DISCUSSION

Proteins are very important structural macromolecules in all animals. Amino acids are the building blocks of proteins for the tissues so it was planned to study the effects of different metal chlorides on amino acid composition of fish muscles and red blood cell.

A wide range of pollutants either physical – chemical – biological and radiological have been observed in the aquatic biota due to urbanization – industrialization and new technological development.

Heavy metals are traced in some areas in sufficient concentration and physico – chemical forms that might create pollution problems. Sources of heavy metals in aquatic environment are from different industrial operation particularly South Gujarat region, which due to alarming industrialization will cause serious depletion in fisheries catch.

During last decades, parallel with rapidly developing technology, increasing population and urbanization we have been witnessing alarming phenomena of pollution all over South Gujarat region.

Several pollutants such as Servin, Melathion cause protein depletion in tissues of aquatic animals. In present study when young fishes *Labeo rohita* were exposed to different heavy metal concentrations, amino acids content (Table 1, 2, 3, 4, 5) of fish muscles were affected.

When fish were exposed to sublethal concentration of copper chloride, certain amino acids were depleted or destroyed. In normal (control) fish muscles both essential and non essential, amino acids which were traced in good quantity, are Histidine, Proline, Glycine, Alanine, Methionine and Valine. Exposure of fishes to 1, 2 and 3 ppm Copper
Chloride for 15, 30 and 45 days, shows that as concentration and duration increases, there is much more effect on optical density (O.D.) of amino acids.

Histidine in normal fish was up to .035 O.D. it is reduced to .033 on 30th and .032 on 45th day in 1 ppm Copper chloride media exposed fish. As concentration increased there was drastic change in O.D. in 2 and 3 ppm on 30th and 45th day. (Graph 1 and fig. 1, 2, 3).

Proline has normally shown .030 O.D. But even during longer days and higher concentration it was traceable only with reduction in O.D. reading (Graph 2 and fig. 1, 2, 3).

Glycine has normally shown .037 O.D. it went down to .026 and .20 O.D. on 45th day in 2 and 3 ppm irrespectively (Graph 3 and fig. 1, 2, 3).

Alanine even in control fish has shown .021 on 15th, 30th and 45th day. In 2 ppm it was .016 and .013 O.D. on 30th and 45th day. But when fish were exposed to 3 ppm for 30 days there was great reduction up to .014 O.D. and on 45th day the reduction was up to .011 O.D. of amino acids (Graph 4 and fig. 1, 2, 3).

Methionine normally ranged at .029 O.D. was depleted up to .013 on 45th day in 3 ppm (Graph 5 and fig. 1, 2, 3).

Valine normally shows .025 O.D. but its reduced O.D. was .024 to .020 on 15th – 30th and 45th day in 1 ppm. In case of 2 ppm it ranged from .022 to 0.18 O.D. on 15th and 45th day of exposure. While in 3 ppm on 30th day presence of Valine was very low the O.D. was .014 and it was .10 on 45th day (Graph 6 and fig. 1, 2, 3).

In case of fish exposed to Zinc Chloride fish were capable of tolerating higher concentrations. Sublethal dose was higher than Copper Chloride. So for the study of effects of Zinc Chloride on amino acid compositions of fish muscles 40, 50 and 60 ppm concentration was reflected.
Proline normally shows .028 O.D. but Zinc Chloride exposed to fish extract have shown reduction in O.D. at 40, 50 and 60 ppm. In 60 ppm on 45th day it went down up to .010. This has shown highly reduced quantity of Proline from fish muscles (Graph 7 and fig. 4, 5, 6).

Glycine normally shows .039 O.D. but Zinc Chloride exposed to fish extract have shown reduction in O.D. at 40, 50 and 60 ppm. In 60 ppm on 45th day it went down up to .008. This has shown highly reduced quantity of Glycine from fish muscles (Graph 8 and fig. 4, 5, 6).

Alanine normally shows .021 O.D. but Zinc Chloride exposed to fish extract have shown reduction in O.D. at 40, 50 and 60 ppm. In 60 ppm on 45th day it went down up to .008. This has shown highly reduced quantity of Alanine from fish muscles (Graph 9 and fig. 4, 5, 6).

Methionine normally shows .029 O.D. but Zinc Chloride exposed to fish extract have shown reduction in O.D. at 40, 50 and 60 ppm. In 60 ppm on 45th day it went down up to .016. This has shown low reduced quantity of Methionine from fish muscles (Graph 10 and fig. 4, 5, 6).

Valine normally shows .023 O.D. but Zinc Chloride exposed to fish extract have shown reduction in O.D. at 40, 50 and 60 ppm. In 60 ppm on 45th day it went down up to .012. This has shown low reduced quantity of Valine from fish muscles (Graph 11 and fig. 4, 5, 6).

Histidine normally shows .036 O.D. but Zinc Chloride exposed to fish extract have shown reduction in O.D. at 40, 50 and 60 ppm. In 60 ppm on 45th day it went down up to .020. This has shown low reduced quantity of Histidine from fish muscles (Graph 12 and fig. 4, 5, 6).
In case of fish exposed to Cadmium Chloride – fish are not capable of tolerating higher concentrations. Sublethal dose was higher than Copper Chloride. So for the study of effects of Cadmium Chloride on amino acid compositions of fish muscles 5, 10 and 15 ppm concentration was reflected.

Histidine normally shows .037 O.D. but Cadmium Chloride exposed to fish extract have shown reduction in O.D. at 5, 10 and 15 ppm. In 15 ppm on 45th day it went down up to .012. This has shown highly reduced quantity of Histidine from fish muscles (Graph 13 and fig. 7, 8, 9).

Proline normally shows .030 O.D. but Cadmium Chloride exposed to fish extract have shown reduction in O.D. at 5, 10 and 15 ppm. In 15 ppm on 45th day it went down up to .011. This has shown highly reduced quantity of Proline from fish muscles (Graph 14 and fig. 7, 8, 9).

Glycine normally shows .036 O.D. but Cadmium Chloride exposed to fish extract have shown reduction in O.D. at 5, 10 and 15 ppm. In 15 ppm on 45th day it went down up to .010. This has shown highly reduced quantity of Glycine from fish muscles (Graph 15 and fig. 7, 8, 9).

Alanine normally shows .020 O.D. but Cadmium Chloride exposed to fish extract have shown reduction in O.D. at 5, 10 and 15 ppm. In 15 ppm on 45th day it went down up to .008. This has shown highly reduced quantity of Alanine from fish muscles (Graph 16 and fig. 7, 8, 9).

Methionine normally shows .028 O.D. but Cadmium Chloride exposed to fish extract have shown reduction in O.D. at 5, 10 and 15 ppm. In 15 ppm on 45th day it went down up to .012. This has shown highly reduced quantity of Methionine from fish muscles (Graph 17 and fig. 7, 8, 9).
Valine normally shows .024 O.D. but Cadmium Chloride exposed to fish extract have shown reduction in O.D. at 5, 10 and 15 ppm. In 15 ppm on 45th day it went down up to .009. This has shown highly reduced quantity of Valine from fish muscles (Graph 18 and fig. 7, 8, 9).

In case of fish exposed to Nickel sulphate – fish are capable of tolerating higher concentrations. Sublethal dose was higher than Copper Chloride. So for the study of effects of Nickel sulphate on amino acid compositions of fish muscles 40, 55 and 70 ppm concentration was reflected.

Glycine normally shows .059 O.D. but Nickel sulphate exposed to fish extract have shown reduction in O.D. at 40, 55 and 70 ppm. In 70 ppm on 45th day it went down up to .039. This has shown low reduced quantity of Glycine from fish muscles (Graph 19 and fig. 10, 11, 12).

Aspartic acid normally shows .042 O.D. but Nickel sulphate exposed to fish extract have shown reduction in O.D. at 40, 55 and 70 ppm. In 70 ppm on 45th day it went down up to .022. This has shown low reduced quantity of Aspartic acid from fish muscles (Graph 20 and fig. 10, 11, 12).

Proline normally shows .028 O.D. but Nickel sulphate exposed to fish extract have shown reduction in O.D. at 40, 55 and 70 ppm. In 70 ppm on 45th day it went down up to .010. This has shown highly reduced quantity of Proline from fish muscles (Graph 21 and fig. 10, 11, 12).

Leucine normally shows .032 O.D. but Nickel sulphate exposed to fish extract have shown reduction in O.D. at 40, 55 and 70 ppm. In 70 ppm on 45th day it went down up to .011. This has shown great reduction in quantity of Alanine from fish muscles (Graph 22 and fig. 10, 11, 12).
In case of fish exposed to Cobalt sulphate - fish are not capable of tolerating higher concentrations. Sublethal dose was higher than Copper Chloride. So for the study of effects of Cobalt sulphate on amino acid compositions of fish muscles 10, 20 and 30 ppm concentration was reflected.

Proline normally shows .032 O.D. but Cobalt sulphate exposed to fish extract have shown reduction in O.D. at 10, 20 and 30 ppm. In 30 ppm on 45th day it went down up to .009. This has shown highly reduced quantity of Proline from fish muscles (Graph 23 and fig. 13, 14, 15).

Glycine normally shows .061 O.D. but Cobalt sulphate exposed to fish extract have shown reduction in O.D. at 10, 20 and 30 ppm. In 30 ppm on 45th day it went down up to .032. This has shown low reduced quantity of Glycine from fish muscles (Graph 24 and fig. 13, 14, 15).

Aspartic acid normally shows .038 O.D. but Cobalt sulphate exposed to fish extract have shown reduction in O.D. at 10, 20 and 30 ppm. In 30 ppm on 45th day it went down up to .022. This has shown low reduced quantity of Aspartic acid from fish muscles (Graph 25 and fig. 13, 14, 15).

Leucine normally shows .029 O.D. but Cobalt sulphate exposed to fish extract have shown reduction in O.D. at 10, 20 and 30 ppm. In 30 ppm on 45th day it went down up to .014. This has shown low reduced quantity of Alanine from fish muscles (Graph 26 and fig. 13, 14, 15).

When fish were exposed to Copper Chloride in low concentration 1 ppm only cell membrane was damaged and this frequency was less but during longer exposure there was (fig. 17, 18, 19) effect on nucleus, vacullation and nucleus shifted from normal position. This type of vacullation caused by disruptive effects of Copper Chloride might cause anemia. Anemic condition of fish will also lead to decrease in oxygen carrying capacity; it may be due to injury caused to the RBCs. The toxic chemicals present in the
pollutants, interfered with respiration, by causing coagulation in gills, which inhibits the enzyme system at mitochondrial levels, resulting in the reduction of oxygen consumption. 2 ppm concentration caused damage to cell membrane and vacuolation on 15\textsuperscript{th} day, nucleus was affected on 30\textsuperscript{th} day and on 45\textsuperscript{th} day the cell membrane was wrinkled and damaged (fig. 20, 21, 22). 3 ppm concentration leads to vacuolation – shifting of nucleus from its normal central position. This might be due to toxic effect on plasmolysis of cell content (fig. 23, 24, 25).

Concentration of Zinc Chloride selected was higher than that of Copper Chloride and Cadmium Chloride. Effects of Zinc Chloride were observed in short and long duration exposure. 40 ppm short duration exposure has shown enlarged and damaged nucleus (fig. 26, 27, 28) in same concentration longer exposure leads to reduction of size of nucleus and wrinkled cell membrane due to plasmolysis effect at longer duration. 50 ppm short duration exposure has shown the nucleus and damaged and (fig.29, 30, 31) in same concentration longer exposure leads to the cell membrane wrinkled and vacuolation observed, the nucleus shift in position and the cell membrane damaged. 60 ppm short duration exposure has shown the cell membrane damaged and nucleus enlarged (fig.32, 33, 34) in same concentration longer exposure leads to the nucleus enlarged and vacuolation observed, the nucleus enlarged and shifts in position.

During the study of Cadmium Chloride on RBCs 5 ppm concentration exposed to fish on 15\textsuperscript{th} day the cell membrane damaged (Fig.35) but in longer duration it was observed on 30\textsuperscript{th} day the cell membrane damaged and vacuolation observed (Fig.36). While on 45\textsuperscript{th} day nucleus damaged and vacuolation observed (Fig.37). 10 ppm of Cadmium Chloride concentration leads to damage cell membrane and enlarge nucleus (Fig.38) even in short term exposure of 15 days. While on 30\textsuperscript{th} and 45\textsuperscript{th} day exposure, there was damaged cell membrane, vacuolation and damaged nucleus, accomplished by wrinkled cells as they are seen in Fig.39 and 40 respectively. 15 ppm concentration leads to wrinkled cell membrane on 15\textsuperscript{th} day (Fig.41) destroyed cell membrane observed on 30\textsuperscript{th} day (Fig.42), while on 45\textsuperscript{th} day there was damaged or destroyed cell membrane and nucleus enlarged and damaged (Fig.43).
During the study of Nickel sulphate on RBCs 40 ppm concentration exposed to fish on 15\textsuperscript{th} day the cell membrane damaged (Fig. 44) but in longer duration it was observed on 30\textsuperscript{th} day the nucleus enlarged and vacuolation observed (Fig. 45). While on 45\textsuperscript{th} day the cell membrane damaged and vacuolation observed (Fig. 46). 55 ppm of Nickel sulphate concentration leads to the nucleus shift in position (Fig. 47) even in short term exposure of 15 days. While on 30\textsuperscript{th} day exposure the cell membrane wrinkled and vacuolation observed (fig. 48). While on 45\textsuperscript{th} day exposure the nucleus damaged (fig. 49). 70 ppm concentration leads to the cell membrane damaged and vacuolation observed 15\textsuperscript{th} day (Fig 50). While on 30\textsuperscript{th} day exposure the nucleus enlarged and shift in position (fig. 51). While on 45\textsuperscript{th} day exposure the cell membrane destroyed and the nucleus enlarged and damaged (fig. 52).

During the study of Cobalt sulphate on RBCs 10 ppm concentration exposed to fish on 15\textsuperscript{th} day the cell membrane damaged and nucleus enlarged (Fig. 53) but in longer duration it was observed on 30\textsuperscript{th} day the nucleus shift in position (Fig. 54). While on 45\textsuperscript{th} day the nucleus shift in position and the cell membrane damaged (Fig. 55). 20 ppm of Cobalt sulphate concentration leads to the nucleus enlarged and damaged (Fig. 56) even in short term exposure of 15 days. While on 30\textsuperscript{th} day exposure the nucleus enlarged and vacuolation observed (fig. 57). While on 45\textsuperscript{th} day exposure the cell membrane damaged and vacuolation observed (fig. 58). 30 ppm concentration leads to the cell membrane damaged and nucleus enlarged 15\textsuperscript{th} day (Fig 59). While on 30\textsuperscript{th} day exposure the nucleus enlarged and shift in position (fig. 60). While on 45\textsuperscript{th} day exposure the nucleus damaged and the cell membrane wrinkled (fig. 61).

These types of condition like wrinkled cell membrane, damaged nucleus or enlarged nucleus, vacuolation lead either to death of cell in long run or are responsible for anemic condition of animals. Anemic conditions and less iron content have been reported by several workers in fish, bird and mammal following exposure to pollutants. It is also reported by Anusha (1994) that RBCs count was declined by higher concentration of endosulfan subjected to fish. Anusha (1994) postulated that the reduction of RBCs count
might be due to inhibition of RBCs production and destruction of RBCs by pollutant. Heavy metals effect on reticulo-endothelial system and haematopoisis.