CHAPTER V

Effects of pollutants on the gonads and hypothalamo-hypophysial neurosecretory system of *Nandus nandus* (Ham.).
Introduction:

Progressive development in the field of mining and industries led to the pollution of rivers by heavy metal salts such as lead, zinc, copper, mercury, cadmium, nickel, etc. through various sources. These are harmful to fish either directly or indirectly by depleting dissolved oxygen, changing carbon dioxide level and significantly increasing or decreasing the pH value of the water. The water polluted with the heavy metal salts upsets the normal metabolism of the fish. Lead is present in the effluents associated with the manufacture of accumulators and lead paints. Zinc is present in the effluents associated with the manufacture of rubber, the process of zinc plating and galvanizing whereas copper is present in effluents associated with the mines and trade waste. The toxic effects of the salts of these metals (i.e. lead nitrate, zinc sulphate and copper sulphate) on the physiology of fish respiration has long been recognised. Important contributions in this field are made by Carpenter (1924, 1925, 1926 and 1930), Dilling et al. (1928), Jones (1938 a, b, 1939, 1940, 1947 and 1958), Affleck (1952), Sudoroff (1952), Lloyd (1960), Crandall and Goodnight (1963), Skidmore (1964, 1965 and 1966), Brungs (1969), Gardiner and Yevich (1970), Pickering and Jast (1972), Smith (1973), Brown et al. (1974), Jinley et al. (1974) Davies et al.
(1976), Holcombe et al. (1976) and Pascoe and Gram (1977).

Dudoroff and Katz (1953), Jones (1964) and Nakim et al. (1973,
1974, 1975, 1976) have reviewed the literature on this subject.

Very little work has been done to investigate the effects
of heavy metal salts on fish reproduction (Affleck, 1952;
Grantall and Goodnight, 1963; Skidmore, 1966, Khosa and
Since copper sulphate is used as algicide in reservoirs and
lakes, it becomes more important from the viewpoint of
fisheries development also to investigate its effect on the
physiology of reproduction.

Khosa and Chandrasekhar have observed the effects of
copper acetate and asphadl on preoptic nucleus of Clarias
batrachus and Ophiocephalus punctatus. Sahari and Sahari (1979)
have seen effects of mercury (heavy metal) on the preoptic-
neurohypophysial system of fresh water fish, Heteronemus
fossiliis. The perusal of available literature shows that no
other work has been done on the effect of heavy metal salts on
the hypothalamic-neurohypophysial system of fishes. In the
present study, effects of lead nitrate (\(\text{Pb(NO}_3\text{)}_2\)), zinc sulphate
(\(\text{ZnSO}_4\cdot\text{H}_2\text{O}\)) and copper sulphate (\(\text{CuSO}_4\cdot\text{H}_2\text{O}\)) have been observed
on the gonads and the hypothalamic-hypophysial system (nucleus
preopticus, nucleus lateralis tuberis and the pituitary gland).
Materials and Methods:

To study the effects of pollutants (heavy metal salts) on the gonads and the hypothalamo-hypophyseal system, experiments were conducted in two phases. In the first phase of experiment, lethal concentration and sub-lethal concentration of the pollutants was calculated whereas in the second phase of experiment, the fishes were kept in sub-lethal concentration of pollutants over a period of two months.

For the first phase of experiment, hundred adult *Harmaja nandus*, ranging between 10 cm to 13 cm in length, were caught in the month of November, 1978 and were acclimatized in different aquaria for seven days at a temperature of 20 ± 3°C. The fishes were kept in different strong concentrations of pollutants and their overturning time (i.e. the time at which the fish looses its sense of balance and floats on its side or upside down) was noted which is 14 hrs. in 20 p.p.m. copper sulphate, 18 hrs. in 100 p.p.m. zinc sulphate and 20 hrs. in 200 p.p.m. lead nitrate solutions. Keeping these observations in mind rest of the fishes were divided into different grades of more diluted solutions of pollutants and their survival time was noted.

It has been found that at sub-lethal concentrations of 1 p.p.m. of copper sulphate, 10 p.p.m. of zinc sulphate and 20 p.p.m. of lead nitrate, fishes survive for a long period of more than two months and as such these concentrations were taken for the second phase of experiments.
During the second phase of experiment, forty five adult male and forty five adult female \textit{Lates calcarifer} from the catch of August, 1979 (spawning season) were acclimatized in the aquaria (measuring 90 cm x 36 cm x 36 cm) for seven days at a temperature of \(28 \pm 2^\circ\text{C}\). As the fishes were caught in the middle of spawning period, they were easily differentiated sexually. Throughout the experiment, tap water stored in earthen pots (at least for 24 hrs.) was used. Five males and five females were sacrificed at the onset of experiment and the brain and gonads were fixed in Aqueous Bouin and Hollande's modified Bouin. The length of the fish, body weight, gonad weight and gonad volume was also recorded. Ten males and ten females were kept as control and rest of the thirty males and thirty females were separated in six aquaria (each having either five males or five females) in above mentioned concentrations of different pollutants. The solutions of the pollutants were changed daily so as to maintain the level of concentration. The fishes were aerated properly and were fed on fresh earthworm pieces on alternate days. The fishes from different experimental groups were sacrificed either on 30th day or on 60th day of experiment. The number of fishes sacrificed from each group along with their length, body weight, gonad weight and gonad volume is shown in Table 7, p. 231 and Table 8, p. 232.

The sections of the brain and gonads were cut at 5 \(\mu\) and 6 \(\mu\) respectively. The sections of the brain were stained in Aldehyde fuchsin and Chrome alum Haematoxylin Phloxine and
those of gonads were stained in Mallorl's triple stain and Ehrlich's acid-alum Haematoxylin eosin stains.

Observations:

A. Ovaries

(i) Ovaries of normal (initial control) fishes:

The ovaries of the fishes of initial control are covered over by a thin layer of peritoneum. The ovarian wall is thin and the ovigerous lamellae are not distinct. The ovary is packed with mature oocytes. A few early stages of oogenesis are also present in between the mature ones. The corpora atretica are very few which are in their early stages of absorption (Fig. 114; p. 180)

(ii) Ovaries of 30 days control fishes:

The ovaries of 30 days control fishes do not show marked changes from those of normal fishes except that the corpora atretica are present in their later stages of absorption (Fig. 115; p. 189).

(iii) Ovaries of 30 days pollutant treated fishes:

In lead nitrate treated fishes, the ovarian wall is thin and the ovigerous lamellae are not distinct. All the stages of oogenesis (i.e. from early chromatin nucleolus stage...
Photomicrograph of Transverse section of the:

**Fig. 114:** Ovary of initial control *Nandus nandus* (Ehrlich's haematoxylin eosin)

**Fig. 116:** Ovary of 30 days control *Nandus nandus* (Mallory triple).

**Fig. 116:** Ovary of *Nandus nandus* treated with lead nitrate (20 p.p.m. soln.) for 30 days (Ehrlich's haematoxylin eosin)

**Fig. 117:** Ovary of *Nandus nandus* treated with zinc sulphate (10 p.p.m. soln.) for 30 days (Ehrlich's haematoxylin eosin).

**Abbreviations:**

CA.I. - Corpora atretica in early stage of absorption.

CA.II. - Corpora atretica in later stage of absorption.

L.PN. - Late peri-nucleolus stage

LY. - Late yolk stage.

L.YV. - Late yolk-vesicle stage.

M.S. - Mature stage.
to mature stage) are present. With the effect of lead nitrate, a few oocytes of later stages have undergone atresia and as such the number of corpora atretica is more from those in the ovaries of 30 days control fishes (Fig. 116; p. 189). In zinc sulphate and copper sulphate treated fishes, the ovarian wall is thick and the ovigerous lamellae are distinct. Oocytes of chromatin nucleolus and perinucleolus stages are many whereas those of early yolk-vesicle stages are few. All the other later stages of oogenesis are not present. In zinc sulphate treated fishes, the number of corpora atretica is further increased which are mostly in their early stages of absorption (Fig. 117; p. 189). In copper sulphate treated fishes most of the corpora atretica are absorbed in the stroma of the ovary and very few are present in the last stage of their absorption (Fig. 118; p. 192).

(iv) Ovaries of 60 days control fishes:

In the ovaries of 60 days control fishes the number of mature oocytes is decreased. Many corpora atretica are found in various stages of their absorption (Fig. 119; p. 192).

(v) Ovaries of 60 days pollutant treated fishes:

In lead nitrate treated fishes, the ovarian wall is thick and the ovigerous lamellae are distinct. Though all the stages of oogenesis are present but the oocytes of yolk
Photomicrograph of transverse section of the :-

Fig. 118 : Ovary of *Nandus nandus* treated with copper sulphate (1 p.p.m. soln.) for 30 days (Mallory trile).

Fig. 119 : Ovary of 60 days control *Nandus nandus* (Mallory trile).

Fig. 120 : Ovary of *Nandus nandus* treated with lead nitrate (10 p.p.m. soln.) for 60 days (Ehrlich's haematoxylin eosin).

Fig. 121 : Ovary of *Nandus nandus* treated with zinc sulphate (10 p.p.m. soln.) for 60 days (Ehrlich's haematoxylin eosin).

Abbreviations:

CA I. = Corpora atretica in early stage of absorption

CA II. = Corpora atretica in later state of absorption.

E.PN. = Early peri-nucleolus stage

L.PN. = Late peri-nucleolus stage.
vesicle stage, yolk stage, prematuration stage and mature stage are very few. The ovary contains all the stages of corona atretica in a large number (Fig. 120; p. 193). In zinc sulphate and copper sulphate treated fishes also the ovigerous lamellae are very distinct. The ovary is loosely packed with oocytes of chromatin nucleolus and perinucleolus stages but the remaining stages of oocytes are not present (Fig. 121; p. 193; Fig. 122, p. 194). All the corona atretica are finally absorbed leaving empty spaces inside the ovigerous lamellae. In zinc sulphate treated fishes a few oocytes of late perinucleolus stage are found undergoing pyknosis. Their cytoplasm shows a few vacuoles (Fig. 123; p. 195). This vacuolization is further increased in the oocytes of late perinucleolar stages in the ovaries of copper sulphate treated fishes (Fig. 124; p. 195).

8. Testes:

(1) Testes of normal (initial control) fishes:

The testes are enclosed in a peritoneal membrane below which lies a thick layer of connective tissue fibres and seminiferous epithelial cells. In the testes a number of seminiferous tubules of various sizes are present which are separated from one another by a very thin layer of interstitial tissue. The seminiferous tubules are closely packed with different stages of spermatogenesis. Primary and secondary
Photomicrograph of transverse section of the :-

Fig. 122 : Ovary of Macacus rhesus treated with copper sulphate (1 p.p.m. soln.) for 60 days (Mallory triple).

Fig. 123 : Ovary of Macacus rhesus treated with zinc sulphate (10 p.p.m. soln.) for 60 days showing pyknotic late peri-nucleolus stage (Ehrlich's haematoxylin eosin).

Fig. 124 : Ovary of Macacus rhesus treated with copper sulphate (1 p.p.m. soln.) for 60 days showing pyknotic late peri-nucleolus stage (Ehrlich's haematoxylin eosin).

Abbreviations :

L.PN. - Late peri-nucleolus stage
N. - Nucleus
PY.JY. - Pyknotic cytoplasm
Y.N. - Yolk nucleus.
spermatogonia are found in the tubules of peripheral region of the testes. The interior of the testis is occupied by seminiferous tubules having advance stages of spermatogenesis including spermatozoa (fig. 125; p. 198).

(iv) Testes of 30 days control fishes:

In the testes of 30 days control fishes the interstitial tissue separating the seminiferous tubules is thick. The seminiferous tubules are loosely packed with different stages of spermatogenesis (fig. 126; p. 198).

(iii) Testes of 30 days pollutant treated fishes:

In all the pollutant treated fishes, the number of early stages of spermatogenesis, i.e., primary and secondary spermatogonia is reduced. Many of these primary and secondary spermatogonia lose their shape and become pyknotic. The pyknotic condition is visible in lead nitrate treated fishes as compared to that in zinc sulphate treated fishes while it is maximum in copper sulphate treated fishes (figs. 127, p. 198; 128 and 129, p. 200).

(iv) Testes of 60 days control fishes:

When compared with the testes of 30 days control fishes, no marked change is observed in the testes of 60 days control fishes (fig. 130; p. 200)
Photomicrograph of the transverse section of :-

**Fig. 125**: Peripheral region of testis of initial control *H. nandus* nandus (Mallory triple).

**Fig. 126**: Peripheral region of testis of 30 days control *H. nandus* nandus (Mallory triple).

**Fig. 127**: Peripheral region of testis of *H. nandus nandus* treated with lead nitrate (20 p.p.m. soln.) for 30 days (Mallory triple).

**Abbreviations**:

- P.SC. - Primary spermatocyte
- P.SG. - Primary spermatogonia
- S.SG. - Secondary spermatogonia
- S.T. - Seminiferous tubules.
Photomicrograph of transverse section of the -

Fig. 128 : Peripheral region of testis of *Hamburga nandus* treated with zinc sulphate (10 p.p.m. soln.) for 30 days (Mallory triple).

Fig. 129 : Peripheral region of testis of *Hamburga nandus* treated with copper sulphate (1 p.p.m. soln.) for 30 days (Mallory triple).

Fig. 130 : Peripheral region of testis of 60 days control *Hamburga nandus* (Mallory triple).

Abbreviations :

- **D.C.**  - Damaged cell wall of primary spermatogonia.
- **P.SC.**  - Primary spermatocyte.
- **PY.P.SC.**  - Pyknotic primary spermatogonia
- **PY.S.SC.**  - Pyknotic secondary spermatogonia
- **SP.**  - Spermatozoa
- **S.SC.**  - Secondary spermatogonia.
(v) Testes of 60 days pollutant treated fishes:

In the fishes treated with lead nitrate and zinc sulphate, the ryknosis of early stages of spermatogenesis (i.e., primary spermatogonia and secondary spermatogonia) is more pronounced (figs. 131, 132; p. 203). In copper sulphate treated fishes, the rate of ryknosis is very high and most of the primary and secondary spermatogonial cells and spermatocytes are distorted and disorganised (fig. 133; p. 203). In lead nitrate treated fishes the tubular walls are least affected whereas in zinc sulphate treated fishes disintegration and damage of the tubular walls is a common feature. The tubular walls are severely damaged and disintegrated in copper sulphate treated fishes. The damaged ryknotic cells appear like a compact tissue mass.

C. Nucleus preopticus

(1) Nucleus preopticus of normal (initial control) fishes:

To investigate the effect of pollutants the parasmagnocellularis cells of nucleus preopticus have been taken into consideration as these cells have been found to exhibit a close correlation with the gonads in their seasonal activity (see Chapter IV). These cells are elongated, spherical, polygonal or multicolor in shape. The cytoplasm is granular with a large nucleus situated near the periphery. The cytoplasm
Photomicrograph of transverse section of the $\rightarrow$

**Fig. 131** : Peripheral region of *Nandus nandus* treated with lead nitrate (20 p.p.m. soln.) for 60 days (Mallory triple).

**Fig. 132** : Peripheral region of testis of *Nandus nandus* treated with zinc sulphate (10 p.p.m. soln.) for 60 days (Mallory triple).

**Fig. 133** : Peripheral region of testis of *Nandus nandus* treated with copper sulphate (1 p.p.m. soln.) for 60 days (Mallory triple).

**Abbreviations** :

- C.T. - Compact tissue mass
- D.C. - Damaged pyknotic cells
- PY.P.S.C. - Pyknotic primary spermatocyte
- PY.P.SG. - Pyknotic primary spermatogonia
- PY.S.SG. - Pyknotic secondary spermatocyte
- PY.S.SG. - Pyknotic secondary spermatogonia.
is deeply stained with AF and CHAM staining methods. The nucleus contains one or two nucleoli with chromatin material (Fig. 134; p. 206).

(ii) **Nucleus preorbitalis of 30 days control fishes**

The nucleus preorbital cells are smaller in size. The cytoplasm is granular, however, in a few cells it is slightly degranulated (Fig. 135; p. 206).

(iii) **Nucleus preorbitalis of 30 days pollutant treated fishes**

The nucleus preorbital cells of the lead nitrate treated fishes and their nuclear size are decreased as compared to their size in 30 days control fishes. The nucleus contains one or two nucleoli and the chromatin material is rarely visible (Fig. 136; p. 206). In zinc sulphate treated fishes the effect is greater than in lead nitrate treated fishes. The nucleus preorbital cells are further reduced in their size and nuclear volume. The cytoplasm has a small round nucleus containing a deeply stained nucleolus. The chromatin granules are rarely visible (Fig. 137; p. 206). In copper sulphate treated fishes nucleus preorbital cells are highly affected. Few NPO cells are hypertrophied with a big round nucleus. Their cytoplasm is depleted or neurosecretory material. The nucleus contains a thick streak-like nucleolus with clumped chromatin granules. Other cells are very much smaller in size with their
Photomicrograph of vertical longitudinal section of the brain of *Monius mordax*.

**Fig. 134**: Showing nucleus pretorius cells of initial control (AF).

**Fig. 135**: Showing nucleus pretorius cells of 30 days control fish (AF).

**Fig. 136**: Showing nucleus pretorius cells of fish treated with lead nitrate (20 p.p.m. soln.) for 30 days (AF).

**Fig. 137**: Showing pretorius cells of fish treated with zinc sulphate (10 p.p.m. soln.) for 30 days (AF).

**Fig. 138**: Showing nucleus pretorius cells of fish treated with copper sulphate (1 p.p.m. soln.) for 30 days (AF).

**Abbreviations**:

- **D.G.R.**: Degranulated cytoplasm
- **D.E.C.Y.**: Cytoplasm depleted of neurosecretory material
- **G.G.R.**: Greater granulated cytoplasm
- **L.G.R.**: Lesser granulated cytoplasm
- **N.**: Nucleus
- **N.U.**: Nucleolus.
cytoplasm depleted of neurosecretory material. They have a small round nucleus containing faintly stained nucleolus. The chromatin granules are not visible (fig. 133; p. 208).

(iv) **Nucleus preopticus of 60 days control fishes**

The size of nucleus preopticus cells along with their nuclei is slightly decreased as compared to their size in 30 days control fishes. The cytoplasm of many cells is degranulated (fig. 139; p. 209).

(v) **Nucleus preopticus of 60 days pollutant treated fishes**

The NPO cells along with their nuclei in lead nitrate treated fishes are further reduced (fig. 140; p. 209) but not to an extent as seen in the zinc sulphate and copper sulphate treated fishes. In the zinc sulphate and copper sulphate treated fishes the nucleus is round and faintly stained and the chromatin granules are not visible. Few cells are also seen undergoing pyknosis. The process of pyknosis is found higher in copper sulphate treated fishes than in the zinc sulphate treated fishes (figs. 141, 142; p. 209).

D. **Nucleus lateralis tubercis**

(i) **Nucleus lateralis tubercis of normal (initial control) fishes**

To investigate the effect of pollutants the
Photomicrograph of vertical longitudinal section of the brain of *Nandus nandus*.

**Fig. 139**: Showing nucleus preopticus cells of 60 days control fish (AF).

**Fig. 140**: Showing nucleus preopticus cells of fish treated with lead nitrate (20 p.p.m. soln.) for 60 days (AF).

**Fig. 141**: Showing nucleus preopticus cells of fish treated with zinc sulphate (10 p.p.m. soln.) for 60 days (AF).

**Fig. 142**: Showing nucleus preopticus cells of fish treated with copper sulphate (1 p.p.m. soln.) for 60 days (AF).

**Abbreviations**:

D. GR. - Degranulated cytoplasm

N. - Nucleus

NU. - Nucleolus

PY.C. - Pyknotic cell.
ventro-lateral cells of nucleus lateralis tuberis have been taken into consideration. These cells are larger in size than the ventro-median cells of nucleus lateralis tuberis and show a close relation with the seasonal changes of the gonads (see Chapter IV). They possess round/oval nucleus surrounded by a thin granular cytoplasm with deposition of neurosecretory material in them. The nucleus contains a centrally placed nucleolus and chromation material (fig. 143; p. 210).

(ii) *Nucleus lateralis tuberis of 20 days control fishes*:

The nucleus lateralis tuberis cells of 30 days control fishes do not show marked changes when compared to the condition in normal fishes (fig. 144; p. 212).

(iii) *Nucleus lateralis tuberis of 30 days pollutant treated fishes*:

In lead nitrate treated fishes the cytoplasm is less depleted of the neurosecretory material as compared to the condition in normal fishes. The nucleus and the chromatin material is visible (fig. 145; p. 212). In zinc sulphate and copper sulphate treated fishes the cells are very much smaller in size without any neurosecretory material in the cytoplasm. The nucleolus is faintly stained and the chromatin material is visible (figs. 146, 147; p. 212).
Photomicrograph of vertical longitudinal section of the brain of *Hendus nandus*.

Fig. 143: Showing nucleus lateralis tuberis cells of initial control fish (JAHP).

Fig. 144: Showing nucleus lateralis tuberis cells of 30 days control fish (JAHP).

Fig. 145: Showing nucleus lateralis tuberis cells of fish treated with lead nitrate (20 p.p.m. soln.) for 30 days (JAHP).

Fig. 146: Showing nucleus lateralis tuberis cells of fish treated with zinc sulphate (10 p.p.m. soln.) for 30 days (JAHP).

Fig. 147: Showing nucleus lateralis tuberis cells of fish treated with copper sulphate (1 p.p.m. soln.) for 30 days (JAHP).

Abbreviations:

D.GR. = Degranulated cytoplasm
DE.CY. = Cytoplasm depleted of neurosecretory material
L.GR. = Lesser granulated cytoplasm
N. = Nucleus
NU- = Nucleolus.
(iv) *Nucleus lateralis tuberis of 60 days control fishes:*

The *nucleus lateralis tuberis* cells of 60 days control fishes are smaller in size than those of 30 days control fishes. The granular cytoplasm around the nucleus is very thin (fig. 149; p. 215).

(v) *Nucleus lateralis tuberis of 60 days pollutant treated fishes:*

In lead nitrate treated fishes the nucleus of a few cells is larger in size but their cytoplasm is very much depleted of neurosecretory material. Most of the cells are smaller in size with small round nucleus containing faintly stained nucleolus. The chromatin granules are rarely visible (fig. 149; p. 215). In zinc sulphate treated fishes the cells are very much smaller in size with small nucleus and clear cytoplasm. The nucleolus is faintly stained and the chromatin granules are not visible (fig. 150; p. 215). In copper sulphate treated fishes the effects are severe and a few cells show pyknotic condition. Other cells are very much smaller in size and the cytoplasm is very much depleted of neurosecretory material. The nucleus is also smaller in size and the nucleolus is rarely visible. The chromatin granules are not visible (fig. 151; p. 215).

E. *Proximal pars distalis of the pituitary gland:*

In *Manius nanus*, the glandular region of the pituitary
Photomicrograph of vertical longitudinal section of the brain of Pandus nandus.

**Fig. 148**: Showing nucleus lateralis tuberis cells of 60 days control fish (CAHT).

**Fig. 149**: Showing nucleus lateralis tuberis cells of fish treated with lead nitrate (20 p.p.m. soln.) for 90 days (CAHT).

**Fig. 150**: Showing nucleus lateralis tuberis cells of fish treated with zinc sulphate (10 p.p.m. soln.) for 90 days (CAHT).

**Fig. 151**: Showing nucleus lateralis tuberis cells of fish treated with copper sulphate (1 p.p.m. soln.) for 90 days (CAHT).

**Abbreviations**:

- **D.CR.** - Degranulated cytoplasm
- **D.E.GY.** - Cytoplasm depleted of neurosecretory material.
- **P.Y.C.** - Pyknotic cell.
gland is divided into three regions, the rostral pars distalis (containing two types of acidophils), proximal pars distalis (containing acidophils, basophils and chromophobes) and the pars intermedia (containing acidophils and basophils). Out of these cells, only basophils of the proximal pars distalis of the pituitary gland show marked cytological effects which are described in the following account.

(i) Basophils of normal (initial control) fishes:

The basophils of proximal pars distalis of the pituitary gland of a normal fish are large elongated cells with a big round nucleus which is situated at one end. The cytoplasm is sparsely granulated and deeply stained. The basophils show different grades of size depending on the plane the cells are cut (Fig. 152; p. 212).

(ii) Basophils of 30 days control fishes:

The basophils of 30 days control fishes do not show marked changes from those of the normal fishes (Fig. 153; p. 218).

(iii) Basophils of 30 days pollutant treated fishes:

The basophils of fishes treated with lead nitrate, zinc sulphate and copper sulphate show degranulation in their cytoplasm. In the fishes treated with lead nitrate
Photomicrograph of the vertical longitudinal section of the pituitary gland of *Nandus nandus*.

**Fig. 152**: Showing basophils of initial control fish (AF).

**Fig. 153**: Showing basophils of 30 days control fish (AF).

**Fig. 154**: Showing basophils of the fish treated with lead nitrate (20 p.p.m.) for 30 days (GAHF).

**Fig. 155**: Showing basophils of the fish treated with zinc sulphate (10 p.p.m.) for 30 days (Ar).

**Fig. 156**: Showing basophils of the fish treated with copper sulphate (1 p.p.m.) for 30 days (Ar).

**Abbreviations**:

- D.GR. - Degranulated basophil
- GR. - Granulated basophil.
the basophils are least affected and show very few vacuoles in their cytoplasm (fig. 154; p. 218). The intensity of degranulation is higher in zinc sulphate treated fishes (fig. 155; p. 218) and maximum in copper sulphate treated fishes of this group, (fig. 156; p. 218). The number of basophils decreases with the effect of these pollutants. The cell size and the nuclear size is also reduced depending on the intensity of degranulation.

(iv) Basophils of 60 days control fishes:

The basophils of 60 days control fishes are smaller in size than those of 30 days control fishes. Their cytoplasm is granulated (fig. 157; p. 221).

(v) Basophils of 60 days pollutant treated fishes:

With 60 days treatment of lead nitrate, zinc sulphate and copper sulphate the rate of degranulation is further increased (figs. 158, 159, 160; p. 221). This is particularly so in the copper sulphate treated fishes in which the cytoplasm of the basophils is smooth and very faintly stained and the stainable neurosecretory granules are completely depleted. The number of basophils, their cell size and nuclear size is further reduced.
Photomicrograph of the vertical longitudinal section of the pituitary gland of *Nandus nandus*.

**Fig. 157** Showing basophils of 60 days control fish (AF).

**Fig. 158** Showing Basophils of the fish treated with lead nitrate (20 p.p.m.) for 60 days (AF).

**Fig. 159** Showing basophils of the fish treated with zinc sulphate (10 p.p.m.) for 60 days (CAHF).

**Fig. 160** Showing basophils of the fish treated with copper sulphate (1 p.p.m.) for 60 days (Ar).

**Abbreviations**

DF.CY. - Cytoplasm of basophil depleted of neurosecretory material

D.Gk. - Degranulated basophil

Gk. - Granulated basophil
Quantitative study of the effects of pollutants on the gonads, nucleus prorecticus and the basophilic of the proximal pars distalis of the pituitary gland:

The effects of pollutants have been assessed quantitatively in thirty and sixty days pollutant treated fishes in respect of the volume of gonads and the gonosomatic index of the fish. Effects on the nuclear volume of nucleus prorecticus and maximum length of basophils have also been assessed quantitatively.

For quantitative study of the volume of gonads and the gonosomatic index, the body weight and gonad weight of the fishes (fixed material) was taken after blotting away extra fixative. The volume of the gonads was also measured by liquid displacement method (Bullock, 1939). The average volume and average weight of testes and ovaries and the average body weight of the male and female fishes of initial control (normal) and 30 and 60 days control fishes and those treated with lead nitrate, zinc sulphate and copper sulphate for 30 and 60 days were calculated separately. The average gonosomatic index and the average volume of the gonads have been shown in Table 7: p. 231 and 9, p. 232 and Figs. 161, 162, 163; 159, p. 236).

For the karyometric study of nucleus prorecticus, nuclear outlines of fifty cell nuclei from ten different planes of nucleus prorecticus of a fish were drawn on the millimeter square graph papers by camera lucida at a magnification of x675
approximately. In this way all the 10 normal (initial control) fishes and 7 to 10 fishes of 30 and 60 days interval (control and pollutant treated fishes) were taken into consideration (Table 7; p. 233). The nuclear area in millimeter squares and maximum nuclear diameter in millimeters were measured. The nuclear volume was calculated by the formula described earlier (see page 163). Standard deviation for the mean nuclear volume of different sample and the standard error was also calculated (Table 9; p. 233) by the formulae given earlier (see pages 164 and 165). The mean nuclear volumes along with their standard deviations are plotted in Fig. 163; p. 229).

The method for the study of maximum length of basophils, used in the present study, is based on the work of Bhargava (1966) followed by Kaur (1968), Raizada (1969), Bhargava and Raizada (1973), Jaksena (1974), Anant Prakash (1976) and Bais (1977). In the present study, only those basophils which abutted on the processes of neurohypophysis were taken into consideration. Such a criterion helps in studying only those cells which are cut longitudinally along their maximum length (Bhargava, 1966). The cells which do not abut on the neurohypophysis are possibly those cells which are cut in planes other than longitudinal and as such would not represent their maximum length.

For this study all the fishes of initial control and all the fishes surviving the treatment with different pollutants were taken into account. Nine equidistant vertical longitudinal
sections of the pituitary gland of each fish were marked (four on either side of the median sagittal section). After a general survey of these sections under a magnification of X675 approximately, five large basophils from each section cut along their maximum length were selected and their length was measured with the help of ocular disc. Thus a total of forty-five cells were measured in the pituitary gland of each fish. The mean maximum length of basophils and their standard deviation and standard error were calculated which are shown in Table 9; p. 233. The mean maximum length of basophils along with their standard deviations are plotted in Fig. 164; p. 235.

Changes in the volume of gonads and the gonosomatic index of the fish:

In the fishes of initial control, the volume of the ovary/testis and the gonosomatic index of female/female fish is 2.20/0.34 ml and 10.0973/1.1720 respectively. It shows an active state of gonads and a high metabolic activity of the fishes of initial control. These values decrease a little in 30 days and 60 days control fishes, however, they decline sharply after the treatment of pollutants for 30 days (Figs. 161, p. 234; 162, p. 235), and show a further decrease in their values in 60 days pollutant treated fishes which indicates a sharp downward trend in the activity of gonads. This trend has also been observed histologically in the ovary in which later
<table>
<thead>
<tr>
<th>Concentration</th>
<th>Duration of Experiment</th>
<th>Number of Fishes Survived</th>
<th>Range of Fish Length in cm.</th>
<th>Average Weight of Fish in gms.</th>
<th>Average Weight of Ovaries in gms.</th>
<th>Average Volume of Ovaries in ml.</th>
<th>Average Gonosomatic Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial control</td>
<td>-</td>
<td>5</td>
<td>11.0-11.5</td>
<td>22.08</td>
<td>2.23</td>
<td>2.20</td>
<td>10.0973</td>
</tr>
<tr>
<td>Control</td>
<td>30 days</td>
<td>5</td>
<td>11.0-12.5</td>
<td>23.77</td>
<td>2.35</td>
<td>2.01</td>
<td>8.0286</td>
</tr>
<tr>
<td>Lead nitrate 20 p.p.m.</td>
<td>30 days</td>
<td>5</td>
<td>12.0-12.5</td>
<td>31.92</td>
<td>1.06</td>
<td>1.08</td>
<td>3.3176</td>
</tr>
<tr>
<td>Zinc sulphate 10 p.p.m.</td>
<td>30 days</td>
<td>5</td>
<td>11.5-12.5</td>
<td>25.60</td>
<td>0.51</td>
<td>0.48</td>
<td>1.9340</td>
</tr>
<tr>
<td>Copper sulphate 1 p.p.m.</td>
<td>30 days</td>
<td>4</td>
<td>11.5-13.0</td>
<td>21.50</td>
<td>0.25</td>
<td>0.25</td>
<td>1.1601</td>
</tr>
<tr>
<td>Control</td>
<td>60 days</td>
<td>5</td>
<td>12.0-12.5</td>
<td>20.73</td>
<td>1.01</td>
<td>1.81</td>
<td>6.4935</td>
</tr>
<tr>
<td>Lead nitrate 20 p.p.m.</td>
<td>60 days</td>
<td>5</td>
<td>12.5-13.0</td>
<td>35.25</td>
<td>0.82</td>
<td>0.80</td>
<td>2.3149</td>
</tr>
<tr>
<td>Zinc sulphate 10 p.p.m.</td>
<td>60 days</td>
<td>5</td>
<td>12.5-13.0</td>
<td>36.05</td>
<td>0.30</td>
<td>0.41</td>
<td>1.5125</td>
</tr>
<tr>
<td>Copper sulphate 1 p.p.m.</td>
<td>60 days</td>
<td>3</td>
<td>10.0-13.0</td>
<td>22.03</td>
<td>0.20</td>
<td>0.17</td>
<td>0.8940</td>
</tr>
</tbody>
</table>

**Explanation:** Table showing the duration of experiment, range of length and average weight of female *Hansalus pandus*, average weight and average volume of ovaries and the gonosomatic index of the normal, controlled and pollutant treated fishes.
Fig. 161: Graph showing the average volume of ovaries and average gono-somatic index of female *Nandus nandus* in different experimental conditions.
Fig. 161

- **NORMAL**
- **CONTROL**
- **LEAD NITRATE**
- **ZINC SULPHATE**
- **COPPER SULPHATE**

**Volume of Ovaries in Millilitres**

- 2.4
- 2.0
- 1.6
- 1.2
- 0.8
- 0.4

- **GONAD-SONOMIC INDEX**

- 11
- 10
- 9
- 8
- 7
- 6
- 5
- 4
- 3
- 2

- 30 Days
- 60 Days
### Table 8

<table>
<thead>
<tr>
<th>Concentration of Pollutants</th>
<th>Duration of Experiment</th>
<th>Number of Fishes Survived</th>
<th>Range of Fish Length in cm.</th>
<th>Average Weight of Fish in gms</th>
<th>Average Weight of Testes in gms</th>
<th>Average Volume of Testes in ml</th>
<th>Average Gono-somatic Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial control</td>
<td>-</td>
<td>5</td>
<td>10.4-13.0</td>
<td>16.77</td>
<td>0.220</td>
<td>0.34</td>
<td>1.1720</td>
</tr>
<tr>
<td>Control</td>
<td>30 days</td>
<td>5</td>
<td>10.5-11.2</td>
<td>19.47</td>
<td>0.196</td>
<td>0.31</td>
<td>1.0822</td>
</tr>
<tr>
<td>Lead nitrate 20 p.p.m.</td>
<td>30 days</td>
<td>5</td>
<td>10.5-11.5</td>
<td>18.30</td>
<td>0.086</td>
<td>0.16</td>
<td>0.4861</td>
</tr>
<tr>
<td>Zinc sulphate 10 p.p.m.</td>
<td>30 days</td>
<td>4</td>
<td>11.5-12.0</td>
<td>18.10</td>
<td>0.041</td>
<td>0.08</td>
<td>0.2261</td>
</tr>
<tr>
<td>Copper sulphate 1 p.p.m.</td>
<td>30 days</td>
<td>4</td>
<td>12.0-13.0</td>
<td>20.21</td>
<td>0.033</td>
<td>0.07</td>
<td>0.1633</td>
</tr>
<tr>
<td>Control</td>
<td>60 days</td>
<td>5</td>
<td>10.0-11.5</td>
<td>16.23</td>
<td>0.168</td>
<td>0.30</td>
<td>1.0304</td>
</tr>
<tr>
<td>Lead nitrate 20 p.p.m.</td>
<td>60 days</td>
<td>4</td>
<td>10.5-11.0</td>
<td>17.18</td>
<td>0.053</td>
<td>0.11</td>
<td>0.3031</td>
</tr>
<tr>
<td>Zinc sulphate 10 p.p.m.</td>
<td>60 days</td>
<td>4</td>
<td>11.0-12.5</td>
<td>17.00</td>
<td>0.032</td>
<td>0.07</td>
<td>0.1706</td>
</tr>
<tr>
<td>Copper sulphate 1 p.p.m.</td>
<td>60 days</td>
<td>4</td>
<td>12.0-13.0</td>
<td>17.51</td>
<td>0.018</td>
<td>0.06</td>
<td>0.1028</td>
</tr>
</tbody>
</table>

**Explanation:** Table showing the duration of experiment, range of length and average weight of male *Nandus nandus*, average weight and average volume of testes and the gono-somatic index of the normal, controlled and pollutant treated fishes.
Fig. 182: Graph showing the average volume of testes and average gono-somatic index of male *Nandus nanus* in different experimental conditions.
Fig. 162
<table>
<thead>
<tr>
<th>Concentration of Pollutant</th>
<th>Duration of Experiment</th>
<th>No. of Fishes</th>
<th>Mean Nuclear Volume of N10 Cells in mm³</th>
<th>Standard Deviation</th>
<th>Standard Error</th>
<th>Mean Maximum Length of Basophil of Proximal pars Distalis in μ</th>
<th>Standard Deviation</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial control</td>
<td>-</td>
<td>10</td>
<td>941.50</td>
<td>145.9773</td>
<td>6.5233</td>
<td>14.335</td>
<td>4.5277</td>
<td>0.2134</td>
</tr>
<tr>
<td>Control</td>
<td>30 days</td>
<td>10</td>
<td>312.03</td>
<td>111.9393</td>
<td>4.9738</td>
<td>13.843</td>
<td>4.1656</td>
<td>0.1867</td>
</tr>
<tr>
<td>Lead nitrate 20 p.p.m.</td>
<td>30 days</td>
<td>10</td>
<td>334.36</td>
<td>20.1446</td>
<td>4.0313</td>
<td>11.907</td>
<td>3.8081</td>
<td>0.1795</td>
</tr>
<tr>
<td>Zinc sulphate 10 p.p.m.</td>
<td>30 days</td>
<td>6</td>
<td>278.38</td>
<td>75.7639</td>
<td>3.5715</td>
<td>9.934</td>
<td>3.2814</td>
<td>0.1630</td>
</tr>
<tr>
<td>Copper sulphate 30 days 1 p.p.m.</td>
<td>60 days</td>
<td>10</td>
<td>238.25</td>
<td>39.3651</td>
<td>1.9632</td>
<td>3.871</td>
<td>3.0943</td>
<td>0.1631</td>
</tr>
<tr>
<td>Control</td>
<td>60 days</td>
<td>10</td>
<td>742.07</td>
<td>106.0141</td>
<td>4.7411</td>
<td>13.921</td>
<td>3.9153</td>
<td>0.1751</td>
</tr>
<tr>
<td>Lead nitrate 20 p.p.m.</td>
<td>60 days</td>
<td>6</td>
<td>272.14</td>
<td>63.1602</td>
<td>2.9774</td>
<td>10.925</td>
<td>3.6079</td>
<td>0.1793</td>
</tr>
<tr>
<td>Zinc sulphate 10 p.p.m.</td>
<td>60 days</td>
<td>6</td>
<td>187.20</td>
<td>34.6466</td>
<td>1.6337</td>
<td>9.537</td>
<td>2.8132</td>
<td>0.1400</td>
</tr>
<tr>
<td>Copper sulphate 30 days 1 p.p.m.</td>
<td>60 days</td>
<td>7</td>
<td>174.04</td>
<td>24.1243</td>
<td>1.2895</td>
<td>6.917</td>
<td>2.5134</td>
<td>0.1416</td>
</tr>
</tbody>
</table>

**Explanation:** Table showing the mean nuclear volume of nucleus preortious cells and mean maximum length of basophils of proximal pars distalis along with their standard deviation and standard error in normal, controlled and pollutant treated *Nanusius nanus*.
Fig. 163: Graph showing the mean nuclear volume of nucleus preopticus of *Drosophila melanogaster* in different experimental conditions.
Fig. 163
Fig. 164: Graph showing the mean maximum length of basophils of the proximal pars distalis of the pituitary gland of Nandus nandus in different experimental conditions.
Fig. 164
stages of oocytes become atretic and get finally absorbed. In the testes also a gradual pyknosis sets in.

Changes in the nuclear volume of the nucleus preantricous cells and in the maximum length of basophils:

The mean nuclear volume of nucleus preantricous and the mean maximum length of basophils is 941.5 μm³ and 14.335 μ respectively. These values show a downward trend (Fig. 163, p. 223; 164, p. 235) and are comparable to those regarding the volume of gonads and gonosomatic index in fishes (Fig. 161, p. 224; 162, p. 226) of 30 and 60 days control and the fishes treated with pollutants for 30 and 60 days. Thus it can be inferred that with the gradual effect of pollutants the activity of these cells is interrupted which is similarly seen in the case of gonads also.

Discussion:

In *Clarias batrachus* and *Orhioderhabia punctatus*, Khosa and Chandrasekhar (1972) observed that the effect of copper acetate and asphalt results in the acceleration of maturation of ova and increase in vitellogenesis which could be exerted by way of nucleus preantricous. In flag fish, *Jordanella florigas*, Spehar (1976) has reported that cadmium and zinc toxicity causes significant decrease in survival, growth and reproduction over the complete life cycle of the fish. Speranza
at al. (1977), in Zebra fish *Brachydanio rerio*, observed that the effect of sub-lethal concentration of zinc causes delay in spawning and the viability of eggs and embryos are lower than control. In *Heteroconger mossii* Maharatna and Medda (1977) observed that with the rise of the concentration of sodium chloride within certain limits, the gonado-somatic index of the fish increases gradually. In *Eleotris batrachus*, Vishwanath Rao and Chandrasekhar (1976) observed that the treatment of clomiphene accelerates oogenesis and the fish could be made to spawn during off season. In *Ihanna punctatus*, Saxena and Sarg (1978) investigated that the corb as treated ovary loses maturing and developing mature oocytes while the number of immature and atretic oocytes increases. Ujyovu and Beatty (1979) found that the chronic exposure to zinc affects reproduction in the guppy, *Poecilia reticulata* and the later stages of oocytes undergo atresia. Such an effect has also been observed in the present fish, *Hanseus mossul*, where the ovaries show greater loss of advance stages of oogenesis as one passes from the lead nitrate to zinc sulphate and copper sulphate treated fish. The effect of zinc sulphate and copper sulphate is found to be maximum where all the stages except chromatin-nucleolus and perinucleolus stages have lost and even the late perinucleolus stages show vacuolization in their cytoplasm.

In *Gryzias laticeps*, Nagori (1977) has reported that the treatment of initiates water causes marked loss of testes weight and the number of primary spermatogonia decreases. The
pyknosis has been observed in primary spermatogonia, secondary spermatogonia and spermatocytes. In brook trout, Jangalani and Malloran (1974) reported that the effect of cadmium (25 p.p.m.) on testes causes extensive neurosis and disintegration of tubule boundary. In Nanilus maniculatus the effect of lead nitrate, zinc sulphate and copper sulphate is noticed on the earlier stages of spermatogenesis (primary and secondary spermatogonia) which undergo pyknosis and this is followed by the later stages except the spermatocytes. The seminiferous tubules also disintegrate and in fishes treated with copper sulphate solution for 60 days the disintegrated tubule walls and pyknotic cells give an appearance of compact tissue mass. Thus in Nanilus maniculatus the effects of lead nitrate, zinc sulphate and copper sulphate have been found destructive in nature and thus causing excessive damage to gonads.

In the teleost, Lomus lacrimal, Haider and Sathyanesan (1973) have seen the effect of thiourea and found initial increase in the stainability of nucleus reactive cells which tends to decrease later. In Clarias batrachus (Haider and Sathyanesan, 1975 a) the injection of formalin depletes 70%–90% of stainable neurosecretory material from all the components of neurosecretory system. They have also described that the chlorpromazine treatment causes quantitative increase in the neurosecretory material throughout the neurosecretory complex. In the same fish, Haider and Sathyanesan (1975 b) found alcohol inhibition of formalin induced depletion of neurosecretory
material. Banerjee (1973) has reported the effect of carbon
tetra chloride in garden lizard, *Calotes versicolor* and observed
depletion of neurosecretory cells. Taheri and Taheri (1970)
have reported the effect of mercuric chloride on the preoptic-
neurohypophysial system in *Heteronotus fossilius* and observed
acute depletion of neurosecretory material in nucleus preopticus.
These findings are similar to the present fish *Hansus nandus*
treated with lead nitrate, zinc sulphate and copper sulphate.
In the fishes treated with copper sulphate for 30 days nuclear
enlargement, karyopyknosis and chromatin clumping have been
observed which are similar to the findings of Taheri and Taheri
(1970). The available literature indicates that so far no work
has been done on the effects of pollutants on the nucleus
lateralis tuberis. In *Hansus nandus* treated with lead nitrate,
zinc sulphate and copper sulphate the nucleus lateralis tuberis
cells become smaller in size and depleted of the neurosecretory
material. In fishes treated with copper sulphate for 60 days,
most of the nucleus lateralis tuberis cells are in pyknotic
condition.

Vishwanath Rao and Jhonsupal (1976) observed that
the clorophylene increases the activity of cyanorhils of the
pituitary gland and the fish was made to spawn in the off
season. In *Elassoma iranica* (Naider and Sethyanesan, 1973) and
*Clarias batrachus* (Naider and Sethyanesan, 1975 a,b), the
effect of different chemicals on neurosecretory system
(nucleus preopticus, neurosecretory tract and neurohypophysis) shows depletion in neurosecretory material. Fahari and Fahari (1970) have observed the effect of mercuric chloride on the hypothalamo neurohypophysial system and observed depletion of the stainable neurosecretory material in the different components of the system. Most of the authors have seen the effects only on the neurohypophysis of the pituitary gland but not on adenohypophysis. In the present study on *Nandus nandus* the treatment of lead nitrate, zinc sulphate and copper sulphate affects the basophils which become degamulated and their number decreases. The stainable neurosecretory material becomes depleted gradually and the basophils stain very faintly with Aldehyde fuchsin and Chrome alum haematoxylin rhodamine stains.

Thus it can be suggested that, in *Nandus nandus*, the effect of lead nitrate, zinc sulphate and copper sulphate causes inactivity in the hypothalamic cells (nucleus preopticus and nucleus lateralis tuberis) and the basophils of proximal pars distalis. As such the secretion and release of hormones decreases and ultimately stops due to which the process of gametogenesis decreases while hypotrophy and pyknosis in the stages of gametogenesis sets in.