CHAPTER VII

DISCUSSION
Heavy metals are being introduced into aquatic environment through industrial processes, sewage disposal, soil leaching and rainfall. These metals are relatively toxic even at fairly low concentrations and affect the survival of fishes and other aquatic organisms. Stebbing and Fandino (1983) reported that, the biological effect of heavy metals in the aquatic environment is adverse mainly due to their complexation. There is a paucity of information on the combined effect of heavy metals on fishes. The present work is a toxicological evaluation of copper, zinc and nickel and their mixtures on *Gambusia affinis*.

The static bioassay tests have been widely used for evaluation of the impacts of these toxic chemicals on aquatic life (Doudoroff et al., 1951). The literature concerning procedures and results of bioassay testing of fish and other organisms was reviewed by Maccorowski et al. (1980). The effects of pollution on freshwater fish from around the world were reviewed by (Stephon and Mount, 1973), Sphehar et al., 1980), Phipps et al. 1984), Roush et al., 1985), Kline et al., 1987), and Pickering et al., 1989), Bellavere and Gorbi (1981) conducted 96 hr static bioassay with Cr, Cd and Cu on three species of aquatic organisms and zebra fish (*Brachidanio*
rerio) of the three metals tested Cu was the most toxic. The 96 h LC<sub>50</sub> values for Cr, Cd and Cu were 58.5, 4.35, 0.21 mg/l respectively. Static bioassay tests at 25°C using Rasbera daniconius neclegeriensis were performed by Durve et al. (1980). The LC<sub>50</sub> values ranged from 2.1 mg/l (12 h test) to 0.20 mg/l for 96 h tests. Kaur and Virk (1980) reported that a concentration of 3.5 to 4 mg CuSO<sub>4</sub>/l resulted in 50% abnormal hatchlings of carp eggs. The 96 h LC<sub>50</sub> values for Claseas gasiepinus (Burchell) ranged from 1.29 ml to 1.38 mg/l Cu (VanDer Merwe et al., 1993). The acute toxicity of chelated Cu to juvenile red drum (Sciaenops ocellatus) was 0.52 mg/l Cu at 96 hr. The toxicity of Cu was influenced by temperature, pH (Liepolt and Weber, 1958), hardness (Tabata, 1969), salinity (Birdsong and Avault, 1971), age and size (Gardner and La Roche, 1973). Acute toxicity of zinc was affected by the interaction of water hardness and pH 96 hr LC<sub>50</sub> value for total zinc ranged from < 0.14 mg/l in alkaline soft water to 3.20 mg/l in acidic hardwater to the brown trout (Salmo trutta) (Everall et al., 1989). Zn was fatal to the developmental stages of Cypnrius carpio (L) and Cirrhinus mrigala (Hamilton) (Sharma and Sharma, 1994, 1995). Ni at its lethal concentration interfered in the carbohydratae metabolism of Oreochromis niloticus (Alkahem, 1995) and
protein metabolism in *Cyprinus carpio* L. (Sreedevi *et al.*, 1992). Pickering and Henderson (1966) compared the toxicities of nickel chloride in water of two hardness characteristics (total hardness 20 mg/l and 300 mg/l as CaCO₃). The 96 h LC₅₀ was 4.9 mg/l for fathead minnow (*Pimaphales promelar*) and 5.3 mg/l for bluegill sunfish (*Leponis macrochirus*) in soft water and 43.5 mg/l and 39.6 respectively in hardwater. Brown and Dalton (1970) measured the 48 h LC₅₀ to rainbow trout of Cu, Zn and Ni and mixtures of the three, in a hardwater (240 mg/l as CaCO₃) in the ratio of 1:1:1 of the respective proportions of their 48 h LC₅₀ values and found that the joint action of such a mixture was approximately additive. Weinstein and Anderson (1978) using zebra fish (*Brachydanio resia*) showed that copper and nickel were more than additive at lethal levels, and also reported (Anderson *et al.*, 1979) that this was markedly affected by the relative proportion of each in the mixture: the higher the proportion of nickel, the lower the percentage mortality. However, Muska and Weber (1977) reported briefly that, in 7-day tests on the effect of copper and nickel and their mixture on the growth and food consumption of juvenile guppy at 7°C and 25°C interaction between copper and Ni was slightly more than additive with a restricted ration. In the present study, the 96 hr LC₅₀
values for male were 4, 4, 140 and 240 mg/l of Cu, Zn and Ni respectively and the 96 hr LC\textsubscript{50} value for female was 2.15, 155 and 285 mg/l of Cu, Zn and Ni respectively. The moderately hardwater (70-110 mg/l CaCO\textsubscript{3}) that was used affected the toxicity of these metals similar to the results of the other workers. Between male and female for Cu, the deviation between parallelism of dosage mortality plots was significant and was also significant for zinc and nickel exposure.

This is due to the independent mode of toxic action that is not similar in both male and female. However, females are more susceptible to copper as indicated by the potency ratio but when exposed to zinc there was no significant difference in its potency between sexes. For the acute exposure to Ni, male were more susceptible as compared to the female and the difference in potency was significant.

The 96 hr LC\textsubscript{50} for mixture of metals was also influenced by the presence of other metals. For Cu/Zn the LC\textsubscript{50} values were antagonistic and for female they were synergistic but the other mixtures of Cu/Ni, Zn/Cu, Zn/Ni, Ni/Cu and Ni/Zn, the toxicity of this mixture was antagonistic greater than additive and was significantly magnified. The antagonistic effect was also observed for the triple mixtures of Cu+Zn/Ni,
Cu+Ni/Zn, Zn+Ni/Cu and Zn+Cu/Ni. However for Ni=Cu/Zn exposure, there was a synergistic effect in female but antagonistic effect in male and in Ni+Zn/Cu exposure, there was synergistic effect for male and antagonistic effect in females.

The potency ratio and deviation in the parallelism of dosage mortality plots was affected by the size and weight of the fish. Acute toxicity is affected by the length and weight of the fish. Increase in size increased the LC$_{50}$ values of *Cyprinus carpio* when exposed to the lethal concentrations of mercury (Hg), lead (Pb), copper (Cu), nickel (Ni) and zinc (Zn) (Alam and Maughan, 1995). The toxicity of similar metal mixtures is additive or greater than additive for Colorado squawfish, bonytail and razorback sucker (Buhl and Hamilton, 1996). In the toxicity of individual metals and their mixtures it is evident that Cu and its mixture are more toxic than Zn and Ni. The toxicity of metals is also size and weight dependent. Acute toxicity of Cu to a freshwater fish *Lepidocephalichthyes guntea* of two different sizes were found to be significantly different. The 96 h LC$_{50}$ values of these fishes weighing 0.334 g and 1.29 g were 2.0 mg/l and 3.7 mg/l respectively (Bengeri et al., 1986b). Acute toxicity also
depends largely on water hardness. At low water hardness (12 mg/l) the incipient lethal concentration (ILC) of dissolved Cu to rainbow trout was not affected by a change in alkalinity of 10-15 mg/l (Miller and Mackay, 1980). However, same change in alkalinity in hardwater (98 mg/l) resulted in a 1.8 fold increase in the ILC. The present investigation was carried out in the moderately hardwater and the complexation of metals caused an antagonistic effect in most of the mixture of metals. The acute toxicity was affected by size in the Zn exposed fishes. The 96 h LC values were 65 and 77.5 mg Zn/l for fish (Labeo rohita) weighing 1.72 and 1.92 g respectively (Bengeri and Patil, 1986a,c). Susceptibility to Zn is largely species dependent. Smith and Heath (1979) found that goldfish were particularly tolerant with a 24 h LC$_{50}$ of 110 mg/l whereas the LC$_{50}$ for rainbow trout was only 5 mg/l. Stage of development also significantly influences toxicity. Average 96 hr LC$_{50}$s for alevins for steelhead trout and chinook salmon were 10-15 times greater than those for swintips (Chapman, 1978). Juvenile rainbow trout were about 3 times more resistant to Zn than eyed eggs (Sinley et al., 1974). The toxicity of nickel chloride was also affected by hardness of water. The 96 h LC$_{50}$ was 4.9 mg/l for fathead minnow (Pimephales promelas) and 5.3 mg/l for bluegill sunfish.
(Lepomis macrochirus) in soft water and 43.5 mg/l and 39.6 respectively in hardwater (Pickering and Henderson, 1966). The size and weight of the fishes is also an influencing factor in the toxicity of Ni as observed in carp, where the 72 h LC$_{50}$ for freshly fertilized egg and one day old larvae were 6.1 and 6.2 mg/l respectively (Blaylock and Frank, 1979).

In the study undertaken, the effect of metal and their mixtures on the rate of oxygen uptake and nitrogen excretion was investigated for male and female of G. affinis.

Fishes exposed to the lethal concentration of individual metals like Cu, Zn and Ni had decreasing rate of oxygen uptake from 24 hr to 96 hr exposure period in both male and female. In fishes exposed to mixture of two metals, the rate of oxygen consumption was relatively decreased as compared to that of control. However, the uptake was irregular and recovery was observed during the 72 and 96 hrs of exposure. In fishes exposed to triple combination of metals similar situation was observed. The initial decline recovered to certain percentage that, at later stages, was greater than the control in some mixtures. However, the consumption was irregular and also the efforts to recover were observed in certain combinations. This situation was observed both in male and female.
The oxygen consumption in catfish, *Mystus gulio* (Ham) exposed to heavy metals Cu and Zn was altered. Zn is found to be potent respiratory inhibitor than other heavy metals (Sultana and Devi, 1995). Similar alteration was observed in *Labeo rohita* exposed to Zn (Bengeri and Patil, 1986e) and *Lepidocephalichthyes guntea* exposed to Cu (Bengeri et al., 1986b). The decrease in oxygen uptake due to heavy metal exposure is due to mucus secretion which clogs the gills, Singh and Singh, 1979, and Rani and Ramamurthi, 1987). Higher concentration of heavy metals might have induced the fish to consume more oxygen particularly during the later part of the tests. This indicates that more oxygen is required by the fish to meet this metabolic demand under heavy metal stress. The sticklebacks when exposed to lethal concentrations of lead, copper and mercury salts also showed impaired opercular movements and decrease in oxygen consumption (Jones, 1947). Other workers have suggested that heavy metals inhibit oxygen consumption at the autochondrial level (O'Hara, 1971). A number of workers have studied the alterations in oxygen consumption under heavy metal stress. Singh and Singh (1979) studied the oxygen consumption of a siluroid fish *Mystus vittatus* exposed to different concentrations of heavy metal
salts. In the study undertaken the decrease in oxygen uptake of the fish under heavy metal stress may be attributed to the reduced gill permeability (Matthiessen and Brafield, 1973) which causes respiratory distress finally leading to the death of the fish (Watenpaugh and Beitinger, 1985) the results obtained are well in agreement with the other workers.

Fishes were also exposed to sublethal concentration of 1/10th and 1/15th of their 96 hr LC50 and sublethal mixtures of double and triple metal mixtures, for a period of 30 days. The oxygen uptake was studied at 4, 10, 20 and 30th days of exposure to the toxicant concentration.

The initial decline in oxygen uptake in the fish exposed to individual metals recovered and was observed to be more than the control fishes at the later days of exposure in both male and female. The uptake was also increased in the fish exposed to mixture of two and three metals inspite some of irregular rate on the 10th day in the triple exposure.

Similar results were obtained following exposure to sublethal levels of Cu to Puntius arulius (Shivaraj et al., 1989). Oxygen consumption was also affected in the common carp (Cyprinus carpio) due to the sublethal levels of Cu
Arterial oxygen tension and the structure of the secondary lamellae of the gills in rainbow trout (*Oncorhynchus mykiss*) were affected on exposure to Zn (Lappivaara et al., 1995). The rate of oxygen consumption in fishes has been identified as an indicator of intensity of metabolism (Singh and Singh, 1979, Watenpaugh and Beitinger, 1985). In fishes any change from normal/control value might reflect an alteration in the respiratory epithelium of the gill. Gills are considered as the main osmoregulatory surface organs in fishes and are the primary site of uptake of water borne pollutants (Evans, 1987). The intimate contact of the gill with water-borne pollutants may lead to alterations in the respiratory surface area (Singh and Singh, 1979), in turn lowering the diffusion capacity of the gills (Cairns et al., 1981; Hughes, 1980). Therefore, the gills may be the first site where sublethal effects of pollutants would be observed (Lauren and McDonald, 1985). It is apparent that many workers have investigated the effect of various substances in metabolic rate of fishes both by whole animal oxygen consumption or by using apercular beat, least rate gill diffusing capacity and isolated gill oxygen consumption (Watenpaugh and Beitinger, 1985; Cairns et al., 1981; Hughes, 1979, 1980. It was suggested that the damage to the gill
tissue responsible for death, because of the breakdown of its vital function leading to the decrease efficiency for gas exchange (Lloyd, 1960, Hughes, 1980). Since the whole animal oxygen consumption may be altered at the gas exchange surface which is directly proportional to the activity in ectotherms. The atrophy of respiratory epithelium, enlargement of water/blood barrier of the gill tissues following sublethal action of metals has been reported (Singh and Singh, 1979; Hughes et al., 1979; Hughes, 1980). Prolonged exposure of fish to heavy metals leads to stress, which disrupts hormonal balance in turn leading to a variety of pathological changes in tissues have been reported. Thus the changes in the oxygen consumption are due to the physiological imbalance caused by the histopathological damages on the gill and due to the metabolic processes that indirectly cause changes due to the metal stress in the fish.

Nitrogen excretion was inhibited on exposure to metals at their lethal concentration in *G. affinis*. Cu, Zn and Ni were effective in causing irregular rate of nitrogen excretion in both male and female. Ni exposure caused increased rate of excretion at their 72 and 96 hrs of exposure in female however, in male there was decrease as compared to the control.
Fishes exposed to mixtures of two metals had an enhanced rate of nitrogen excretion at later stages but initially the rate of excretion was irregular. Female were more affected as compared to the male. Triple metal mixture also affected the nitrogen excretion in a critical way with decrease and later recovery in the rate of excretion. In fishes exposed to sublethal concentration of individual metals there was reduction in the excretion rate in both male and female. At a mixture of two metals the initial reduction increased gradually to a rate relatively more than that of control in both male and female. Also triple metal mixture exposure the rate of excretion was irregular.

Fish excrete about 85% of their waste nitrogen as ammonia down a concentration gradient through the gills into water with little or no energy cost (Goldstein and Forester, 1970). However, the pathological effects of metals are extensively worked out. Lloyd, 1960 and Mount, 1964 observed the epithelial cells of the gill secondary lamellae became swollen, separated from the pillar cells and finally sloughed off in rainbow trout (Salmo gairdneri) on exposure to Zn. Effects on the gill at lower concentrations are less severe (Dabrouska, 1976) compared to higher concentrations of Cu.
exposure, the epithelial layer showing thickening of the apical cells, vacuolization and containing myelin-like bodies and increased number of chloride cells (Baker, 1969). Ni also is known to reduce the diffusion capacity of the gills and promotes an increase in the thickness of lamellar membrane in rainbow trout (Moore and Ramamurthy, 1984).

Ammonia is diffused in its unionised state due to the greater lipid solubility to cross biological membrane (Jacobs, 1940). The damage on the gill epithelium would inhibit the ammonia excretion. Katz (1977) has demonstrated increased sodium efflux in *G. affinis* within few hours in the presence of high concentratin of Zn and a decrease in the efflux of ammonia. And ammonia excretion takes place as an exchange component at the gills in fishes (Maetz, 1964).

On prolonged exposure tolerance is developed and the pathological damages would recover causing increase in diffusion. However, this is temporary and the decline is observed again. As this process repeats there is an irregular rate of nitrogen excretion.

Liver and Kidney are vital sites of ammonia synthesis (Colin and Walton, 1989). However in rudd (*Scardinius*
erythropthalmus) very low levels of fat and glycogen were reported on exposure to Zn (Ministry of Technology, U.K., 1966) common carp on prolonged exposure to copper showed elevated accumulation of the metal in liver with disturbed protein synthesis and reduced serum globulins (Semcaik and Avdusev, 1972). Ni if present in excess, interferes with the detoxification process and metabolism (Moore and Ramamoorthy, 1984).

Ammonia production is due to amino and amide groups of amino acids. The amination and deamination involves the action of enzymes such as transaminases and glutamate dehydrogenase. However, Cu is known to inhibit the enzymic activity (Jackim et al., 1970; Christensen et al., 1972; McKim and Bennt, 1971). Zn and Ni cause dysfunction of kidney tissue and enzymes (Moore and Ramamoorthy, 1984). Thus the nitrogen excretion is affected by the combined presence of these metals.

It is also established that nitrogen excretion and the rate of oxygen consumption are interdependent (DeBoeck et al., 1995) and the results obtained in the present investigation affirm the interrelated effect of metal and metal mixtures on oxygen consumption and nitrogen excretion.
Among the myriad of organic and inorganic substances that are released into the environment, heavy metals have received a considerable attention, due to their toxicity and potential bioaccumulation in aquatic organisms. Although a number of aquatic organisms have been used as monitors of heavy metal contamination most studies have relied on fish as the sensitive indicators of metal pollution. The main routes of uptake of metals by fish is through food and water or both and they bioaccumulate and increase toxicity of heavy metals in fresh water fishes.

In the present study, the accumulation of metal and metal mixtures was investigated at the end of the 96 hr acute exposure.

The accumulation was not additive with increase in exposure period in individual exposure of metals for both sexes certain degree of detoxification and elimination was evident in the results obtained. Higher exposure concentration caused higher accumulation of Ni and Cu in male and Zn in female. However, there was a decreasing rate of accumulation of Cu and Ni in female and Zn in male. In fishes exposed to mixture of two and three metals, there was a significant relationship existing between the metals and their
accumulation. The relationship is statistically established with the regression equations for the rate of accumulation of Cu due to its highest toxicity to fishes as compared to Zn and Ni. The accumulation of Cu is affected by the presence of Zn and Ni in the aquatic media. The regression analysis affirms and establishes the relationship existing between the exposed metals.

The acute exposure of fathead minnous to Cu caused the accumulation that was influenced by water hardness, complexation and pH (Playle et al., 1992). The accumulation in the present study is influenced by water hardness, size and weight of the fish which is evident in the results for Cu accumulation. The susceptibility of adult and juvenile Clarius gariepinus (Burschell) for Cu accumulation as a factor of weight and size has been investigated (Van Der Merwe et al., 1993). The variation in zinc accumulation is due to the differential absorption rate as a factor of physiology and sex. This is investigated for common carp and other fishes by Jeng and Lian (1994) and they suggest that the absorption rate is an important factor in the metal accumulation. The accumulation increased with increase in exposure concentration of Zn in Cirrhinus mrigala (Hamilton) (Gupta and Sharma,
1994). Ni accumulation also was affected by exposure concentration for *Cyprinus carpio* (Sreedevi *et al.*, 1992a). The results obtained by an investigation are our conformity to that of the earlier workers.

Fishes have also been exposed to sublethal concentration of 1/10th and 1/15th of 96 hr LC$_{50}$ values of individual metals and the active ingredients of these metals in double and triple combination for 30 days.

Fishes exposed to Cu had an initial accumulation that was eliminated by 10th day and there was no accumulation of Cu in both male and female of *G. affinis* exposed to 0.293 and 0.440 mg/g of Cu. There was an increasing rate of accumulation for Zn and a decreasing trend in Ni accumulation in the male. In female there was an increasing rate of accumulation of Zn and Ni with exposure period. In a mixture of two metals the accumulation was affected by the presence of other metal and relation existed between the metals. The relationship of other metals on Cu accumulation was investigated statistically and the regression equations were established. Fishes were exposed to three metal mixtures with relative increase in the component metals of the mixture and the accumulation was dependent on the inter elemental interactions. The
relationship existing between the metals exposed was investigated statistically with the regression analysis and there was a significant relationship existing between the metals.

Accumulation of Cu increased with time in *Tilapia nilotica* (L.) exposed to sublethal doses of Cu (Erdem Cahit, 1990). Similar rate of accumulation was seen in *Clarias anguillaris* L. and *Oreochromis niloticus* L. (Daramola and Oladimeji, 1989). Accumulation of Zn in *Brachydanio rerio* increased with exposure period and stabilized after certain days (Memmert Ulrich, 1987). The Ni accumulation increased with concentration and exposure time for *Clarias batrachus* (Ray et al., 1990). The results obtained in our investigation are similar to the work carried out by earlier workers.

The relationship between Cu and Zn has been investigated for white sucker (*Catostomus commersoni*) (Miller et al., 1992). Experimental study of interaction between Cu and Zn has confirmed a complex set of actions and interactions between elements exposed to *Brachydanio rerio* (Ribeyre et al., 1995). The interactions of major ions in aquatic animals is reviewed by Wright (1995). Correlation existing between elements in aquatic organisms is investigated and the relationship
established by our investigation between the inter elemental toxicity and accumulation is confirmed by the investigators.

Behaviour has long been recognised as an excellent way to assess the condition and well being of a particular organism. Behavioural characteristics may be associated with normality and well being while others may be associated with illness, injury, fatigue and the like. In fish, behavioural indications of substandard performance or lack of general well being might include difficulty in maintaining equilibrium, inability to keep pace with the school, loss of swimming speed or clamping of the fins. As a consequence, behaviour is recognized as an integrator of physiological condition.

At lethal exposure, the symptoms of poisoning in fish were observed. For individual exposure to Cu, Zn and Ni, the fish were restless and aggressive swimming was observed with attempts to jump out of the media. Loss of sensitivity was also observed with reduced excitation and loss of equilibrium. There was excessive mucus secretion and fish died with mouths wide open.

Similar observations were made for the fishes exposed to double metal mixtures and the symptoms of extreme stress were
evident with increased mucus production and proportional increase in mortality. Fish died with mouth open and haemorrhage beneath pectoral fins. In fishes exposed to triple combination of metals there was an intense excitation in the beginning and increased mucus secretion followed with lethargic behaviour. Loss of sensitivity and equilibrium were common. Fishes rolled over and many died with bent bodies and wide open mouths.

The observations made are similar for the lethal exposure of Cu to *Puntius aurilis* (Shivaraj and Patil, 1988) and *Leidocephalichthyes guntea* (Bengeri et al., 1986). Fishes exposed to Zn also exhibited similar symptoms of poisoning in *Lepidocephalichthyes guntea* and *Labeo rohita* (Bengeri and Patil, 1986c). Nickel also reduces the diffusion capacity of gills and promotes an increase in thickness of lamellae inducing the symptoms of poisoning. This leads to death by asphyxiation and is consistent with high Ni residues in gills (Moore and Ramamorthy, 1984). The effect of metal mixtures in inducing the symptoms of poisoning are due to the joint action of these metals on the gill surface and accumulation within the body. The toxicity of Cu salts to fish is attributed to the precipitation of mucus on the gills (Ellis, 1937) thereby
causing suffocation and also to direct damage of the gill. Acute lethal concentrations of Cu cause a temporary reduction in the number of mucus cells in common carp (Cyprinus carpio) (Labat et al., 1974) and rainbow trout (Salmo gairneri) (Peqvignot et al., 1975) and extensive breakdown of the gill of the latter. The lamellae tending to collapse and overlap each other showing hypertrophy and hyperplasia (D.S.I.R. 1961) affects on the gills of lower concentration are less severe (Dabrowska, 1976). The epithelial layer showing thickening of the apical cells, vacuolization and containing myelin-like bodies and increased numbers of chloride cells (Baker, 1969).

Zinc salts also precipitated the mucus on the gills of fish, causing them to die from suffocation, although calcium salts inhibited precipitation. However, Lloyd (1960) and Mount (1966) observed little or no precipitation of mucus of rainbow trout (Salmo gairdneri) at low, lethal concentrations and histological examinations showed that the epithelial cells of the gill secondary lamellae became swollen, separated from the pillar cells and finally sloughed off Skidmore (1970) and Skidmore and Tovell (1972) concluded that under such conditions the fish died from asphyxia, rather than osmotic stress, an hypothesis supported by the increased concentration
of lactic acid and glycogen utilization (Hodson, 1976) and reduced concentration of pyruvic acid in the tissues of trout killed by high concentration of zinc (Burton et al., 1972).

Hughes, Perry and Brown (1979) found damage to the structural components of the gill secondary lamellae of rainbow trout (Salmo gairdneri) with a consequent impairment of the ability of fish to transfer oxygen from the water to their blood on exposure to nickel. Thus the cumulative action of metal mixtures on the fish is similar to the individual metal action, however, the threshold is much lowered in the concentration of metal components in inducing symptoms of death.

Chemical agents may be behaviourally toxic even though symptoms of structural or biochemical toxicity are not detectable (Thompson and Lilja, 1964). The introduction of a chemical substance may (1) increase the time required to learn to escape or avoid noxious stimuli (2) decrease the animal's sensitivity to subtle changes in its environment or (3) interfere with animal's ability to retain previously learned behaviour. In the investigation undertaken by us, efforts were made to study the effect of metal and metal mixtures in affecting the behaviour of the fishes.
Fishes avoided the lethal concentration of individual metals. However, it was observed that at sublethal exposure fishes preferred the polluted zone relatively over the zone with food odour. Juvenile sockeye salmon, *Oncorhynchus nerka*, responded by evoking exploratory and feeding behaviour to aqueous extracts of foods to which they had been previously conditioned but failed to respond to similar foods which had not previously been in their diet (McBride et al., 1962). But in *G. affinis* it is evident that the metal toxicants lured the fishes to the toxicant zone inspite of the food odour to which they had been previously conditioned.

Higher concentration of Cu was avoided by male but females preferred the toxicant zone. Similar response was observed for Zn, higher concentration was avoided but females avoided the toxicant zone and preferred the uncontaminated zone. The Ni zone was preferred by male. Female preferred Ni at higher concentration and avoided the lower concentrations.

In a mixture of Cu and Zn, both male and female preferred the toxicant zone. Cu and Ni, and Ni and Zn mixture were preferred over unpolluted zone. Study on preference and avoidance of sublethal doses of three metal mixture was
undertaken to evaluate the effect of increase in mixture concentrations and it was found that the higher concentration of metals lured the fish to the polluted region.

However fishes preferred Zn over Cu when given a choice in male but female preferred Cu over Zn at this sublethal concentration. At higher concentration however both male and female preferred Zn over Cu. Cu is thus understood to be an irritant and repellent agent. Between Cu and Ni, male avoided Cu but female preferred it over Ni. At higher concentration, male avoided Cu but female avoided Ni. For a choice between Zn and Ni, the female preferred the mixing zone over Ni and Zn. The female preferred the mixing zone over Ni and Zn. At higher concentration male preferred Ni and female preferred Zn. It is evident from these results that the fish are able to discriminate the metal plumes and avoid and prefer.

The ability of fishes to discriminate between changes in concentration of metals was verified. The higher concentration of Cu was avoided by both male and female. Similar condition was observed for Zn. But male preferred the higher concentration of Ni and female were not distinct in their choice. In a mixture of three metals, higher concentration of such mixture was avoided in general.
In the observation the preference to mixing zone is considered to be the most adverse response since the toxicant mixture and concentration increases these before draining (Cherry and Cairns, 1982).

Experiments on the effects of heavy metals on the palatal chemo-reception of common carp showed that a concentration of 6.4 mg Cu/1 depressed the response of the sugar and salt receptors (Hidaka, 1970). Also olfactory responses of sockeye salmon, *Coho salmon* (*Oncorhynchus kisutch*) and rainbow trout to food extracts, amino acids and hand rinses were extinguished after more than 12 hr exposure of the fish to a concentration of 40 mg Cu/1 (Hara, 1972). The results obtained by our study are in agreement. Sprague (1964b) showed that in the absence of other stimuli, Atlantic Salmon (*parr*) could detect levels of Cu in soft water (20 mg/l as CaCO₃) as low as 2.4 µg/l, which was 0.05 of the threshold lethal concentration; in these laboratory experiments the fish were given a choice of clean or polluted water in a short tube with a sharp interface between the two solutions. Rainbow trout apparently avoided even lower concentrations (0.1 µg Cu/l) in a Y-shaped maze in water having a hardness of 89.5 mg/l as CaCO₃ (Fomar, 1976).
Sprague (1964b) used Atlantic salmon (parr) in a soft water (hardness 18 mg/l as CaCO₃) in an avoidance trough in the laboratory. The lowest concentrations causing the average fish to show significant avoidance (EC₅₀) were 0.053 mg Zn/l for zinc sulphate alone and 6 µg Zn/l for Zn in the presence of 0.4 µg Cu/l. The EC₅₀ for rainbow trout avoidance of Zn under laboratory conditions at 9.5°C and 17°C was 5 µg Zn/l (0.01 of the 7-day LC₅₀) and was not changed when acclimation levels were increased from 3 to 13 µg Zn/l (Sprague, 1968a) Syazuki (1964) and Ishio (1966) report avoidance at 0.3 and 0.45 of concentrations lethal to common carp (Cyprinus carpio) and gold fish, (Jones, 1947) however found that stickleback (Pygosteus pungitius) showed avoidance only of lethal concentrations.

There is not much work carried out in the behavioural responses of fish to Ni. Hypoactivity was induced by sublethal concentrations of it in goldfish, Carassius auratus (Ellgard et al., 1995). Avoidance was also observed in fishes exposed to a blend of four metals in fat head minnows (Pinephales promelas) (Hartwell et al., 1987). The results obtained by our investigation are in agreement with those of earlier workers. And the results obtained support the concepts
proposed by Thomapson and Schuster (1968) that first those chemical agents that produce only behavioural changes that have serious and possibly irreversible deleterious effects on the animals ability to adapt may be identified and controlled. Second, the identification of the behaviourally toxic effects of chemical agents may provide an early warning system which may allow the detection of toxicity before irreversible structural and biochemical damage has occurred.

The present findings are consistent with the results of several other investigators on the mixture toxicity of various types of toxic substances on fish and other aquatic organisms. Thus it is concluded that whenever experimental data on the toxicity of a specific mixture are not available concentration additivity may be a reasonable assumption it hazard assessment procedures.