INTRODUCTION

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Physiological changes and toxicological effects on the blood and the behaviour of fish exposed to various degrees and types of pesticides have been measured by several investigators (Abegg, 1950; Schiffman and Fromm, 1959 & Jackim et al., 1970). Furthermore, Liebmann (1966), pointed out that fish are the most important indicators for water quality and their long term exposure are of much greater importance than short term one.

Derek (1971) reported that some herbicides are generally less toxic to fish, although a few may constitute a potential danger in their normal use. However, Schultz (1972) studied the effect of herbicide "karmex" with different concentrations on *Cyprinus carpio* and found that the highest concentration (6,12 ppm) lead to coma and death. The experiment showed gross exophthalmus, abdominal enlargement hypotrophic and febrile liver, with considerable loss of body weight. The same author also, discussed the dynamics of the herbicide 2,4-Dichlorophenoxy acetic acid amine salt (2,4 - D) in fish. The fish were exposed to 0.5, 1 and 2 mg/l concentration of 2,4 - D for up to two months, residues were found in all fish tissues and organs. He also added, that the actual 2,4 - D content was negligible in muscles indicating that most of the residues consisted of one or more metabolites. Also Schultz and Whitney (1974) proved that among 60 samples of fish treated with 2,4 -D, 3 had

residue less than 0.01 mg/kg, 16 had more than 0.01 mg/kg and the remainder had no detectable levels. He also added, this herbicide apparently caused no ill effect on fish.

Walsh (1975) found that the only diagnostic changes produced in ***Oncorhynchus kisutch*** by herbicide 2,4 - D were congestion of the blood vessels within the brain.

Also the blood picture of fish is considered as a mirror for the effect of external or internal changes **(Reichenbach, 1966)**. Elevation in the erythrocyte number, haemoglobin concentration and haematocrit readings in ***Cyprinus carpio*** after application of 2,4 -D was reported by **Cope (1970)**.

According to **Finlayson** and **Faggella (1986)** survival, hemoglobin and hematocrit levels of ***Cyprinus carpio*** were significantly reduced after 14 days exposure to different concentration of the rice herbicide "molinate" and the mortality was occurred after 28 days of exposure. They added none of the molinate concentration significantly affected channel catfish ***Ictalurus punctatus*** survival or its hemoglobin level after 12 days,but significantly reduced after 28 days.

Pathological changes attributable to cadmium poisoning were observed in the ***fundulus heteroclitus*** after exposure to 50 ppm of the metal **(Gardner and Yevich, 1970)**, the circulating blood elements revealed

rapid and striking changes among cells of the eosinophil lineage and the abundance of eosinophils increased to a level approximating 45% above the normal count.

McLeay (1973) reported that the number of circulating immature erythrocytes increased in effluent exposed coho salmon ***Oncorhynchus kisutch*** in both the 12 hour and 25 day exposure. However, the circulating small lymphocytes decreased in its number markedly after 12 hour exposure. He also added that after the prolonged exposure, the number of small lymphocytes returned to normal, while the number of circulating neutrophils increased. However, significant leucopenia as the result of a large reduction in lymphocyte number was observed in rainbow trout ***Salmo gairdneri*** exposed to 301 ugl copper for 24 hour **(Dick and Dixon, 1985)** while after chronic exposure to 118 ugl copper for 16 weeks, neutrophilia was observed but other blood values had returned to the control levels.

Drezewina (1911) reported the rare presence of basophilic granulocyte in the blood of a few species of the 68 fish species studied and he described the occurrence of eosinophilic granulocytes in a large number of species. **Saunders (1968)** described the range and morphology of thrombocyte in over than 225 species of fish and found great variations in shape, cell size and staining characteristics of the nucleus and cytoplasm among the various species.

Fey (1966 a,b,c) studied the esterases activity in ***Carassius auratus***, ***C.carassius***, ***Cyprinus carpio*** and ***Rutilus rutilus***, and they found that lymphocytes are weak; monocytes, eosinophils and thrombocytes (except in ***R.rutilus***) are weak to moderate, while neutrophils are moderate and the ***R.rutilus*** thrombocytes are strong in esterase activity.

Neutrophils were absent from cyprinodonts while the eosinophil considered to be the only granulocyte present in the circulating blood of ***Fundulus heteroclitus***, ***F. majalis*** and ***Cyprinodon variegatus*** forming 3%, 5.3% and 2.3% respectively of all circulating leucocytes **(Gardner and Yevich, 1969)**. Although **Kelenyi and Nemeth (1969)** described the fine structure of neutrophils, in the carp ***Cyprinus carpio***, tench ***Tinca tinca*** and river bleak ***Alburmus alburmus*** and found this cell not staind byperoxidase. They also identified eosinophil in the blood of carp, but neutrophils was the commonest granulocyte in this species. The presence of periodic acid schiff (PAS) postivity in the neutrophil and basophil of the spanish mackerel, ***Scomber japanicus Colias*** was reported by **Pitombeira and Martins (1970)**.

Blaxhall and Daisley (1973) found that thrombocytes of brown trout ***Salmo trutta*** were negative for PAS, sudan black - B and peroxidase, in contrast to the positive reaction of these enzymes in neutrophils. They mentioned that the monocytes were absent in this species.

Ellis (1976) found in plaice ***Pleuronectus platessa*** that peroxidase reaction was positive in neutrophils and monocytes. Acid phosphatase was positive in all leucocytes except the lymphocyte. All leucocytes showed strong lipid content, alkaline phosphatase was positive in neutrophils. They also added that PAS positivity was observed in lymphocytes, thrombocytes and neutrophil.

The cytoplasmic granules of neutrophil in channel catfish ***Ictalurus punctatus*** have been shown to exhibit peroxidase activity while monocytes and thrombocytes did not exhibit reactivity, eosinophils and basophils are absent or extremely rare (Cannon et al., 1980).

Elarifi (1982) reported peroxidase and acid phosphatase staining in neutrophils of whiting ***Merlangius merlangius*** but not in lymphocytes. However, tests on neutrophil alkaline phosphatase and for all the three enzymes in monocytes and thrombocytes were inconclusive.

Page and Rowley (1983) observed that the granulocyte of lamprey ***Lampetra fluviatilis*** were positive for alkaline phosphatase and negative for peroxidase, neutrophils and lymphocytes were acid phosphatase positive. The acid esterase and acid phosphatase staining in brown trout lymphocytes which are separated into the two fractions T and B showed equal enzyme activity. Thus

they suggested that fish lymphocytes do not separate into T and B cells according to density but the fractions may indicate groups of cells at different stages of maturity.

Leucocytes of ***Scyliorhinus canicula*** contain esterase, alkaline and acid phosphatase which are strong in heterophil, moderate in eosinophil and thrombocyte, and weak in monocytes and lymphocytes **(Mainwaring and Rowley, 1985)**. According to **Parish et al.(1986)** the lymphocytes and thrombocytes are the commonest cell types among the blood leucocytes of the dog fish, ***Scyliorhinus canicula***, peroxidase staining was detected in neutrophil, absent in monocyte, lymphocyte, eosinophil and basophil while esterase reaction was strong in monocyte, weak in granulocyte, absent in lymphocyte but PAS and acid phosphatase reaction were positive in granulocyte, negative in lymphocyte and all leucocyte showed little lipid content.

Hendrick et al.(1986) reported that the ***Cyprinus carpio*** has a circulating eosinophil cells strongly positive for peroxidase. They added that this cell contains dense electron granules while circulating neutrophils have less electron dense homogenous granules.

Hine et al.(1986 a) noticed the lack of peroxidase and alkaline phosphatase in most circulating neutrophils of ***Anguilla anguilla, A. australis*** and ***A.dieffenbachii*** but it was rich in acid phosphatase; eosinophils showed close similarities to eel neutrophils in enzyme content

but stained more strongly for peroxidase and acid phosphatase, although alkaline phosphatase was not observed. The eel granulocytes produce, contain and secrete large quantities of esterases, peroxidase reaction of eel monocyte was extremely weak or usually absent and this type of cells lack alkaline phosphatase, while lymphocyte lack peroxidase but acid phosphatase and esterases were heterogenous, most being negative. ***A. dieffenbachii*** lymphocyte very rarely contained acid phosphatase granules and thrombocyte cytochemistry was similar to that of lymphocyte. Also, Hine et al.(1986 b) identified the peroxidase positive cell in peripheral blood of ***Anguilla australis*** and ***A.dieffenbachii*** as eosinophil and as neutrophil in ***A.anguilla***, while in New Zealand eel, some granules of neutrophils are positive peroxidase while others are negative.

Acid phosphatase and alpha-naphthyl acetate esterase activity have been observed in lymphocytes of rainbow trout ***Salmo gairdneri*** (Blaxhall and Doggett, 1987).

In the Tilapia ***Oreochromis mossambicus***, PAS, alpha-naphthyl acetate esterase and acid phosphatase positive staining were reported in lymphocyte, monocyte and neutrophil while sudan black - B, peroxidase and alkaline phosphatase reaction were negative in lymphocyte and monocyte and positive in neutrophil. Two forms of thrombocyte were identified in this species with PAS

positive, while sudan black - B, acid and alkaline phosphatase, non specific esterase and peroxidase were negative (Doggett et al., 1987).

Hine et al. (1987) made an observation on the leucocyte enzyme cytochemistry of different fish species. They found that peroxidase was not found in blood granulocytes of holocephalans, uncommon and weak in eosinophils of elasmobranchs, variable in neutrophils and eosinophils of elopomorphs and clupeiforms, rare and weak in eosinophils of higher teleosts; alkaline phosphatase was strong in holocephalan fine granulocytes and occurred intermittently in teleost neutrophils, but in elasmobranchs it was most often seen in lymphocytes of dog fish; acid phosphatase was common in all leucocytes lysosomes. Esterases were strong intra- and extracellular in elasmobranch, granulocytes often strong in elopomorphs, but of variable strength in higher groups.

The major role for prolactin in teleostean fishes is related to osmotic regulation in fresh water, and even in man prolactin release can be induced following alterations in blood osmotic pressure (Butler, 1966). The same author declared that fresh water eels Anguilla spp. and gold fish *Carassius auratus* exhibit increase in sodium loss after hypophysectomy. Another major effect of prolactin in teleosts is its influence on water permeability, hypophysectomy of fresh water adapted *Salmo trutta*, is followed by a decrease in water

turnover that is returned to normal by prolactin treatment **(Oduleye, 1975)**. A third important action of prolactin in teleosts is the effect on water excretion via the kidney, prolactin treatment causes an increase in glomerular filteration rate indirectly by altering the anatomical relationship between the vascular system and the kidney **(Lam and Leatherland, 1969)**.

Ball and Ensor (1969) suggested that in Gambusia and ***Fundulus kansae*** prolactin may act like ACTH in stimulating the interrenal gland but experimental evidence showed that ovine prolactin do not stimulate the interrenal in ***Fundulus heteroclitus*** or ***Poecilia latipinna*** and of course in both species ACTH did not mimic prolactin in promoting electrolyte conservation. Eel ***Anguilla anguilla*** after hypophysectomy suffers a slow reduction in plasma sodium, potassium and calcium which can be retarded by maintenance therapy with ovine prolactin **(Olivereau and Chartier - Baraduc, 1966)** .

Spieler and Meier (1976) noted that serum prolactin concentration in serially sampled gold fish ***Carassius auratus*** did not vary significantly between 30 seconds and 3 minutes after initial capture. A marked decrease in prolactin concentration was noted 9 - 17 minutes after initial capture followed by a recovery at 30 - 48 minutes. Although there was a circadian variation in prolactin concentration, the pattern of response to capture and sampling did not vary significantly among the times tested .

It is documented that insulin is one of the most important anabolic hormone in vertebrates which directs the metabolic fluxes towards the storage of energy in the form of glycogen or lipid **(Plisetskaya et al., 1987).**

Plasma immunoreactive glucagon (IRG) were analysed in ***Cyprinus carpio*** subjected to short and long term temperature changes, (11 hours and 21 months), the high temperature (28 °C) produced significant increase in IRG in both short and long term experiments but low temperature did not provoke any changes **(Blasco et al., 1988).**

Many workers have used blood corticosteroid levels as indicators of stress because of the extreme sensitivity of the hypothalamo - pituitary - interrenal (HPI) axis **(Ball and Olivereau, 1966).** Also, elevation of plasma corticoid concentration appears to be in many vertebrates a generalized response to a variety of disturbances and potentially, corticoids might be used as an indicator of stress in fish **(Selye, 1976).**

Spieler (1974) reported an elevation (67%) in **plasma** cortisol in gold fish ***Carassus auratus*** observed from 10 to 22 minute after initial net capture and restraint. However, he observed no significant elevation in adreno-corticosteroids during restraint from 30 second to 10 minute.

Serum cortisol was found to constitute 77.6% of the adrenocorticosteroids in gold fish ***Carassius auratus*** (Fryer, 1975), after swimming in shallow water or a thermal shock without handling disturbance, circulating levels of corticosteroids were significantly higher than undisturbed fish.

Plasma corticoid concentrations in juvenile chinook salmon ***Oncorhynchus tshawytscha*** netted and confined in a small live-cage elevated from approximately 100 ng/ml to about 500 ng/ml in 24 hour, then fall to 250 ng/ml at 48 hour. In juvenile of the same species dip netted into a bucket containing aerated water and sampled serially at 90 second intervals, plasma corticoids increased from more than 10 ng/ml to 100 ng/ml in 20 min. In the same study juvenile cutthroat trout ***Salmo clarki Clarki*** acclimated to 13 °C and subjected to a rapid increase in water temperature 26 °C, plasma corticoid concentration increased from about 20 ng/ml to 70 ng/ml in 25 min and remained elevated for more than 3 hours (Strange et al., 1977).

Mazeaud et al. (1977) found high corticosteroid concentrations after struggling and hypoxia in male coho salmon ***Oncorhynchus kisutch***, while in females, there was no difference in plasma corticosteroid concentration after 10 minutes of stress. However even in unstressed females, the corticosteroids levels were higher than

stressed or unstressed male. They suggested that this difference reflects the increased interrenal activity in females.

The response of the brown trout ***Salmo trutta*** to a single, short incidence of handling stress was monitored for one month post stress (**Pickering et al., 1982**), there was a significant elevation in plasma cortisol of the handled fish and the peak of cortisol was 130 ng/ml at 2 hour post handling which declined to control values within 4 hour post handling .

Wedemeyer (1969) reported elevation of serum cortisol in coho salmon ***Oncorhynchus kisutch*** stressed for 15 minute at + 7 °C, temperature change or by 15 minute forced exertion, marked reduction of kidney ascorbic acid observed, which reflects activation of the pituitary interrenal axis and is thus ACTH mediated. Plasma cortisol increased when ***Tilapia aurea*** were exposed to 11 - 12 °C for 60 minutes, 11 days, and 5 week period, acclamatization to 35 °C did not significantly affect cortisol compared to the control, 22 °C (**Kindle and Whitmore, 1986**).

Hill and Fromm (1968) reported significant elevation of plasma cortisol in rainbow trout ***salmo gairdneri*** exposed to environmental hexavalent chromium for one week which returned to normal level after 2 and 3

weeks. However, larger doses (20-30 mg/l) for three days, elevated plasma cortisol and returned to control levels after 6, 7 and 10 days exposure.

Singley and Chavin (1971, 1972) recorded increase of 375% and 600% in cortisol levels of gold fish ***Carassius auratus*** sampled 30 seconds, and 15 seconds respectively after the addition of crystalline sodium chloride to the aquarium water.

When socheye salmon ***Oncorhynchus nerka*** were exposed to cupric sulfate in fresh water aquaria for 1 - 24 hour, cortisol levels were significantly higher than control concentrations after one hour of exposur, and fish exposed to 10^{-5} molar copper died between 8 and 24 hour **(Donaldson and Dye, 1975)**. Furthermore **Schreck and Lorz** **(1978)** recorded that exposure of coho salmon ***Oncorhynchus*** ***Kisutch*** to copper produced a marked dose dependent serum cortisol elevation and stressing salmon with sublethal levels of copper or handling, plus close confinement, resulted in ideal compensation returned to prestress levels. They also reported that treatment with cadmium did not elicit a cortisol elevation even in moribund fish. However, exposure of rainbow trout, ***Salmo gairdneri*** to acid PH 4.8 for 72 hour caused no mortality, significantly elevated blood cortisol level, addition of aluminum to this exposure caused 100% mortality with a mean survival time of only 27 hour and there was a sharp rise in plasma cortisol which was greatly increased as the fish approached death **(Goss and Wood, 1988)**.

Mature ***Salmo gairdneri*** receiving sublethal doses of organophosphorus insecticide endrin for 163 days and then forced to swim for one hour, trout given 14.5 ug/kg or more had lowered serum cortisol levels whereas the lowest dose had elevated cortisol level **(Grant and Mehrle, 1973).**

According to **Forlin** et al. **(1986)** the strict ion regulation is necessary for aquatic organisms if they are to maintain water and ion haemostasis and disturbances, in ion regulation, induced by pollutants, are manifested by altered plasma ion concentrations. **Eisler and Edmunds (1966)** reported a fall in liver sodium content and concomitant elevated serum sodium in a marine teleost northern puffers ***Sphoeroides maculatus*** exposed to the organophosphorus "endrin".

Gold fish ***Carassius auratus*** exposed to "endrin" in the 4.3, 14.3, 43 and 143 ug/kg, showed significant elevated serum sodium except for the 14.3 ug/kg dose **(Grant and Mehrle, 1970)**, while the potassium values did not differ significantly. Also, **Grant and Mehrle (1973)** recorded higher serum sodium level in rainbow trout ***Salmo gairdneri*** similarly treated with endrin and direct but low degree of correlation existed between serum sodium and the endrin dose, while the increase in potassium level was non significant.

Larsson et al. (1976) reported that flounders *Platichthys flesus L.* exposed for 15 days to cadmium in concentration of 0.1, 1.0, and 10 mg/l caused an elevation of plasma sodium level in the two lowest cadmium concentrations, however the plasma potassium content was reduced at all. Goss and Wood (1988), found that rainbow trout *Salmo gairdneri* exposed to acid PH has significant decrease in plasma sodium over the first 7 hour, thereafter, the levels were more or less stable, they found that addition of aluminum to this exposure cause marked decrease in plasma sodium.

Glucose level in mature rainbow trout *Salmo gairdneri* receiving sublethal doses of insecticide "endrin" for 163 days and then forced to swim were increased about 50% by 145 ug/kg, but was not effected by lower doses. Forced swimming elevated serum lactate in all groups, but there was no correlation between the increase and endrin dose (Grant and Mehrle, 1973). Silbergeld (1974) reported significant glucose level in fresh water fish *Etheostoma nigrum* exposed to "dieldrin" insecticide for 5 days and the increase persisted for 10 days. After 15 and 30 days of exposure, the glucose levels remained elevated significantly. The Indian catfish *Heteropneustes fossilis* which exposed to a high sublethal concentration of "methyl parathion" for 96 hours showed an elevation of blood glucose after 3 and 6 hours, blood lactate was also elevated after 3 hours but hypolactaemia was observed after 48 and 96 hours of treatment (Srivastava and Singh, 1981).

The level of plasma glucose increased in coho salmon ***Oncorhynchus kisutch*** exposed to effluent for 12 hour, and decreased in fish exposed for 25 days **(McLeay, 1973)**.

Larsson et al. (1976) found that flounder fish ***Platichthys flesus L.*** exposed to subacute concentrations of cadmium caused elevation of blood glucose with a maximum change (75%) at 10 mg cd/1, but diminshed blood lactate was reported. Although, **Dheer et al.(1986)** reported a steady decrease in glucose level of ***Channa punctatus*** when exposed to 1.2, 3.2, and 6.2 gm/l sodium chloride for 8 weeks. He also added, the decrease was highly significant for the two higher concentrations but not for the lower ones and two weeks of recovery under control conditions restored the glucose levels almost to control values.

Rainbow trout, ***Salmo gairdneri*** exposed to acid PH for 72 hours showed no change in blood glucose levels, while there was a mild increases in lactate concentrations and addition of aluminum to this exposure caused hyperglycemia and lactate accumulation **(Goss and Wood 1988)**.

In rainbow trout ***Salmo gairdneri*** after 20 minutes of hypoxia, there was a slight increase of blood glucose **(Mazeaud et al., 1977)**, while in carp, 2 hours of hypoxia

led to a marked increase in blood glucose, the highest values recorded after 10 hour at 10 °C, recovery occur within 26 hour.

Perrier et al. (1978) reported marked increase of plasma lactate in rainbow trout ***Salmo gairdneri*** at the end of the hooking period, maximal level was seen after 5 minute recovery and remained unchanged for 4 hour, also plasma glucose increased gradually to arrive the top levels at 4 hour and 16 hour and returned to the basal level after 64 hour.

Pickering et al. (1982) studied the response of the brown trout ***Salmo trutta*** to a single, short handling stress monitored for one month post stress. He reported that there was a significant elevation in plasma glucose and lactate of the handled fish. The same author found that plasma glucose was significantly greater at 2 and 4 hours and significantly lower at 30 hour in the handled fish, recovery and stabilization of plasma glucose levels occurred at 72 hour post handling; lactate levels elevated at 2 and 30 hours of handling, but reduced at 8 hours post handling.

Plasma glucose increased when ***Tilapia aurea*** were exposed to 11 - 12 °C for 6 minute, 11 days, and 5 week period, and acclimation to 35 °C did not significantly affect glucose compared to control 22 °C (Kindle and Whitmore, 1986).