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# Histopathological Effect of 2, 4-Dichlorophenoxy Acetic Acid on Gill Arch Epithelium of The Fish *Anabas testudineus*

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**ABSTRACT**

Effect of sub-lethal exposure of 2,4-dicholophenoxy acetic acid has been studied on the Gill arch epithelium of *Anabas testudineus*.2, 4- Dichlorophenoxyacetic acid (2,4-D) is used in herbicides. 2,4-D, a chlorinated phenoxy compound functions as a systemic herbicide and is used to control many types of weeds. *Anabas testudineus* (Bloch) is a carnivorous air breathing fish, inhabiting hypoxic swampy waters of India. From 3 hour (h) to 24 h treatment, there was no significant change in epithelial cells. At 48h treatment epithelial cells showed degenerative changes with the appearance of prominent inter cellular spaces. In subsequent treatments from 72h to 10 days, epithelial cells undergoing necrosis in general appear characteristically dilated and vacuolated. At 3 h exposure mucous cells appeared elongated. At 6h treatment mucous cells in superficial layer became enlarged and acquired flask shape. At 12h treatment mucous cells became round and appear relatively close to each other. At 48h exposure, enormous increase in number of mucous cells was observed. At 72h mucous cells at some places lied close together to form clusters. At 96 h treatment there was instant decline in the number and size of mucous cells. At 5d and 6d exposures there was tremendous increase in the number of mucous cells. In subsequent treatment gradual decline in number of mucous cells was observed. Chloride cells were found to be absent in Gill arch epithelium in untreated fish. Chloride cells were observed at 96 h, 5d, 6d and 7d treatments. At 96 h exposure these cells appeared small in size; however their size increase at 5d and 6d exposure which on further treatments at 7d decline. At 8d, 9d and 10d exposure they all together disappeared. Increase in density and dimensions of mucous cells are related to enhanced mucous secretion which is an inbuilt defence mechanism of fish against a disturbed aquatic environment. The acid glycoprotein content of mucous may act as receptor sites for binding the exogenous micromolecules of bacterial and viral etiology. The mitochondria rich chloride cells are associated to provide energy for ionic regulation in disturbed aquatic environment.

## 1. Introduction

2, 4, D is 2, 4, Dichlorophenoxyacetic acid used in herbicides. 2,4, D, a chlorinated phenoxy compound functions as a systemic herbicide and is used to control many types of weeds. This compound is used in cultivated agriculture and pasture and large land applications, forest management, home and garden situations and for the control of aquatic vegetation.

*Anabas testudineus* (Bloch) is a carnivorous air breathing fish, inhabiting hypoxic swampy waters of India. The peculiar amphibious mode of the life of an air breathing teleost *Anabas testudineus* has been a matter of great interest to the biologists, especially due to its faculty of moving for some distance over land. This fish is also reported to ascend palm trees near a pond to a height of 5 feet above the water (Gunther 1880) and hence often called 'Climbing Perch'. Physiologists have been trying to unravel the role played by the various organs for its survival outside the water. Munshi et al (1984) have made extensive investigation on the role of gills and other accessory respiratory organs of this fish facilitating its amphibious mode of life. It belongs to the family Anabantidae and order Perciformes.

In teleosts, gill arches are located at the boundary between the pharynx and opercular chamber on either side of the head and extend from the otic region of the neuro cranium to join ventrally near the basihyal. The first four pairs of gill arches are respiratory and bear gill filaments on their opercular side and gill raker towards their pharyngeal side. The fifth pair of gill arches lack gill filaments.

Several workers working on fish gills have given attention to the study of gill filament and gill lamellae (Hughe 1984; Munshi et. al. 1984; Zaccone el. al. 1985). Effects of toxicants on gill



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filaments and gill lamellae have also been studied. (Weatherly et.al. 1980; Ojha 1999; Singh et.al 2007). The study of structure and histochemistry and effect of different toxicants on different cellular components of gill arch is sporadic and scanty. In this communication the structural organization of gill arch epithelium of *Anabas testudineus* and impact of herbicide 2,4-D on different cellular components of gill arch using a battery of histochemical techniques described in this paper.

## 2. Materials and Methods

Specimens *Anabas testudineus* were collected from local ponds at Singramau, Jaunpur, India (2017) and were acclimated at optimum laboratory conditions for 15 days experimentation. Fish during acclimatization were fed with minced goat liver on alternate days.The fishes were exposed to sub lethal concentration 60mg/L of 2, 4-D (IFFCO-MC, Varanasi). Fishes were cold anaesthetized and gills of the fishes were excised, rinsed in physiological saline and were fixed in 10% neutral formalin and aqueous Bouin's fluid at 3h, 6h, 12h, 1d, 2d, 3d, 4d, 5d, 6d, 7d, 8d, 9d, and 10d of chlordecone treatment. Paraffin sections were cut at 5µm thick and stained with Ehrlich's haemotoxylin-eosin (H/E), Verhoef's Haematoxylin eosin (VHE) and Papanicolaou's stain (PS) to study structural organization. Sections were subjected to various histochemical tests for carbohydrate and proteins following Lillie (1954) and Stevens (1982).

## 3. Results and Discussion

### 3.1 Control

Gill arch region (GAR) is externally covered by a thick epithelium which is composed of epithelial cells (EC), mucous cells (MC), acidophil cells (AC) and basement membrane (BM). Epithelial cells divided into middle, outer and basal layer epithelium cells (PLATE Ia) form the outermost region of gill arch with various gland cells lie to perform their specific functions. Middle and outer layer epithelial cells were polyhedral, having well differentiated centrally placed nucleus. Basal epithelial cells were cuboidal in shape, resting on a thin non-cellular basement membrane. Each cell is provided with prominent centrally placed oval nucleus. The cytoplasm is homogenous in middle and outer layer as well as basal epithelial cells. In HE and PS their nuclei stain blue, the cytoplasm is eosinophilic and the cell peripheries stain purplish blue, however, in VHE, their nuclei stain moderately black, the cytoplasm stains weakly pink and the cell peripheries stain moderately blue-black. Epithelial cells of GAR stain moderately magenta with PAS, with and without prior diastase/saliva treatment blocked by prior acetylation and restored by deacetylation. In control few glycogen granules were dispersed in between the epithelial cells of middle layer (PLATE I f).

In control, these cells remain unstained with alcian blue (AB) at pH 2.5 and AB at pH 1.0, however showed moderate magenta with AB/PAS. The cytoplasmic contents of epithelial cells showed weak reaction, whereas, the nuclei and cell peripheries showed moderate reaction with mercury bromophenol blue (Hg-BPB) method for general proteins, acid solochrome cyanine method for basic proteins and ninhydrin-Schiff method for protein bound-NH2 groups. With the other histochemical techniques employed, the epithelial cells of the gill arch region do not showed positive reactions for other protein end groups.

Acidophil cells of GAR are large, oval, eosinophilic cells found in middle layer epithelial cells. In control acidophil cells of GAR are elliptical or oval in shape with homogenous cytoplasm having strong affinity for eosin. The small nucleus is not easily differentiated due to dark staining of cytoplasm. In HE and PS, their nuclei stain weakly blue, the cytoplasm stains moderately pink and the cell peripheries stain purplish blue, however in VHE, their nuclei stain weakly black, the cytoplasm stain weakly pink and the cell peripheries stain moderately blue-black. Acidophil cells in GAR do not stain magenta with PAS, with and without prior diastase/saliva treatment. Acidophil cells do not stain with AB at pH 2.5, pH 1.0 and AB/PAS. These reactions suggest that acidophil cells, in general, do not contain neutral or acid Gps. Acidophil cells show strong reactions with (Hg-BPB) method for general proteins, acid solochrome cyanine method for basic proteins and ninhydrin-Schiff method for protein bound- NH2 groups. With the other histochemical techniques, acidophil cells do not show positive reaction for protein end groups.Mucous cells are mostly flask-shaped opening to the exterior by small pores to void their secretions and extending up to middle layer of epithelial cells.

In HE and PS the secretory contents of the mucous cells, in general, appears weakly basophilic and remains unstained in VHE. However, with routine histological stains difficulties are experienced in the localization of the mucous cells and these are often, not clearly distinguished. With specific histochemical techniques, however, the mucous cells are readily differentiated and these techniques are thus utilized to locate them. Mucous cells in GAR showed strong reactions, magenta with PAS with and without prior diastase treatment which was abolished by acetylation and restored by deacetylation(PLATE I f). Flask shaped mucous cells in the GAR opened to exterior by small pores, along with their secretory contents as a whole, in general showed very strong reactions, greenish-blue with AB at pH 2.5 that was blocked by prior high temperature methylation and restored by subsequent. Saponification even with prior mild methylation, and purple with AB/PAS indicating the presence of a mixture of neutral and acid (sulphated) GPs. The cytoplasm was pushed at the periphery forming a thin rim and stains strongly as compared to secretory contents inside the gland. The mucous cells stained weakly with Hg-BPB method for general proteins and light pink with acid solochrome cyanine method for basic proteins and with ninhydrin-Schiff method for protein bound - $NH_2$ groups. With other histochemical techniques the contents of the mucous cells general, do not show positive reactions for the protein end groups.

## Plate I

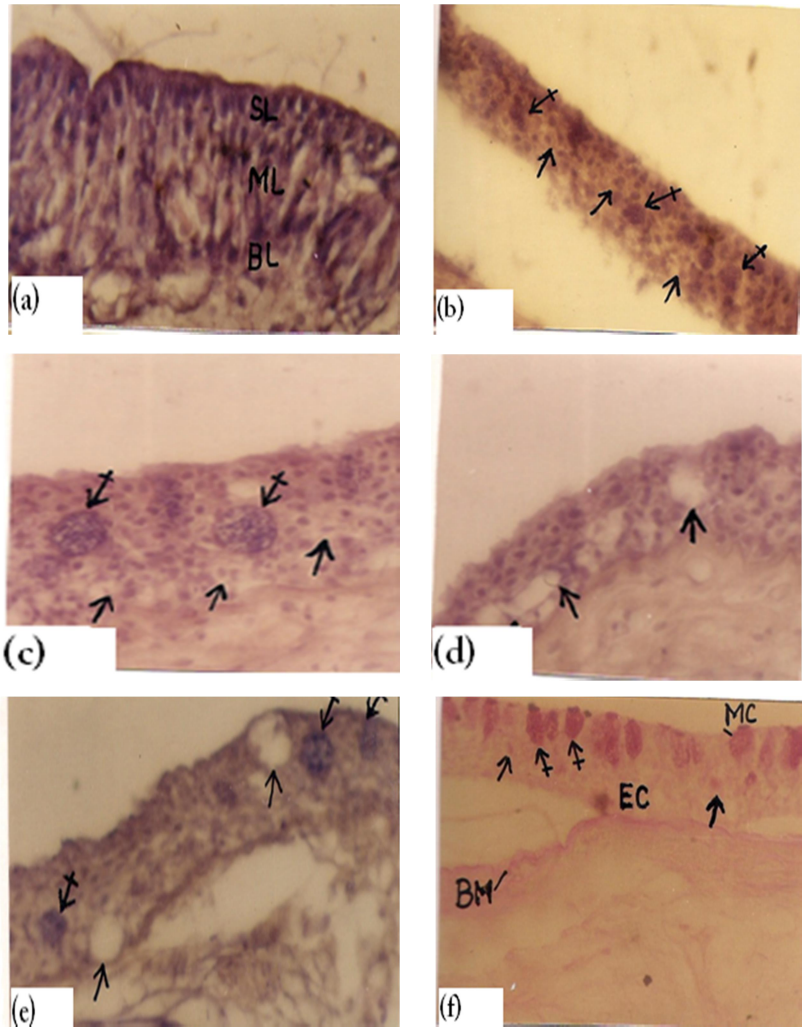


**PLATE I (a)-(f) Photomicrographs of the transverse sections of the gill arch epithelium o*f Anabas testudineus*at different duration of 2, 4-D treatment. MC, mucous cell; AC, acidophil cell; BM, basement membrane; EC, epithelial cell; CL chloride cell.**

(a) Showing superficial (SL) and middle layer (ML) epithelial cells polyhedral in outline and basal layer (BL) epithelial cells cuboidal in shape with a prominent centrally placed nucleus (Control, HE)x400.

(b) Showing degenerative changes in epithelial cells (arrow) and, appearance of chloride cells (barred arrow) (96h, HE)x400.

(c) Showing epithelial cells undergoing necrosis, in general appear characteristically dilated and vacuolated (arrow). An increase in dimension of chloride cell is also observed (barred arrow) (5 d, HE)x400.

(d) Showing an increase in vacuolization (arrow) (6 dHE)x400.

(e) Showing vacuolization (arrow) in epithelial cells and reduction in size of chloride cells (barred arrow). (7 d HE)x400.

(f) Showing positive reactions for neutral glycoproteins in the epithelial cells (EC), basement membrane (BM), and mucous cells (MC). Note the presence of glycogen granules (arrow) and strong reactions for neutral glycoproetins in few mucous cells (barred arrow) (Control; PAS)x 400.

## Plate II

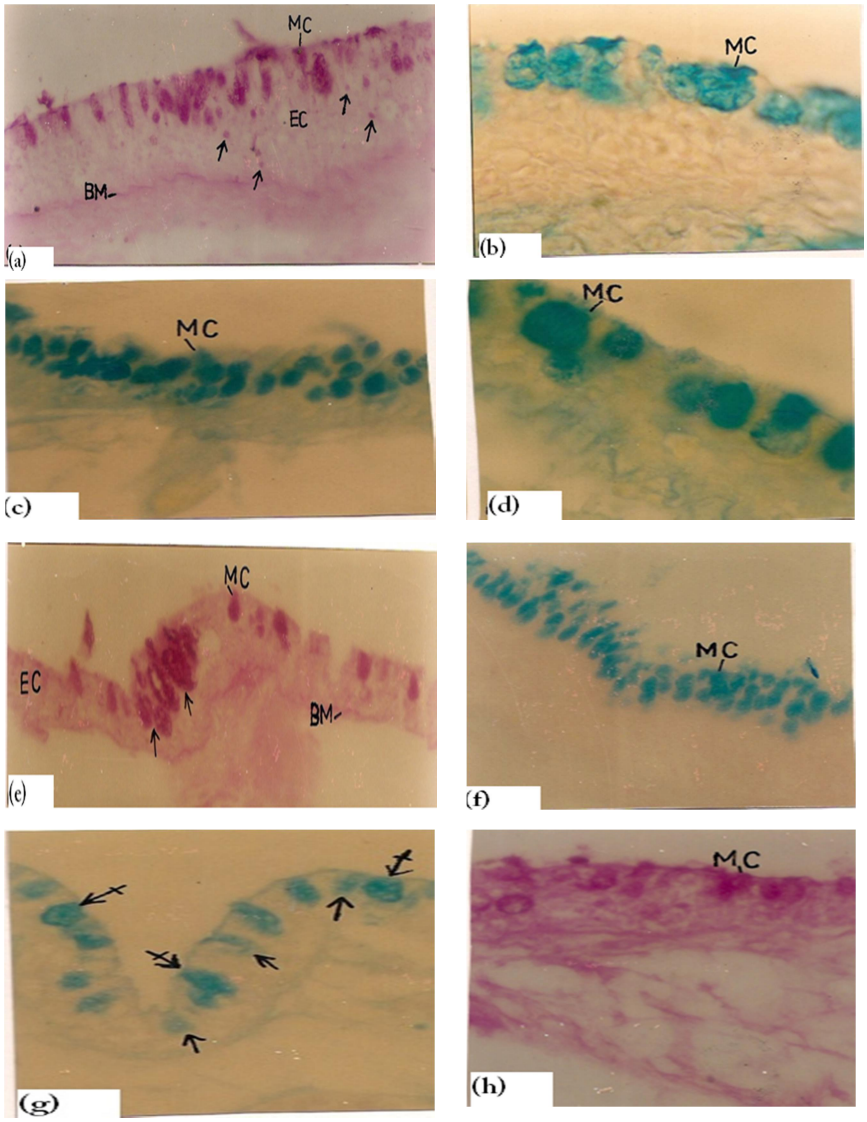


**PLATE II (a)-(h) Photographs of the transverse sections of GAR of *Anabas testudineus* at different durations of 2, 4-d treatment.**

(a) Showing strongly positive reactions for neutral Gps in mucous cells (MC), weak reaction in epithelial cells (EC) and moderate reaction in basement membrane (BM). Note an increase in number of glycogen granules dispersed in epithelium (arrow) (6h; PAS)x400.

(b) Showing roughly round mucous cells (arrow) which appeared relatively clise to each other (12h; AB)x1000.

(c) and (d) Showing enormous increase in the number of mucous cells (MC) [48h; (c) AB x400, (d) AB x 1000].

(e) Showing positive reactions for neutral GPs in mucous cells (MC), epithelial cells (EC) and basement membrane (BM). Note few mucous cells lie in clusters (arrow) (72h; PAS)x600.

(f) Showing an increase in the number of (MC) stain weakly (arrow) however, most of the mucous cells showed strong reactions for acidic Gps (barred arrow) (7d; AB)x600.

(g) Showing decline in the number of mucous cells (7d, AB pH2.5)x400.

(h) Showing evacuation of mucous cells (9d, PAS)x400.

Basement membrane is a thin, non-cellular membrane, on which gill arch epithelium rests. In HE and PS, it stains weakly pink showing eosinophilic nature. This membrane stains black in VHE. The basement membrane of GAR stains strongly magenta with PAS, with and without prior diastase/saliva treatment blocked b prior acetylation and restored by deacetylation. The basement membrane remained unstained with AB at pH 2.5 and AB at pH 1.0. These reactions suggest that basement membrane, in general, contain neutral GPs, and are absent. The basement membrane shows moderate reaction with Hg-BPB method for general proteins, weak reaction with acid solochrome cyanine method for basic proteins and ninhydrin-Schiff method for protein bound-$NH_2$ groups. With the other histochemical techniques, basement membrane does not show positive reactions for protein end groups. Mitochondria rich chloride cells also called ionocytes were not observed in GAR control fish.

### 3.2 Herbicide Treatment

At 3h, 6h, 12h, and 24h treatments there was no significant change in epithelial cells. At 48h treatment epithelial cells showed degenerative changes and inter-cellular spaces appeared dilated and prominent. In subsequent treatments (72h, 96h, 5d, 6d, 7d, 8d and 9d) epithelial cells undergoing necrosis in general appeared characteristically dilated and vacuolated (PLATE I b,c,d,e).

Epithelial cells responded quickly to the dye treatment, however this reaction persisted till the end of the experiment. The reaction of these cells, in general, is reflected through abrupt change in their intensity of staining which is negative for acid glycoprotiens in control but shows weakly positive reaction for the same at 3h exposure which remained unaltered at 12h, 24h, 48h, 72h and 5d exposures. Further at 6d, 7d, 8d, 9d and 10d exposures, epithelial cells did not give positive reaction acid glycoproteins (GPs). At 3h exposure no significant change was observed in the number of glycogen granules, however, at 6h treatment there was increase in number of glycogen granules dispersed in the whole thickness of epithelium which was further intensified at 24h treatment. There was gradual decline in number of glycogen granules in subsequent treatment. The epithelial cells of GAR, in general, did not show significant changes in the histochemical characterization, as well as in the intensity of the reaction or neutral glycoprotein and protein moieties at different durations of the dye treatment.

At 3h and 6h treatments acidophil cells of GAR did not show significant changes, however, appeared polygonal with round, centrally placed nuclei. At 12h exposure acidophil cells of GAR lied close to each other, their nuclei appeared strongly eosinophilic, showing degenerative changes. At 24h, 48h, 72h, and 96h exposure the size of acidophil cells was gradually reduced. At 5d and 6d acidophil cells were not clearly distinguished; however large vacuoles were present in the GAR. Almost similar condition persists in the subsequent treatments (7d, 8d, 9d, and 10d). Necrotic acidophil cells appeared few in number and were located at irregular intervals showing a gradual decline in their number.

Acidophil cells, in general, did not show significant changes in the histochemical characterization, as well as in the intensity of the reactions for carbohydrate and protein moieties at different durations of the treatment however; in control they showed relatively strong reactions with Hg- BPB Method for general proteins and with acid-solochrome cyanine method for basic proteins.

Mucous cells showed quick responses in terms of their shape, size and density. At 3h exposure mucous cells at GAR appeared elongated. At 6h treatment mucous cells in superficial layer became enlarged and acquired flask shape. In addition to flask-shaped mucous cells, some round mucous cells were also discernible in middle layer (PLATE II a). At 12h exposure mucous cells in GAR became round and appeared relatively close to each other (PLATE II b). At 24h treatment a few small round mucous cells were observed arising from basal layer epithelial cells. At 48h exposure there was enormous increase in the number of mucous cells occupying middle and superficial layer having mostly round shape (PLATE IIc,d). At 72h exposure mucous cells at some places on the GAR lied close together to form clusters (PLATE II e,f). At 96h treatment there was instant decline in the number and size of mucous cells.At 5d and 6d exposures there was tremendous increase in the number of mucous cells occupying middle and superficial layer. In subsequent treatment gradual decline in number of mucous cells was observed (PLATE II g,h).

Mucous cells stain moderately at 3h, 6h, 12h and 24h treatment, and show no increase or decrease in the intensity of reactions for acidic and neutral GPs. Mucous cells stain strongly at 48h, 72h and 96h exposure, showing gradual increase in the intensity for acid GPs at these durations. At 5d, 6d, 7d, 8d, 9d and 10d exposures gradual decline in the intensity of reaction for acid GPs is observed. No significant change in histological organization was noticed in basement membrane at different durations of treatment.

There was slight increase in the intensity of reaction for acidic GPs. It did not stain for acid GPs in control; on the contrary, it showed weak reaction for acid GPs at 3h, 6h and 12h exposure. It stained moderately for acid GPs at 24h treatment however, it showed gradual decline in the intensity of acid GPs in the subsequent exposures at 48h, 72h, 96h, 5d, 6d, 7d, 8d, 9d and 10d. It showed moderate reaction for neutral GPs at 3h, 6h and 12h treatment as compared to strong reaction in control, however at 24h, 48h, 96h, 5d, 6d, 7d, 8d, 9d and 10d it showed strong reaction for neutral GPs. The basement membrane, in general, does not show significant changes in the histochemical characterization, as well as in the intensity of the reactions for protein moieties at different durations of the herbicide treatment.

Chloride cells were observed in GAR at 96h, 5d, 6d, 7d treatment (PLATE I b,c,d,e), however, for rest of the duration they remain absent from the tissue completely. These cells were few in number and frequently extend deep into the middle layer of GAR. At 96h exposure these cells appeared small in size (PLATE I b) however, their size increased at 5d (PLATE I c) and 6d (PLATE Id) exposure which on further treatment at 7d (PLATE I e) declined. After 96h exposure their number was reduced significantly at 5d and 6d treatment.

The chloride cells were small, almost spherical with slightly eosinophilic cytoplasm which appeared to be homogenous or finely granular in HE at 96h exposure (PLATE I b). The cytoplasmic contents of these cells were packed with numerous spherical mitochondria which were stained black in FLEMMING'S (without acetic acid) method, HEIDENHAIN'S and REGAUD'S iron taematoxyline methods. The chloride cells gave weak PAS positive reaction for GPs which resist one hour treatment with saliva/diastase at 37$^{o}$C. The cells remained unstained when treated with Schiff's reagent without prior oxidation with periodic acid indicating that the PAS positive reaction was not due to free aldehydes.

Degenerative changes in epithelial cell of gill arch, different durations of herbicide exposure in *Anabas testudineus* in present study are interesting. Phola-Gubo and Adam (1982) in the epidermis of juvenile *Salmogairdneri* exposed to anionic detergent, have reported the degeneration of epithelial cells, which at electron microscopic level, is marked by the degeneration of cell organells-mitochondria, is marked by the degeneration of cell organelles-mitochondria, golgi cisternae, loss of nuclear membranes and vacuolization of cytoplasam. Abel and Skidmore (1975) and Abel (1976) in the gills o*f Salmogairdneri* and *Salmotrutta*, respectively pointed out an extensive evidences of cellular death including nuclear pyknosis, cytoplasmic disintegration and the wide-spread sloughing of the epithelial cells at an early stage.

The present investigations show an enlargement of intercellular spaces between the epithelial cells during different exposures of the herbicide treatment. Enlarged intercellular spaces have also been reported in the epidermis of juvenile *Salmogairdneri* under the influence of an anionic detergent (Phola-Gubo and Adams, 1982), in *Heteropneustes fossilis* during repair of cutaneous wounds (Mittal, et al. 1978), in *Barvussophore* subjected to hyper osmotic stress (Agarwal, et al. 1979) in the stressed fish or those with parasitic or virus infections. (Whitear and Mittal 1986) in *Salmogairdneri* and *Salmotrutta* gills under the influence of anionic detergent (Abel and Skidmore, 1975, Abel, 1976) in the mucosal side of frog urinary bladder under osmotic stress (Chevalier, et al. 1974). Spearman (1968) considered that the fine spaces containing tissue fluid between the epidermal cells in amphibians constitute an important pathway for movement of nutrient and respiratory gases through the skin. The enlarged intercellular spaces have also been correlated to act as passage of nutrient (Agarwal et al. 1979) and in the osmotic balance and oxygen supply for the general requirements and notably for the high metabolic rhythm of the epidermal cells (Lodi and Bani, 1971). Further investigations are, however, needed to ascertain the functional significance of the enlarged intercellular spaces in the gills under stress.

Degeneration of epithelial cells under the effect of herbicide prominent feature resulted in the appearance of intercellular spaces forming an invasion site for pathogens. Increase in density and dimension of mucous cells containing sulphated GPs on gill arch at different exposure of herbicide of slimy covering over the gill arch present investigation is significant. Sulphation of complex carbohydrates has also increased resistance to their enzymatic breakdown by bacterial glycosidase (Mian et al. 1979; Tasi et al. 1992) to play a role in defense against pathogens (Solanke and Benjamin, 1982) and to prevent the proliferation of pathogenic micro-organisms on the epithelial surfaces has been advocated by Tsukise and Yamada (1981), Suprasert et al. (1986, 1987). Mittal et al. (2003) in operculum of *Garralamta* and Mittal et al. (2004) in operculum of *Lepidocephalichthys guntea* have also reported high proportions of sulphated glycoproteins conferring high resistance against pathogens to protect the fish The slimy covering consisting of acid glycoproteins in the gill lamellae of *Anabas testudineus*at 96h treatment may prevent entry of pathogens through intercellular spaces caused by degeneration of epithelial cells.

Although the function mucous on teleost gills is not fully understood (Hocutt and Tinely, 1985), mucous may also provide on the gills a defence mechanism against toxic substances such as heavy metals (Burton et al. 1972). The additional amount of mucous provides an uninterrupted protective coating as the slime is known to act as lubricant and provides mechanical protection, has osmoregulatory function, prevents colonization of pathogens, contains various types of compounds that can support several chemical and immunological reactions (Van Oosten, 1957, Fletcher and Grant, 1969, Rosen and Cornford, 1971, Cameron and Endean, 1973, Pickering, 1974) and is known to have detoxifying action against ambient toxicants (Arillo and Melodia, 1990). Heat stress also induces secretions of thicker mucous layer or chemically different mucous which have lower oxygen permeability (Jacobs et al. 1981, Hocutt and Tinely, 1985). Jacobs et al. (1981) hypothesized that hypoxia at elevated temperature was a function of morphological changes in gill structure resulting in reduced surface area for gaseous exchange, in addition to other possible cellular or sub-cellular events. Cold shock on the other hand induced milder or insignificant injury to the gills (Tinley and Hocutt, 1987).

Sudden appearance of mitochondria rich chloride cells, in the gill arch region of *Anabas testudineus*, is associated with the defence mechanism against the toxicant. Possibly the appearance of chloride cells in the GAR at 96h, 5d, 6d and 7d of the herbicide is to protect the fish from the irritant present in the environment, either by providing energy or by faciliting the tissue to excrete nitrogenous or other toxic wastes by active ion extrusion method. The identity of cells responsible for ion transport was suggested

by Keys and Willmer (1932) who observed the presence of certain spherical acidophilic granulated cells-the chloride secreting cells in the gills epithelium. Chloride cells are found in good numbers in the gills of many air-breathing fishes (Munshi, 1964; Hughes and Munshi, 1979).

In most teleosts the so called chloride cell occurs in two readily distinguishable forms, viz. active and inactive. The active chloride cells are rich in mitochondria that are typically small and rounded inactive type of chloride cells possess an electron transparent cytoplasm and a large basally located nucleus, both inactive and active type of chloride cells have been reported from the gills of Hoplerythrinusa facultative air-breather of Amazon, *Monopterus cuchia*, *Clarias magur* and *Channa punctata* (Munshi, 1964).

Significant structural changes in the chloride cells occur when the fish is transferred from freshwater to sea water (Karnaky et al; 1976) (Dunel and Laurent, 1973, Dunel, 1975) and vice-versa (Copeland 1948; Pettengill and Copeland, 1948; Getman, 1950; Threadgold and Houston, 1964; Conte, 1969; Coten and Lin 1967; Olivereau, 1970; Dunel, 1975; Doyle, 1977; Laurent and Dunel, 1980; Pisam et al. 1980; Hossler, 1980 and Zaccone, 1981). These authors have clearly shown that chloride cells increase in size, number and exhibit darkening of cytoplasm when the fish is transferred from the freshwater to sea water, thus supporting the observation in present investigation that when *Anabas testudineus* is transferred from fresh water to test solution, favouring an increased ion loss, chloride cells develop on the gill arch epithelium. Increase in the number of chloride cells has been noted by Baker (1969) in some cases of heavy metal poisoning. Presumably the chloride cells increase in number and size to cope with the influx of herbicide. Matthiessen and Brafield (1973, 1977) observed increase in the number of chloride cells in the gills of stickle-black exposed to zinc. It is assumed that chloride cells which appear in the gill arch epithelium arise from existing basal cells. Subsequent decline in chloride cell numbers suggest that they, are the cells from which they arise, were being poisoned by the herbicide and their effectiveness in removing the herbicide can be lessened by long term exposure to high concentration.

## 4. Conclusion

These results emphasized that the use of herbicides has become a necessary evil for developing countries like India. Unfortunately herbicides lack target specificity and can cause severe, and long – lasting population effects on terrestrial and aquatic non – target species. Herbicides create a great economic loss through fish mortalities on one hand and on the other, can cause health hazards for those who utilize these fish. It is concluded that herbicides of almost all the groups do have acute toxic effects on various fish species. Therefore, it is recommended that the expensive use of herbicides should be avoided near waterbodies and applications should be judicious and rationalized.

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**Conflicts of Interest**

The author declares that there are no conflicts of interest.

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