in the cadmium treated crabs were statistically significant (P<0.00001) in both the sublethal concentrations at both the exposure periods.

3.12.1.2 Spermatheca

In control crabs, the mean protein content in spermatheca tissues were 71.65 and 71.69 mg/g wet tissue for 15 and 30 days respectively (Table 2, Fig.31). In the crabs treated with lower sublethal concentration (0.013 ppm) of cadmium the protein content in the spermatheca tissues were 60.34 and 54.20 mg/g wet tissue and in higher sublethal concentrations (0.04 ppm), it was 56.10 and 45.80 mg/g wet tissue for 15 and 30 days of exposure periods. In lower and higher sublethal concentrations of cadmium treated crabs the mean of protein content decreased at both the exposure periods. The maximum decrease was observed on the 30th day of experiment in both the sublethal concentrations. The mean decrease in protein content in both the sublethal concentrations were statistically significant (P<0.00001) at both the exposure periods.
Table 2: Effect of sublethal concentration of cadmium on protein content in different tissues of *U. annulipes*

<table>
<thead>
<tr>
<th>Exposure period in days</th>
<th>Tissues</th>
<th>Control. Mean ± SD</th>
<th>Lower sublethal concentration (0.013 ppm) Mean ± SD</th>
<th>Higher sublethal concentration (0.04 ppm) Mean ± SD</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>Ovary</td>
<td>90.31 ± 2.3</td>
<td>71.89 ± 4.7†</td>
<td>66.30 ± 3.6†</td>
<td>69.7</td>
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<tr>
<td></td>
<td>Spermatheca</td>
<td>71.65 ± 3.0</td>
<td>60.34 ± 4.5†</td>
<td>56.10 ± 2.9†</td>
<td>31.07</td>
<td>&lt; 0.00001†</td>
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<td>Hepatopancreas</td>
<td>61.46 ± 3.7</td>
<td>43.57 ± 3.1†</td>
<td>38.14 ± 4.3†</td>
<td>64.04</td>
<td>&lt; 0.00001†</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>63.14 ± 2.7</td>
<td>51.28 ± 3.8†</td>
<td>48.26 ± 3.4†</td>
<td>33.19</td>
<td>&lt; 0.00001†</td>
</tr>
<tr>
<td></td>
<td>Gill</td>
<td>57.32 ± 5.0</td>
<td>44.61 ± 3.7†</td>
<td>40.12 ± 4.0†</td>
<td>26.05</td>
<td>&lt; 0.00001†</td>
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<td>Haemolymph</td>
<td>61.44 ± 3.6</td>
<td>56.15 ± 3.8†</td>
<td>52.25 ± 3.4†</td>
<td>9.69</td>
<td>&lt; 0.002†</td>
</tr>
<tr>
<td>30</td>
<td>Ovary</td>
<td>90.74 ± 5.3</td>
<td>60.56 ± 3.9†</td>
<td>53.31 ± 3.6†</td>
<td>127.0</td>
<td>&lt; 0.00001†</td>
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<td>Spermatheca</td>
<td>71.69 ± 3.4</td>
<td>54.20 ± 4.3†</td>
<td>45.80 ± 3.6†</td>
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<td>Hepatopancreas</td>
<td>61.55 ± 4.2</td>
<td>36.41 ± 3.2†</td>
<td>28.26 ± 5.8†</td>
<td>87.6</td>
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<td>Muscle</td>
<td>63.22 ± 1.8</td>
<td>43.10 ± 3.8†</td>
<td>37.52 ± 6.5†</td>
<td>54.4</td>
<td>&lt; 0.00001†</td>
</tr>
<tr>
<td></td>
<td>Gill</td>
<td>57.35 ± 5.1</td>
<td>37.15 ± 4.5†</td>
<td>29.31 ± 5.0†</td>
<td>52.2</td>
<td>&lt; 0.00001†</td>
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<tr>
<td></td>
<td>Haemolymph</td>
<td>61.57 ± 3.0</td>
<td>50.23 ± 4.1†</td>
<td>44.25 ± 3.9†</td>
<td>33.8</td>
<td>&lt; 0.00001†</td>
</tr>
</tbody>
</table>

Mean ± SD of six individual observations.
Values are expressed mg/g wet tissue and mg/ml haemolymph
*Statistically significant (By one-way analysis of variance)
†Statistically significant (By Duncan's multiple range test)
Fig. 31 Effect of sublethal concentration of cadmium on protein content in different tissues of *U. annulipes*

**Lower sublethal concentration (0.013 ppm)**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>15 days</th>
<th>30 days</th>
</tr>
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<tbody>
<tr>
<td>Ovary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spermatheca</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatopancreas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gill</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemolymph</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Control ■ Experimental □

**Higher sublethal concentration (0.04 ppm)**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>15 days</th>
<th>30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spermatheca</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatopancreas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gill</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemolymph</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Control ■ Experimental □
3.12.1.3 Hepatopancreas

The *U. annulipes* treated with lower sublethal concentration (0.013 ppm) of cadmium was analysed to find out the protein content in the hepatopancreas tissues. The protein content was 43.57 and 36.41 mg/g wet tissue, whereas in higher sublethal concentration (0.04 ppm) it was 38.14 and 28.26 mg/g wet tissue for 15 and 30 days of exposure periods, respectively (Table 2, Fig.31). In control crabs the mean of protein content in the hepatopancreas tissues was 61.46 and 61.55 mg/g wet tissue for 15 and 30 days of experimental periods, respectively. It was noticed that the protein content of treated crabs registered an decrease, when compared to control. The maximum decrease was observed on 30th day of exposure in both the sublethal concentrations. The decrease in protein content were statistically significant (P<0.00001) in both the sublethal concentrations at both the exposure periods.

3.12.1.4 Muscle

The mean protein content in the muscle of control crabs were 63.14 and 63.22 mg/g wet tissue for 15 and 30 days of experiment. In the crabs treated with lower sublethal concentration (0.013 ppm) of cadmium it was 51.28 and 43.10 mg/g wet tissue and in higher sublethal concentration (0.04 ppm), it was 48.26 and 37.52 mg/g wet tissue for 15 and 30 days of exposure periods respectively (Table 2, Fig.31). In cadmium treated crabs, the protein content progressively decreased at both exposure periods. The maximum decline was observed on the 30th day in both the sublethal
concentrations. The test of significance indicated that the protein content was significantly \( P < 0.00001 \) decreased in both the sublethal concentrations at both the exposure periods.

3.12.1.5 Gill

In control crab, the protein content in gill tissues were 57.32 and 57.35 mg/g wet tissues for 15 and 30 days respectively. In the crabs treated with lower sublethal concentration (0.013 ppm) of cadmium, the protein content in the gill tissues were 44.61 and 37.15 mg/g wet tissue and in higher sublethal concentration (0.04 ppm), it was 40.12 and 29.31 mg/g wet tissue for 15 and 30 days of exposure periods respectively (Table 2, Fig.31). In lower and higher sublethal concentrations of cadmium treated crabs, the mean protein content decreased at both the exposure periods. The maximum decrease was observed on 30th day of experiment in both the sublethal concentrations. The decrease in protein content were statistically significant \( P < 0.00001 \) in both the sublethal concentrations at both the exposure periods.

3.12.1.6 Haemolymph

The protein content in the haemolymph of control crab \( U. \textit{annulipes} \) were 61.44 and 61.57 mg/ml for 15 and 30 days of experimental periods respectively (Table 2, Fig.31). In the crabs treated with lower sublethal concentration (0.013 ppm) of cadmium, it was 56.15 and 50.23 mg/ml and in higher sublethal concentration (0.04 ppm), it was 52.25
and 44.25 mg/ml for 15 and 30 days of exposure periods respectively. The protein content was found to decline drastically in both the sublethal concentrations at both exposure periods. The decline in protein content was statistically significant (P<0.00001) in both the sublethal concentrations at both the exposure periods.

3.12.2 Effect of sublethal concentration of cadmium on the carbohydrate content in different tissues of *U. annulipes*

3.12.2.1 Ovary

The carbohydrate content in the ovary of control crabs *U. annulipes* were 18.14 and 18.17 mg/g wet tissue for 15 and 30 days of experimental periods. In the crabs treated with lower sublethal concentration (0.013 ppm) of cadmium the carbohydrate contents was 13.87 and 11.34 mg/g wet tissue, whereas in higher sublethal concentration (0.04 ppm), the carbohydrate content was 11.62 and 10.42 mg/g wet tissue for 15 and 30 days of exposure periods respectively (Table 3, Fig.32). The carbohydrate content was found to decline drastically in both the sublethal concentrations at both the exposure periods. The maximum decrease was observed on 30th day of exposure in both the sublethal concentrations. The decrease in mean carbohydrate levels were statistically significant in both the sublethal concentrations on 15th day of exposure period (P<0.0024) and 30th day of exposure period (P<0.00001).
Table 3: Effect of sublethal concentration of cadmium on carbohydrate content in different tissues of *U. annulipes*

<table>
<thead>
<tr>
<th>Exposure period in days</th>
<th>Tissues</th>
<th>Control</th>
<th>Lower sublethal concentration (0.013 ppm)</th>
<th>Higher sublethal concentration (0.04 ppm)</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>Ovary</td>
<td>18.14 ± 3.7</td>
<td>13.87 ± 1.5†</td>
<td>11.62 ± 2.3†</td>
<td>9.24</td>
<td>0.0024*</td>
</tr>
<tr>
<td></td>
<td>Spermatheca</td>
<td>15.36 ± 3.0</td>
<td>12.25 ± 2.9</td>
<td>11.56 ± 2.1</td>
<td>3.36</td>
<td>0.06NS</td>
</tr>
<tr>
<td></td>
<td>Hepatopancreas</td>
<td>10.11 ± 2.5</td>
<td>6.21 ± 1.9†</td>
<td>5.94 ± 0.7†</td>
<td>9.28</td>
<td>0.002*</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>11.40 ± 3.0</td>
<td>8.58 ± 1.0†</td>
<td>7.73 ± 0.44†</td>
<td>6.36</td>
<td>0.010*</td>
</tr>
<tr>
<td></td>
<td>Gill</td>
<td>10.58 ± 1.9</td>
<td>7.20 ± 1.3†</td>
<td>6.57 ± 1.6†</td>
<td>10.6</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td>Haemolymph</td>
<td>13.07 ± 2.1</td>
<td>10.86 ± 1.8</td>
<td>10.22 ± 2.1</td>
<td>3.37</td>
<td>0.062NS</td>
</tr>
<tr>
<td>30</td>
<td>Ovary</td>
<td>18.17 ± 1.6</td>
<td>11.34 ± 1.6†</td>
<td>10.42 ± 2.2†</td>
<td>32.04</td>
<td>&lt; 0.00001*</td>
</tr>
<tr>
<td></td>
<td>Spermatheca</td>
<td>15.39 ± 2.8</td>
<td>11.13 ± 1.2†</td>
<td>10.55 ± 1.8†</td>
<td>10.24</td>
<td>0.0016*</td>
</tr>
<tr>
<td></td>
<td>Hepatopancreas</td>
<td>10.14 ± 2.3</td>
<td>5.62 ± 1.4†</td>
<td>4.73 ± 0.9†</td>
<td>7.56</td>
<td>0.01*</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>11.42 ± 1.7</td>
<td>7.23 ± 1.2†</td>
<td>6.82 ± 0.99†</td>
<td>22.1</td>
<td>&lt; 0.00001*</td>
</tr>
<tr>
<td></td>
<td>Gill</td>
<td>10.61 ± 1.9</td>
<td>6.35 ± 2.1†</td>
<td>5.74 ± 1.1†</td>
<td>13.85</td>
<td>0.0004*</td>
</tr>
<tr>
<td></td>
<td>Haemolymph</td>
<td>13.10 ± 1.5</td>
<td>9.95 ± 1.0†</td>
<td>9.60 ± 1.9†</td>
<td>9.54</td>
<td>0.002*</td>
</tr>
</tbody>
</table>

Mean ± SD of six individual observations.
Values are expressed mg/g wet tissue and mg/ml haemolymph
*Statistically significant (By one-way analysis of variance)
†Statistically significant (By Duncan’s multiple range test)
NS - Not Significant
Fig. 32 Effect of sublethal concentration of cadmium on carbohydrate content in different tissues of *U. annulipes*

Lower sublethal concentration (0.013 ppm)

15 days  
30 days

mg/g wet tissue  
mg/ml haemolymph

- Ovary  
- Spermatheca  
- Hepatopancreas  
- Muscle  
- Gill  
- Haemolymph

Control  
Experimental

Higher sublethal concentration (0.04 ppm)

15 days  
30 days

mg/g wet tissue  
mg/ml haemolymph

- Ovary  
- Spermatheca  
- Hepatopancreas  
- Muscle  
- Gill  
- Haemolymph

Control  
Experimental
3.12.2.2 Spermatheca

In control crabs, the carbohydrate content in the spermatheca tissues were 15.36 and 15.39 mg/g wet tissue for 15 and 30 days of experimental periods respectively (Table 3, Fig.32). In the crabs treated with lower sublethal concentration (0.013 ppm) of cadmium, the carbohydrate content was 12.25 and 11.13 mg/g wet tissue and in higher sublethal concentration (0.04 ppm), it was 11.56 and 10.55 mg/g wet tissue for 15 and 30 days of exposure periods respectively. The carbohydrate content was found to decline drastically in both the sublethal concentrations at both the exposure periods. The maximum decrease was observed on 30th day of experiment in both the sublethal concentrations. The changes in carbohydrate content in cadmium treated crabs were statistically insignificant (P>0.05) in both sublethal concentrations on 15th day of exposure, whereas statistically significant (P<0.0016) on 30th day of exposure.

3.12.2.3 Hepatopancreas

When *U. annulipes* was subjected to lower sublethal concentration (0.013 ppm) of cadmium the carbohydrate content in the hepatopancreas was 6.21 and 5.62 mg/g wet tissue and in higher sublethal concentration (0.04 ppm), it was 5.94 and 4.73 mg/g wet tissue for 15 and 30 days of exposure periods respectively (Table 3, Fig.32). In control crabs, the mean of carbohydrate content in the hepatopancreas was 10.11 and 10.14 mg/g wet tissue for 15 and 30 days of exposure. The maximum decrease was
observed on the 30th day of experiment in both the sublethal concentrations. The decrease in carbohydrate content in the cadmium treated crabs were statistically significant in both the sublethal concentrations on 15th day exposure period (P<0.002) and 30th day of exposure period.

3.12.2.4 Muscle

The carbohydrate content in the muscle of control crabs of *U. annulipes* were 11.40 and 11.42 mg/g wet tissue for 15 and 30 days of exposure periods respectively (Table 3, Fig.32). In the crabs treated with lower sublethal concentration (0.013 ppm) of cadmium, it was 8.58 and 7.23 mg/g wet tissue and in higher sublethal concentration (0.04 ppm), it was 7.73 and 6.82 mg/g wet tissue for 15 and 30 days of exposure periods respectively. The carbohydrate content was found to decline drastically in both the sublethal concentrations at both the exposure periods. The maximum decline was observed on the 30th day in both the sublethal concentrations. The decrease in carbohydrate levels were statistically significant in both the sublethal concentrations on 15th day of exposure period (P<0.01) and 30th day of exposure period (P<0.00001).

3.12.2.5 Gill

The *U. annulipes* treated with lower sublethal concentration (0.013 ppm) of cadmium was analysed to find out the carbohydrate content in the gill tissues and it was 7.20 and 6.35 mg/g wet tissue, whereas in higher sublethal concentration (0.04 ppm) it was 6.57 and 5.74 mg/g wet
tissue for 15 and 30 days of exposure periods respectively (Table 3, Fig.32). In control crabs, the mean of carbohydrate content in the gill was 10.58 and 10.61 mg/g wet tissue for 15 and 30 days of experimental periods. In cadmium treated crabs, the carbohydrate content drastically decreased at both exposure periods. It was noticed that the carbohydrate content of the treated crabs registered an decrease, when compared to control. The decline in carbohydrate content was statistically significant in both the sublethal concentrations on 15th day exposure period (P<0.001) and 30th day of exposure period (P<0.0004).

3.12.2.6 Haemolymph

In control crabs, the mean of carbohydrate content in the haemolymph was 13.07 and 13.10 mg/ml for 15 and 30 days of experimental periods, respectively (Table 3, Fig.32). In the crabs treated with lower sublethal concentration (0.013 ppm) of cadmium, the carbohydrate content was 10.86 and 9.95 mg/ml and in higher sublethal concentration (0.04 ppm), it was 10.22 and 9.60 mg/ml for 15 and 30 days of exposure periods respectively. The carbohydrate content declined drastically in both the sublethal concentrations at both the exposure periods. The maximum decrease was observed on the 30th day of the experiment in both the sublethal concentrations. The decline in carbohydrate content was statistically insignificant (P>0.05) on 15th day of exposure, whereas statistically significant (P<0.002) on 30th day of exposure.
3.12.3  Effect of sublethal concentrations of cadmium on lipid content in different tissues of *U. annulipes*

3.12.3.1  Ovary

The lipid content in the ovary of control crabs were 70.15 and 70.20 mg/g wet tissues, for a period of 15 and 30 days. In the crabs treated with lower sublethal concentration (0.013 ppm) of cadmium, the lipid content was 46.41 and 41.75 mg/g wet tissue, whereas in higher sublethal concentration (0.04 ppm), it was 44.36 and 36.41 mg/g wet tissue for 15 and 30 days of experimental periods (Table 4, Fig.33). The lipid content of ovary drastically decreased in both sublethal concentrations at both the exposure periods. The maximum decline was observed on the 30th day of exposure. The mean decline in lipid content in both the sublethal concentrations were statistically significant (P<0.00001) at both the exposure periods.

3.12.3.2  Spermatheca

The lipid content in the spermatheca tissues of the control crabs of *U. annulipes* was 62.42 and 62.45 mg/g wet tissue for 15 and 30 days of experimental periods respectively (Table 4, Fig.33). In the crabs treated with lower sublethal concentration (0.013 ppm) of cadmium, it was 48.11 and 44.20 mg/g tissue and in higher sublethal concentration (0.04 ppm), it was 45.17 and 39.73 mg/g wet tissues for 15 and 30 days of exposure periods respectively. In cadmium treated crabs, the lipid content drastically decreased at both the exposure periods. The maximum decline was observed on the 30th day in both the sublethal concentrations. The decline in lipid
Table 4: Effect of sublethal concentration of cadmium on lipid content in different tissues of *U. annulipes*

<table>
<thead>
<tr>
<th>Exposure period in days</th>
<th>Tissues</th>
<th>Control</th>
<th>Lower sublethal concentration (0.013 ppm)</th>
<th>Higher sublethal concentration (0.04 ppm)</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Ovary</td>
<td>70.15 ± 5.4</td>
<td>46.41 ± 4.9†</td>
<td>44.36 ± 3.3†</td>
<td>57.3</td>
<td>&lt; 0.00001*</td>
</tr>
<tr>
<td></td>
<td>Spermatheca</td>
<td>62.42 ± 3.8</td>
<td>48.11 ± 4.6†</td>
<td>45.17 ± 2.6†</td>
<td>36.7</td>
<td>&lt; 0.00001*</td>
</tr>
<tr>
<td></td>
<td>Hepatopancreas</td>
<td>59.84 ± 4.3</td>
<td>36.61 ± 2.4†</td>
<td>33.23 ± 3.3†</td>
<td>105.9</td>
<td>&lt; 0.00001*</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>55.37 ± 4.0</td>
<td>40.26 ± 4.8†</td>
<td>37.44 ± 3.9†</td>
<td>30.3</td>
<td>&lt; 0.00001*</td>
</tr>
<tr>
<td></td>
<td>Gill</td>
<td>52.56 ± 2.0</td>
<td>34.18 ± 3.7†</td>
<td>31.25 ± 3.6†</td>
<td>77.9</td>
<td>&lt; 0.00001*</td>
</tr>
<tr>
<td></td>
<td>Haemolymph</td>
<td>50.14 ± 4.4</td>
<td>41.63 ± 2.6†</td>
<td>40.20 ± 4.4†</td>
<td>11.6</td>
<td>0.0009†</td>
</tr>
</tbody>
</table>

| 30                      | Ovary            | 70.20 ± 3.9      | 41.75 ± 3.8†                             | 36.41 ± 5.2†                             | 104.2   | < 0.00001* |
|                         | Spermatheca      | 62.45 ± 3.4      | 44.20 ± 2.9†                             | 39.73 ± 3.0†                             | 90.1    | < 0.00001* |
|                         | Hepatopancreas   | 59.85 ± 3.6      | 29.16 ± 4.6†                             | 25.15 ± 5.0†                             | 108.9   | < 0.00001* |
|                         | Muscle           | 55.39 ± 2.7      | 34.27 ± 3.1†                             | 31.16 ± 3.3†                             | 97.8    | < 0.00001* |
|                         | Gill             | 52.55 ± 2.1      | 29.50 ± 4.7†                             | 25.80 ± 3.2†                             | 104.6   | < 0.00001* |
|                         | Haemolymph       | 50.15 ± 5.5      | 39.61 ± 3.7†                             | 34.31 ± 4.6†                             | 18.1    | 0.0001†  |

Mean ± SD of six individual observations.

Values are expressed mg/g wet tissue and mg/ml haemolymph

*Statistically significant (By one-way analysis of variance)
†Statistically significant (By Duncan's multiple range test)
Fig. 33 Effect of sublethal concentration of cadmium on lipid content in different tissues of *U. annulipes*

**Lower sublethal concentration (0.013 ppm)**

- 15 days
- 30 days

**Higher sublethal concentration (0.04 ppm)**

- 15 days
- 30 days

- Control
- Experimental
content was statistically significant (P<0.00001) in both the sublethal concentrations at both the exposure periods.

3.12.3.3 Hepatopancreas

In control crabs, the mean of lipid content in the hepatopancreas was 59.84 and 59.85 mg/g wet tissue for 15 and 30 days of experimental periods, respectively (Table 4, Fig.33). In the crabs treated with lower sublethal concentration (0.013 ppm) of cadmium, the lipid content was 36.61 and 29.16 mg/g wet tissues and in higher sublethal concentration (0.04 ppm), it was 33.23 and 25.15 mg/g wet tissue for 15 and 30 days of exposure periods respectively. The lipid content declined drastically in both the sublethal concentrations at both the exposure periods. The maximum decrease was observed on the 30th day of experiment in both the sublethal concentrations. The decline in lipid content in both the sublethal concentrations were statistically significant (P<0.00001) at both the experimental periods.

3.12.3.4 Muscle

When *U. annulipes* was treated with lower sublethal concentration (0.013 ppm) of cadmium the lipid content in the muscle was 40.26 and 34.27 mg/g wet tissue and in higher sublethal concentration (0.04 ppm), it was 37.44 and 31.16 mg/g wet tissue for 15 and 30 days of experimental periods. In control crabs, the mean of lipid content in the muscle was 55.37 and 55.39 mg/g wet tissue for 15 and 30 days of exposure
respectively (Table 4, Fig.33). The maximum decrease was observed on the 30th day of experiment in both the sublethal concentrations. The decline in lipid content in both the sublethal concentrations were statistically significant (P<0.0001) at both the exposure periods.

3.12.3.5 Gill

In control crabs of *U. annulipes*, the lipid content in gill tissues were 52.56 and 52.55 mg/g wet tissue for 15 and 30 days of experiment respectively. In the crabs treated with lower sublethal concentration (0.013 ppm) of cadmium, the lipid content was 34.18 and 29.50 mg/g wet tissue and in higher sublethal concentration (0.04 ppm), it was 31.25 and 25.80 mg/g wet tissue for 15 and 30 days of exposure, respectively (Table 4, Fig.33). The maximum decrease was observed on the 30th day of exposure in both the sublethal concentrations. The mean decline in lipid content in cadmium treated crabs were statistically significant (P<0.00001) in both the sublethal concentrations at both the exposure periods.

3.12.3.6 Haemolymph

The lipid content in the haemolymph of the control crabs of *U. annulipes* was 50.14 and 50.15 mg/ml for 15 and 30 days of experimental periods respectively (Table 4, Fig.33). In the crabs treated with lower sublethal concentration (0.013 ppm) of cadmium it was 41.63 and 39.61 mg/ml and in higher sublethal concentration (0.04 ppm) it was 40.20 and 34.31 mg/ml for 15 and 30 days of exposure periods. In cadmium treated
crabs, the lipid content drastically decreased at both the exposure periods respectively. The decline in lipid content was statistically significant in both the sublethal concentrations on 15th day of exposure period (P<0.00001) and 30th day of exposure period.

3.13 Effect of sublethal concentration of cadmium on the enzymatic parameters of *Uca annulipes*

3.13.1 Effect of sublethal concentration of cadmium on succinate dehydrogenase (SDH) activity in different tissues of *U. annulipes*

3.13.1.1 Ovary

The mean of SDH activity in the ovary of the control crabs were 7.38 and 7.40 MIU/min/mg protein, respectively for 15 and 30 days of exposure. In crabs treated with lower sublethal concentration (0.013 ppm) of cadmium, the mean SDH activity was 5.34 and 4.42 MIU/min/mg protein, whereas in higher sublethal concentration (0.04 ppm), it was 4.91 and 3.72 MIU/min/mg protein for 15 and 30 days of exposure periods respectively (Table 5, Fig.34). The decrease in mean SDH activity was statistically significant in both the sublethal concentrations on 15th day of exposure period (P<0.033) and 30th day of exposure period (P<0.00001).

3.13.1.2 Spermatheca

In the control crabs, the mean SDH activity was 10.75 and 10.77 MIU/min/mg protein respectively for 15 and 30 days of exposure
Table 5:  Effect of sublethal concentration of cadmium on succinate dehydrogenase (SDH) activity in different tissues of *U. annulipes*

<table>
<thead>
<tr>
<th>Exposure period in days</th>
<th>Tissues</th>
<th>Control</th>
<th>Lower sublethal concentration (0.013 ppm)</th>
<th>Higher sublethal concentration (0.04 ppm)</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD  †</td>
<td>Mean ± SD  †</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Ovary</td>
<td>7.38 ± 1.2</td>
<td>5.34 ± 1.9  †</td>
<td>4.91 ± 1.4  †</td>
<td>4.31</td>
<td>0.033*</td>
</tr>
<tr>
<td></td>
<td>Spermatheca</td>
<td>10.75 ± 1.5</td>
<td>8.91 ± 1.4  †</td>
<td>7.84 ± 1.4  †</td>
<td>5.8</td>
<td>0.014*</td>
</tr>
<tr>
<td></td>
<td>Hepatopancreas</td>
<td>6.51 ± 2.0</td>
<td>4.20 ± 1.5  †</td>
<td>3.86 ± 0.8  †</td>
<td>5.5</td>
<td>0.016*</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>9.80 ± 2.5</td>
<td>7.62 ± 2.0  †</td>
<td>6.85 ± 0.9  †</td>
<td>3.83</td>
<td>0.04*</td>
</tr>
<tr>
<td></td>
<td>Gill</td>
<td>5.34 ± 1.9</td>
<td>3.74 ± 0.97  †</td>
<td>3.45 ± 1.4</td>
<td>2.75</td>
<td>0.09NS</td>
</tr>
<tr>
<td></td>
<td>Haemolymph</td>
<td>3.72 ± 1.0</td>
<td>3.22 ± 1.4</td>
<td>2.63 ± 1.3</td>
<td>1.2</td>
<td>0.34NS</td>
</tr>
<tr>
<td>30</td>
<td>Ovary</td>
<td>7.40 ± 1.2</td>
<td>4.42 ± 0.6  †</td>
<td>3.72 ± 0.8  †</td>
<td>26.9</td>
<td>&lt; 0.00001*</td>
</tr>
<tr>
<td></td>
<td>Spermatheca</td>
<td>10.77 ± 1.4</td>
<td>7.27 ± 1.6  †</td>
<td>6.80 ± 1.5  †</td>
<td>12.4</td>
<td>0.0007*</td>
</tr>
<tr>
<td></td>
<td>Hepatopancreas</td>
<td>6.55 ± 1.4</td>
<td>3.52 ± 1.0  †</td>
<td>3.02 ± 1.2  †</td>
<td>14.5</td>
<td>0.0003*</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>9.81 ± 1.9</td>
<td>6.21 ± 1.9  †</td>
<td>5.54 ± 1.3  †</td>
<td>10.6</td>
<td>0.0013*</td>
</tr>
<tr>
<td></td>
<td>Gill</td>
<td>5.36 ± 1.5</td>
<td>3.14 ± 1.3  †</td>
<td>2.68 ± 0.6  †</td>
<td>8.33</td>
<td>0.004*</td>
</tr>
<tr>
<td></td>
<td>Haemolymph</td>
<td>3.74 ± 0.7</td>
<td>2.84 ± 1.2  †</td>
<td>1.82 ± 0.4  †</td>
<td>8.17</td>
<td>0.004*</td>
</tr>
</tbody>
</table>

Mean ± SD of six individual observations.
Values are expressed MIU/min/mg protein
*Statistically significant (By one-way analysis of variance)
†Statistically significant (By Duncan’s multiple range test)
NS - Not Significant
Fig. 34 Effect of sublethal concentration of cadmium on succinate dehydrogenase (SDH) activity in different tissues of *U. annulipes*

Lower sublethal concentration (0.013 ppm)

![Graph showing SDH activity in different tissues over 15 and 30 days at a lower sublethal concentration.](image)

Higher sublethal concentration (0.04 ppm)

![Graph showing SDH activity in different tissues over 15 and 30 days at a higher sublethal concentration.](image)
periods. When exposed to lower sublethal concentration (0.013 ppm) of cadmium, the SDH activity was 8.91 and 7.27 MIU/min/mg protein, whereas in higher sublethal concentration (0.04 ppm) it was 7.84 and 6.80 MIU/min/mg protein for 15 and 30 days of exposure periods (Table 5, Fig.34). The decrease in mean SDH activity was statistically significant in both sublethal concentrations on 15th day of exposure period (P<0.014) and 30th day of exposure period (P<0.0007).

3.13.1.3 Hepatopancreas

The *U. annulipes* subjected to lower sublethal concentration (0.013 ppm) of cadmium was analysed for SDH activity which was 4.20 and 3.52 MIU/min/mg protein, whereas in higher sublethal concentration (0.04 ppm) it was 3.86 and 3.02 MIU/min/mg protein for 15 and 30 days of exposure periods respectively (Table 5, Fig.34). In the control crabs the mean SDH activity was 6.51 and 6.55 MIU/min/mg protein for 15 and 30 days of experimental periods. The decrease in enzyme activity was statistically significant in both the sublethal concentrations on 15th day of exposure period (P<0.016) and 30th day of exposure period (P<0.0003).

3.13.1.4 Muscle

In the control crabs, the mean SDH activity in the muscle was 9.80 and 9.81 MIU/min/mg protein respectively for 15 and 30 days of exposure. In the crabs treated with lower sublethal concentration (0.013 ppm) of cadmium, the mean SDH activity was 7.62 and 6.21
MIU/min/mg protein, whereas in higher sublethal concentration (0.04 ppm) it was 6.85 and 5.54 MIU/min/mg protein for 15 and 30 days of exposure periods (Table 5, Fig.34). The decrease in SDH activity in cadmium treated crabs were statistically significant in both the sublethal concentrations on 15th day of exposure period (P<0.04) and 30th day of exposure period (P<0.0013).

3.13.1.5 Gill

In gills of *U. annulipes*, the SDH activity in the crabs treated with lower sublethal concentration (0.013 ppm) of cadmium was recorded as 3.74 and 3.14 MIU/min/mg protein, and in higher sublethal concentration (0.04 ppm) it was 3.45 and 2.68 MIU/min/mg protein for 15 and 30 days of exposure periods respectively (Table 5, Fig.34). In control crabs the mean SDH level in the gills were 5.34 and 5.36 MIU/min/mg protein for 15 and 30 days of experiment periods respectively. The decrease in the mean SDH activity was statistically insignificant (P>0.05) on 15th day of exposure, whereas statistically significant (P<0.004) on 30th day of exposure.

3.13.1.6 Haemolymph

The mean SDH activity in the haemolymph of the control crabs were 3.72 and 3.74 MIU/min/mg protein, respectively for 15 and 30 days of exposure. In crabs treated with lower sublethal concentration (0.013 ppm) of cadmium, the mean SDH activity was 3.22 and 2.84 MIU/min/mg protein, whereas in higher sublethal concentration (0.04 ppm), it was 2.63 and
1.82 MIU/min/mg protein for 15 and 30 days of exposure periods (Table 5, Fig.34). The decrease in mean SDH activity was statistically insignificant (P<0.05) on 15th day of exposure, whereas statistically significant (P<0.004) on 30th day of exposure period.

3.13.2 Effect of sublethal concentration of cadmium on lactate dehydrogenase (LDH) activity in different tissues of *U. annulipes*

3.13.2.1 Ovary

In the ovary of control crabs, the mean LDH activity was 6.30 and 6.32 µg/100 mg respectively for 15 and 30 days of experimental periods. In the crabs treated with lower sublethal concentration (0.013 ppm) of cadmium the LDH activity was 7.85 and 8.19 µg/100 mg, whereas in higher sublethal concentration (0.04 ppm) it was 8.13 and 8.46 µg/100 mg for 15 and 30 days of exposure periods respectively (Table 6, Fig.35). The increase in LDH activity in cadmium treated crabs were statistically insignificant (P>0.05) in both the sublethal concentrations at both exposure periods.

3.13.2.2 Spermatheca

The *U. annulipes* subjected to lower sublethal concentration (0.013 ppm) of cadmium, was analysed for lactate dehydrogenase activity and it was 6.90 and 7.22 µg/100 mg, whereas in higher sublethal concentration (0.04 ppm), it was 7.16 and 7.45 µg/100 mg for 15 and 30 days of exposure periods respectively (Table 6, Fig.35). In the control crabs the
Table 6: Effect of sublethal concentration of cadmium on lactate dehydrogenase (LDH) activity in different tissues of *U. annulipes*

<table>
<thead>
<tr>
<th>Exposure period in days</th>
<th>Tissues</th>
<th>Control</th>
<th>Lower sublethal concentration (0.013 ppm)</th>
<th>Higher sublethal concentration (0.04 ppm)</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ovary</td>
<td>6.30 ± 1.7</td>
<td>7.85 ± 1.4</td>
<td>8.13 ± 1.6</td>
<td>2.27</td>
<td>0.137&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Spermatheca</td>
<td>5.88 ± 1.7</td>
<td>6.90 ± 1.2</td>
<td>7.16 ± 1.6</td>
<td>1.21</td>
<td>0.32&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Hepatopancreas</td>
<td>7.20 ± 1.6</td>
<td>9.31 ± 1.5†</td>
<td>9.61 ± 1.4†</td>
<td>4.50</td>
<td>0.029&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>9.33 ± 2.3</td>
<td>12.45 ± 1.6†</td>
<td>12.82 ± 0.8†</td>
<td>7.5</td>
<td>0.006&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Gill</td>
<td>5.15 ± 1.7</td>
<td>6.53 ± 1.3</td>
<td>6.97 ± 1.5</td>
<td>2.38</td>
<td>0.127&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Haemolymph</td>
<td>2.10 ± 1.4</td>
<td>2.54 ± 0.8</td>
<td>2.75 ± 0.8</td>
<td>0.61</td>
<td>0.55&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Ovary</td>
<td>6.32 ± 1.3</td>
<td>8.19 ± 1.4</td>
<td>8.46 ± 2.0</td>
<td>3.1</td>
<td>0.078&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Spermatheca</td>
<td>5.89 ± 1.0</td>
<td>7.22 ± 1.6</td>
<td>7.45 ± 1.4</td>
<td>2.2</td>
<td>0.14&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Hepatopancreas</td>
<td>7.22 ± 1.1</td>
<td>9.73 ± 1.9†</td>
<td>10.11 ± 1.7†</td>
<td>5.6</td>
<td>0.015&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>9.31 ± 2.3</td>
<td>13.08 ± 2.2†</td>
<td>14.12 ± 2.0†</td>
<td>8.0</td>
<td>0.0043&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Gill</td>
<td>5.17 ± 1.5</td>
<td>6.82 ± 1.0</td>
<td>7.18 ± 1.6</td>
<td>3.5</td>
<td>0.06&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Haemolymph</td>
<td>2.13 ± 1.4</td>
<td>2.82 ± 0.5</td>
<td>3.07 ± 0.8</td>
<td>1.5</td>
<td>0.258&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean ± SD of six individual observations.

Values are expressed µg/100 mg tissue and µg/ml haemolymph

*Statistically significant (By one-way analysis of variance)
†Statistically significant (By Duncan’s multiple range test)

NS - Not Significant
Fig. 35 Effect of sublethal concentration of cadmium on lactate dehydrogenase (LDH) activity in different tissues of *U. annulipes*

**Lower sublethal concentration (0.013 ppm)**

- **15 days**
- **30 days**

- Ovary
- Spermatheca
- Hepatopancreas
- Muscle
- Gill
- Haemolymph

**Higher sublethal concentration (0.04 ppm)**

- **15 days**
- **30 days**

- Ovary
- Spermatheca
- Hepatopancreas
- Muscle
- Gill
- Haemolymph

- Control
- Experimental
lactate dehydrogenase activity was 5.88 and 5.89 µg/100 mg protein for 15 and 30 days of experimental periods. The increase in enzyme activity was statistically insignificant (P>0.05) in both the sublethal concentrations, at both exposure period.

3.13.2.3 Hepatopancreas

The mean LDH activity in the hepatopancreas of the control crabs were 7.20 and 7.22 µg/100 mg respectively for 15 and 30 days of experimental periods. In the crabs treated with lower sublethal concentration (0.013 ppm) of cadmium, the LDH activity was 9.31 and 9.73 µg/100 mg, whereas in higher sublethal concentration (0.04 ppm) it was 9.61 and 10.11 µg/100 mg for 15 and 30 days of exposure periods (Table 6, Fig.35). The increase in LDH activity was statistically significant in both sublethal concentrations on 15th day of exposure period (P<0.029) and 30th day of exposure period (P<0.015).

3.13.2.4 Muscle

The lactate dehydrogenase content in the muscle of the control crabs were 9.33 and 9.31 µg/100 mg for 15 and 30 days of experimental periods. In the crabs treated with lower sublethal concentration (0.013 ppm) of cadmium, the LDH content was 12.45 and 13.08 µg/100 mg and in higher sublethal concentration (0.04 ppm) it was 12.82 and 14.12 µg/100 mg for 15 and 30 days of exposure periods (Table 6, Fig.35). The result clearly indicated that the LDH content was relatively high in the experimental
groups, when compared to the control. The increase in LDH content was statistically significant in both the sublethal concentrations (P<0.006) on 15th day of exposure and (P<0.0043) on 30th day of exposure period.

3.13.2.5 Gill

The LDH activity in the crabs treated with lower sublethal concentration (0.013 ppm) of cadmium was recorded as 6.53 and 6.82 μg/100 mg and in higher sublethal concentration (0.04 ppm) it was 6.97 and 7.18 μg/100 mg for 15 and 30 days of exposure periods respectively. In control crabs the LDH activity in the gills were 5.15 and 5.17 μg/100 mg for 15 and 30 days of experimental periods. The increase in the LDH activity was statistically insignificant (P>0.05) in both the sublethal concentrations at both the exposure periods (Table 6, Fig.35).

3.13.2.6 Haemolymph

The U. annulipes subjected to lower sublethal concentration (0.013 ppm) of cadmium, was analysed for LDH activity in the haemolymph and it was 2.54 and 2.82 μg/ml, whereas in higher sublethal concentration (0.04 ppm), it was 2.75 and 3.07 μg/ml for 15 and 30 days of exposure periods respectively (Table 6, Fig.35). In the control crabs the LDH activity was 2.10 and 2.13 μg/ml for 15 and 30 days of experimental periods. The increase of enzyme activity in cadmium treated crabs were statistically insignificant (P>0.05) in both the sublethal concentrations at both the exposure periods.
3.13.3 Effect of sublethal concentration of cadmium on acid phosphatase activity in different tissues of *U. annulipes*

3.13.3.1 Ovary

In control crabs, the mean acid phosphatase level in ovary was 8.51 and 8.53 µg PNPP to PNP/100 mg for 15 and 30 days of exposure periods respectively (Table 7, Fig.36). In the crabs treated with lower sublethal concentration (0.013 ppm) of cadmium, the acid phosphatase levels in the ovary was 10.60 and 11.16 µg PNPP to PNP/100 mg and in higher sublethal concentration (0.04 ppm), it was 11.02 and 12.01 µg PNPP to PNP/100 mg for 15 and 30 days of exposure periods respectively. The increase in the enzyme acid phosphatase activity was statistically significant in both the sublethal concentrations on 15th day of exposure period (P<0.036) and 30th day of exposure period (P<0.016).

3.13.3.2 Spermatheca

The mean acid phosphatase activity in the spermatheca of the control crabs were 4.35 and 4.38 µg PNPP to PNP/100 mg for 15 and 30 days of experimental periods respectively (Table 7, Fig.36). In the crabs treated with lower sublethal concentration (0.013 ppm) of cadmium, the mean acid phosphatase activity was 5.11 and 5.18 µg PNPP to PNP/100 mg and in higher sublethal concentration (0.04 ppm), it was 5.15 and 5.94 µg PNPP to PNP/100 mg for 15 and 30 days of experimental periods respectively. The result clearly indicated that the acid phosphatase activity was relatively high in the experimental groups, when compared to the
Table 7: Effect of sublethal concentration of cadmium on acid phosphatase (ACP) activity in different tissues of *U. annulipes*

<table>
<thead>
<tr>
<th>Exposure period in days</th>
<th>Tissues</th>
<th>Control</th>
<th>Lower sublethal concentration (0.013 ppm)</th>
<th>Higher sublethal concentration (0.04 ppm)</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Ovary</td>
<td>8.51 ± 1.5</td>
<td>10.60 ± 1.7†</td>
<td>11.02 ± 1.6†</td>
<td>4.19</td>
<td>0.036atura</td>
</tr>
<tr>
<td></td>
<td>Spermatheca</td>
<td>4.35 ± 1.4</td>
<td>5.11 ± 1.8</td>
<td>5.15 ± 1.9</td>
<td>0.41</td>
<td>0.67NS</td>
</tr>
<tr>
<td></td>
<td>Hepatopancreas</td>
<td>5.08 ± 1.5</td>
<td>6.62 ± 1.2</td>
<td>6.84 ± 1.0</td>
<td>3.45</td>
<td>0.058NS</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>3.22 ± 0.8</td>
<td>3.87 ± 0.6</td>
<td>4.05 ± 0.7</td>
<td>2.52</td>
<td>0.114NS</td>
</tr>
<tr>
<td></td>
<td>Gill</td>
<td>4.38 ± 1.8</td>
<td>5.56 ± 1.4</td>
<td>5.75 ± 0.9</td>
<td>1.64</td>
<td>0.23NS</td>
</tr>
<tr>
<td></td>
<td>Haemolymph</td>
<td>2.04 ± 0.97</td>
<td>2.80 ± 0.4†</td>
<td>3.08 ± 0.3†</td>
<td>4.47</td>
<td>0.03†</td>
</tr>
<tr>
<td>30</td>
<td>Ovary</td>
<td>8.53 ± 1.2</td>
<td>11.16 ± 2.4†</td>
<td>12.01 ± 1.8†</td>
<td>5.54</td>
<td>0.016atura</td>
</tr>
<tr>
<td></td>
<td>Spermatheca</td>
<td>4.38 ± 0.8</td>
<td>5.18 ± 1.6</td>
<td>5.94 ± 1.1</td>
<td>2.4</td>
<td>0.126NS</td>
</tr>
<tr>
<td></td>
<td>Hepatopancreas</td>
<td>5.10 ± 1.8</td>
<td>7.01 ± 1.5</td>
<td>7.42 ± 1.6</td>
<td>3.42</td>
<td>0.06NS</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>3.25 ± 1.2</td>
<td>4.10 ± 1.0</td>
<td>4.46 ± 1.1</td>
<td>3.47</td>
<td>0.06NS</td>
</tr>
<tr>
<td></td>
<td>Gill</td>
<td>4.40 ± 0.8</td>
<td>5.90 ± 1.2</td>
<td>6.25 ± 1.5</td>
<td>1.55</td>
<td>0.29NS</td>
</tr>
<tr>
<td></td>
<td>Haemolymph</td>
<td>2.06 ± 0.8</td>
<td>3.11 ± 0.73†</td>
<td>3.58 ± 0.7†</td>
<td>9.48</td>
<td>0.002†</td>
</tr>
</tbody>
</table>

Mean ± SD of six individual observations.
Values are expressed µg of PNPP to PNP/100 mg tissue.
µg of PNPP to PNP/ml haemolymph.
†Statistically significant (By one-way analysis of variance)
*Statistically significant (By Duncan's multiple range test)
NS - Not Significant
Fig. 36 Effect of sublethal concentration of cadmium on acid phosphatase (ACP) activity in different tissues of *U. annulipes*

**Lower sublethal concentration (0.013 ppm)**

- **15 days**
- **30 days**

**Higher sublethal concentration (0.04 ppm)**

- **15 days**
- **30 days**

**Control** □  □**Experimental**
control. The increase in acid phosphatase activity was statistically insignificant (P>0.05) in both the sublethal concentrations at both the exposure periods.

3.13.3.3 Hepatopancreas

The *U. annulipes* subjected to lower sublethal concentration (0.013 ppm) of cadmium was analysed for acid phosphatase activity and it was 6.62 and 7.01 μg PNPP to PNP/100 mg, whereas in higher sublethal concentration (0.04 ppm), it was 6.84 and 7.42 μg PNPP to PNP/100 mg for 15 and 30 days of exposure periods respectively (Table 7, Fig.36). In the control crabs, the mean acid phosphatase activity was 5.08 and 5.10 μg PNPP to PNP/100 mg for 15 and 30 days of experimental periods. The increase in enzyme activity was statistically insignificant (P>0.05) in both the sublethal concentrations at both exposure periods.

3.13.3.4 Muscle

The acid phosphatase activity in the crabs treated with lower sublethal concentration (0.013 ppm) of cadmium was recorded as 3.87 and 4.10 μg PNPP to PNP/100 mg and in higher sublethal concentration (0.04 pm) it was 6.84 and 4.46 μg PNPP to PNP/100 mg for 15 and 30 days of exposure periods respectively (Table 7, Fig.36). In control crabs, the acid phosphatase activity in the muscle was 3.22 and 3.25 μg PNPP to PNP/100 mg for 15 and 30 days of experimental periods respectively. The
increase in the acid phosphatase activity was statistically insignificant (P>0.05) in both the sublethal concentrations at both the exposure periods.

3.13.3.5 Gill

In control crabs, the mean acid phosphatase activity in the gill tissues were 4.38 and 4.40 μg PNP to PNP/100 mg for 15 and 30 days of exposure. In crabs treated with lower sublethal concentration (0.013 ppm) of cadmium, the mean acid phosphatase activity was 5.56 and 5.90 μg PNPP to PNP/100 mg, whereas in higher sublethal concentration (0.04 ppm) it was 5.75 and 6.25 μg PNPP to PNP/100 mg for 15 and 30 days of exposure periods (Table 7, Fig.36). The increase in enzyme activity was statistically insignificant (P>0.05) in both the sublethal concentrations at both the exposure periods.

3.13.3.6 Haemolymph

The mean acid phosphatase activity in the haemolymph of the control crabs were 2.04 and 2.06 μg PNPP to PNP/ml for 15 and 30 days of experimental periods respectively (Table 7, Fig.36). In the crabs treated with lower sublethal concentration (0.013 ppm) of cadmium, the mean acid phosphatase activity was 2.80 and 3.11 μg PNPP to PNP/ml and in higher sublethal concentration (0.04 ppm) it was 3.08 and 3.58 μg PNPP to PNP/ml for 15 and 30 days of experimental periods respectively. The increase in mean acid phosphatase activity was statistically significant in both the
sublethal concentrations on 15th day of exposure period (P<0.03) and 30th day of exposure period (P<0.002).

3.13.4 Effect of sublethal concentration of cadmium on alkaline phosphatase activity in different tissues of *U. annulipes*

3.13.4.1 Ovary

The crab *U. annulipes* subjected to lower sublethal concentration (0.013 ppm) of cadmium was analysed for alkaline phosphatase activity and it was 5.48 and 4.98 µg PNPP to PNP/100 mg, whereas in higher sublethal concentration (0.04 ppm), it was 5.20 and 4.65 µg PNPP to PNP/100 mg for 15 and 30 days of exposure periods respectively (Table 8, Fig.37). In the control crabs, the mean acid phosphatase activity was 6.30 and 6.32 µg PNPP to PNP/100 mg for 15 and 30 days of exposure periods respectively. The decrease in alkaline phosphatase activity was statistically significant (P<0.0174) in both the sublethal concentrations on 15th day of exposure, whereas statistically insignificant (P>0.05) on 30th day of exposure.

3.13.4.2 Spermatheca

The mean alkaline phosphatase activity in the spermatheca of the control crabs were 2.55 and 2.56 µg PNPP to PNP/100 mg for 15 and 30 days of experimental periods respectively (Table 8, Fig.37). In the crabs treated with lower sublethal concentration (0.013 ppm) of cadmium, the mean alkaline phosphatase activity was 2.28 and 2.16 µg PNPP to PNP/100 mg and in higher sublethal concentration (0.04 ppm), it was 2.22 and
Table 8: Effect of sublethal concentration of cadmium on alkaline phosphatase (ALP) activity in different tissues of *U. annulipes*

<table>
<thead>
<tr>
<th>Exposure period in days</th>
<th>Tissues</th>
<th>Control</th>
<th>Lower sublethal concentration (0.013 ppm)</th>
<th>Higher sublethal concentration (0.04 ppm)</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Ovary</td>
<td>6.30 ± 1.4</td>
<td>5.48 ± 1.8†</td>
<td>5.20 ± 2.3†</td>
<td>5.37</td>
<td>0.0174*</td>
</tr>
<tr>
<td></td>
<td>Spermatheca</td>
<td>2.55 ± 0.6</td>
<td>2.28 ± 1.3</td>
<td>2.22 ± 0.7</td>
<td>0.20</td>
<td>0.82NS</td>
</tr>
<tr>
<td></td>
<td>Hepatopancreas</td>
<td>4.83 ± 1.1</td>
<td>3.95 ± 1.0</td>
<td>3.60 ± 1.3</td>
<td>1.82</td>
<td>0.196NS</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>4.37 ± 1.6</td>
<td>3.88 ± 0.5</td>
<td>3.64 ± 1.2</td>
<td>0.56</td>
<td>0.58NS</td>
</tr>
<tr>
<td></td>
<td>Gill</td>
<td>4.73 ± 1.2</td>
<td>3.94 ± 0.7</td>
<td>3.70 ± 0.9</td>
<td>1.73</td>
<td>0.21NS</td>
</tr>
<tr>
<td></td>
<td>Haemolymph</td>
<td>2.85 ± 0.9</td>
<td>2.31 ± 1.4</td>
<td>2.22 ± 1.0</td>
<td>0.54</td>
<td>0.59NS</td>
</tr>
<tr>
<td>30</td>
<td>Ovary</td>
<td>6.32 ± 1.9</td>
<td>4.98 ± 0.9</td>
<td>4.65 ± 0.7</td>
<td>3.3</td>
<td>0.06NS</td>
</tr>
<tr>
<td></td>
<td>Spermatheca</td>
<td>2.56 ± 0.8</td>
<td>2.16 ± 0.8</td>
<td>2.13 ± 0.8</td>
<td>0.51</td>
<td>0.61NS</td>
</tr>
<tr>
<td></td>
<td>Hepatopancreas</td>
<td>4.84 ± 1.0</td>
<td>3.34 ± 0.9†</td>
<td>2.82 ± 0.5†</td>
<td>8.93</td>
<td>0.003*</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>4.39 ± 1.5</td>
<td>3.60 ± 1.0</td>
<td>3.36 ± 0.8</td>
<td>2.2</td>
<td>0.143NS</td>
</tr>
<tr>
<td></td>
<td>Gill</td>
<td>4.78 ± 1.1</td>
<td>3.50 ± 1.6†</td>
<td>2.99 ± 0.2†</td>
<td>3.9</td>
<td>0.041*</td>
</tr>
<tr>
<td></td>
<td>Haemolymph</td>
<td>2.88 ± 0.5</td>
<td>2.16 ± 1.2</td>
<td>1.90 ± 0.3</td>
<td>2.4</td>
<td>0.12NS</td>
</tr>
</tbody>
</table>

Mean ± SD of six individual observations.
Values are expressed μg of PNPP to PNP/100 mg tissue.
μg of PNPP to PNP/ml haemolymph.
*Statistically significant (By one-way analysis of variance)
†Statistically significant (By Duncan's multiple range test)
NS - Not Significant
Fig. 37 Effect of sublethal concentration of cadmium on alkaline phosphatase (ALP) activity in different tissues of *U. annulipes*

**Lower sublethal concentration (0.013 ppm)**

**Higher sublethal concentration (0.04 ppm)**
2.13 μg PNPP to PNP/100 mg for 15 and 30 days of exposure periods respectively. The decrease in alkaline phosphatase activity was statistically insignificant (P>0.05) in both the sublethal concentrations at both the exposure periods.

### 3.13.4.3 Hepatopancreas

The alkaline phosphatase activity in the crabs treated with lower sublethal concentration (0.013 ppm) of cadmium was recorded as 3.95 and 3.34 μg PNPP to PNP/100 mg and in higher sublethal concentration (0.04 ppm) it was 3.60 and 2.82 μg PNPP to PNP/100 mg for 15 and 30 days of exposure periods respectively (Table 8, Fig.37). In control crabs, the alkaline phosphatase activity in the hepatopancreas was 4.83 and 4.84 μg PNPP to PNP/100 mg for 15 and 30 days of experimental periods respectively. The decline in alkaline phosphatase activity in cadmium treated crabs were statistically insignificant (P>0.05) in both sublethal concentrations on 15th day of exposure, whereas statistically significant (P<0.003) on 30th day of exposure.

### 3.13.4.4 Muscle

In control crabs, the mean alkaline phosphatase activity in muscle was 4.37 and 4.39 μg PNPP to PNP/100 mg for 15 and 30 days of exposure periods respectively (Table 8, Fig.37). In the crabs treated with lower sublethal concentration (0.013 ppm) of cadmium, the alkaline phosphatase activity in the muscle was 3.88 and 3.60 μg PNPP to PNP/100 mg and in
higher sublethal concentration (0.04 ppm), it was 3.64 and 3.36 µg PNPP to PNP/100 mg for 15 and 30 days of exposure periods respectively. The decrease in enzyme activity was statistically insignificant (P>0.05) in both the sublethal concentrations at both the exposure periods.

3.13.4.5 Gill

The *U. annulipes* subjected to lower sublethal concentration (0.013 ppm) of cadmium, was analysed for alkaline phosphatase activity and it was 3.94 and 3.50 µg PNPP to PNP/100 mg, whereas in higher sublethal concentration (0.04 ppm), it was 3.70 and 2.99 µg PNPP to PNP/100 mg for 15 and 30 days of exposure periods respectively (Table 8, Fig.37). In the control crabs the mean alkaline phosphatase activity was 4.73 and 4.78 µg PNPP to PNP/100 mg for 15 and 30 days of exposure periods respectively. The decrease in enzyme alkaline phosphatase activity was statistically insignificant (P>0.05) in both the sublethal concentrations on 15th day of exposure, whereas statistically significant (P<0.04) on 30th day of exposure.

3.13.4.6 Haemolymph

In control crabs, the mean alkaline phosphatase activity in the haemolymph was 2.85 and 2.88 µg PNPP to PNP/ml for 15 and 30 days of exposure. In crabs treated with lower sublethal concentration (0.013 ppm) of cadmium the mean alkaline phosphatase activity was 2.31 and 2.16 µg/PNPP to PNP/ml, whereas in higher sublethal concentration (0.04 ppm) it was 2.22 and 1.90 µg/PNPP to PNP/ml for 15 and 30 days of exposure
periods (Table 8, Fig.37). The decrease in alkaline phosphatase activity was statistically insignificant (P>0.05) in both the sublethal concentrations at both the exposure periods.

3.14 **Bioaccumulation of cadmium in different tissues of* U. annulipes **exposed to sublethal concentrations

The accumulation of cadmium in different tissues namely ovary, spermatheca, hepatopancreas, muscle, gill and haemolymph of *U. annulipes* exposed to lower sublethal concentration (0.013 µg/l) and higher sublethal concentration (0.04 µg/l) after long term exposure (15 and 30 days) are given in Table 9, Fig.38.

3.14.1 **Ovary**

The accumulation of cadmium in ovary showed a gradual increase in lower and higher sublethal concentration (0.013 and 0.04 µg/l), after 15 and 30 days. In the crabs treated with lower sublethal concentration (0.013 µg/l), the accumulation of cadmium recorded in ovary was 5.92 and 7.33 µg/g wet weight of tissue, whereas in higher sublethal concentration (0.04 µg/l), it was 8.51 and 11.28 µg/g wet weight of tissue for 15 and 30 days exposure periods respectively (Table 9, Fig.38). In control crabs the mean accumulation of cadmium in ovary was 3.40 and 3.48 µg/g wet weight of tissue for 15 and 30 days of experimental periods. The increase in cadmium content was statistically significant (P<0.00001) in both the sublethal concentrations at both the exposure periods.
Table 9: Bioaccumulation of cadmium in different tissues of *U. annulipes* exposed to sublethal concentrations

<table>
<thead>
<tr>
<th>Exposure period in days</th>
<th>Tissues</th>
<th>Control</th>
<th>Lower sublethal concentration (0.013 μg/l)</th>
<th>Higher sublethal concentration (0.04 μg/l)</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Ovary</td>
<td>3.40 ± 0.6</td>
<td>5.92 ± 1.0†</td>
<td>8.51 ± 0.07†</td>
<td>59.1</td>
<td>&lt; 0.00001*</td>
</tr>
<tr>
<td></td>
<td>Spermatheca</td>
<td>2.02 ± 0.2</td>
<td>2.83 ± 0.6†</td>
<td>4.53 ± 0.04†</td>
<td>47.0</td>
<td>&lt; 0.00001*</td>
</tr>
<tr>
<td></td>
<td>Hepatopancreas</td>
<td>21.42 ± 5.2</td>
<td>85.10 ± 6.2†</td>
<td>105.66 ± 3.2†</td>
<td>474.4</td>
<td>&lt; 0.00001*</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>4.14 ± 1.2</td>
<td>7.80 ± 1.2†</td>
<td>10.26 ± 2.4†</td>
<td>19.85</td>
<td>&lt; 0.0001*</td>
</tr>
<tr>
<td></td>
<td>Gill</td>
<td>15.25 ± 5.8</td>
<td>70.92 ± 3.9†</td>
<td>86.27 ± 6.7†</td>
<td>338.7</td>
<td>&lt; 0.0001*</td>
</tr>
<tr>
<td></td>
<td>Haemolymph</td>
<td>0.22 ± 0.07</td>
<td>0.36 ± 0.05†</td>
<td>0.44 ± 0.04†</td>
<td>26.6</td>
<td>&lt; 0.00001*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>Ovary</td>
<td>3.48 ± 0.44</td>
<td>7.33 ± 1.5†</td>
<td>11.28 ± 1.8†</td>
<td>49.3</td>
<td>&lt; 0.00001*</td>
</tr>
<tr>
<td></td>
<td>Spermatheca</td>
<td>2.10 ± 0.17</td>
<td>4.24 ± 0.8†</td>
<td>6.94 ± 0.09†</td>
<td>77.1</td>
<td>&lt; 0.00001*</td>
</tr>
<tr>
<td></td>
<td>Hepatopancreas</td>
<td>21.70 ± 4.3</td>
<td>124.50 ± 4.8†</td>
<td>147.35 ± 5.6†</td>
<td>1106.81</td>
<td>&lt; 0.00001*</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>4.23 ± 1.0</td>
<td>8.81 ± 0.31†</td>
<td>13.39 ± 2.6†</td>
<td>47.6</td>
<td>&lt; 0.0001*</td>
</tr>
<tr>
<td></td>
<td>Gill</td>
<td>15.41 ± 3.5</td>
<td>96.42 ± 3.6†</td>
<td>130.70 ± 5.9†</td>
<td>1070.8</td>
<td>&lt; 0.00001*</td>
</tr>
<tr>
<td></td>
<td>Haemolymph</td>
<td>0.24 ± 0.05</td>
<td>0.51 ± 0.07†</td>
<td>0.78 ± 0.05†</td>
<td>118.9</td>
<td>&lt; 0.00001*</td>
</tr>
</tbody>
</table>

Mean ± SD of six individual observations.
Values are expressed μg/g wet tissue and μg/ml haemolymph.
*Statistically significant (By one-way analysis of variance)
†Statistically significant (By Duncan’s multiple range test)
Fig. 38 Bioaccumulation of cadmium in different tissues of *U. annulipes* exposed to sublethal concentrations

**Lower sublethal concentration (0.013 ppm)**

- **15 days**
- **30 days**

![Graph showing bioaccumulation in different tissues](image)

**Higher sublethal concentration (0.04 ppm)**

- **15 days**
- **30 days**

![Graph showing bioaccumulation in different tissues](image)
3.14.2 Spermatheca

The accumulation of cadmium in spermatheca showed a gradual increase when compared with the control, after 15 and 30 days of exposure period. In the crabs treated with lower sublethal concentration (0.013 µg/l) of cadmium, the accumulation of cadmium was 2.83 and 4.24 µg/g wet weight of tissues, whereas in higher sublethal concentration (0.04 µg/l) it was 4.53 and 6.94 µg/g wet weight of tissue for 15 and 30 days of exposure period respectively (Table 9, Fig.38). On the other hand, in the control crabs the cadmium accumulation was 2.02 and 2.10 µg/g wet weight of tissue for 15 and 30 days of experimental periods. The increase in cadmium content was statistically significant (P<0.00001) in both the sublethal concentrations at both the exposure periods.

3.14.3 Hepatopancreas

The mean of cadmium accumulation in the hepatopancreas tissues of control crabs, *U. annulipes* were 21.42 and 21.70 µg/g wet weight of tissues for 15 and 30 days of experimental periods respectively (Table 9, Fig.38). In the crabs treated with lower sublethal concentration (0.013 µg/l) of cadmium, the accumulation of cadmium was 85.10 and 124.50 µg/g wet weight of tissue, whereas in higher sublethal concentration (0.04 µg/l) it was 105.66 and 147.35 µg/g wet weight of tissue for 15 and 30 days of exposure periods respectively. The accumulation of cadmium content in both the sublethal concentrations were statistically significant (P<0.00001) at both the exposure periods.
3.14.4 Muscle

In control crabs the mean cadmium accumulation in muscle tissues were 4.14 and 4.23 µg/g wet weight of tissue for 15 and 30 days. In the crabs exposed to lower sublethal concentration (0.013 µg/l) of cadmium, the accumulation of cadmium was 7.80 and 8.81 µg/g wet weight of tissue, whereas in higher sublethal concentration (0.04 µg/l) it was 10.26 and 13.39 µg/g wet weight of tissue for 15 and 30 days of exposure periods respectively (Table 9, Fig.38). The accumulation of cadmium content in both the sublethal concentrations were statistically significant (P<0.0001) at both the exposure periods.

3.14.5 Gill

The accumulation of cadmium in gill tissues of control crabs, *U. annulipes* were 15.25 and 15.41 µg/g wet weight of tissues for 15 and 30 days of experimental periods respectively (Table 9, Fig.38). In the crabs treated with lower sublethal concentration (0.013 µg/l) of cadmium, the accumulation of cadmium was 70.92 and 96.42 µg/g wet weight of tissue, whereas in higher sublethal concentration (0.04 µg/l) it was 86.27 and 130.70 µg/g wet weight of tissue for 15 and 30 days of exposure periods respectively. The increase in cadmium content was statistically significant (P<0.00001) in both the sublethal concentrations at both the exposure periods.
3.14.6 Haemolymph

The mean cadmium accumulation in the haemolymph of control crabs, *U. annulipes* were 0.22 µg/ml and 0.24 µg/ml for 15 and 30 days of experimental periods respectively (Table 9, Fig.38). In the crabs treated with lower sublethal concentration (0.013 µg/l) of cadmium, the accumulation of cadmium was 0.36 and 0.51 µg/ml, whereas in higher sublethal concentration (0.04 µg/l) it was 0.44 and 0.78 µg/ml for 15 and 30 days of exposure periods respectively. The accumulation of cadmium content in both the sublethal concentrations were statistically significant (*P*<0.00001) at both the exposure periods.