

Summary

This Study aimed to Studying the effects of contaminated food with heavy metals (Cu or Cd or Cu and Cd) on Nile tilapia (*Oreochromis niloticus*) :

- 1-Throwing lights over the proplem of metals contaminated diet.
- 2- Investigate the effect of contaminated diet on the total protein content in muscle and blood plasma, plasma albumin and globulin.
- 3-clarify the effect of contaminated diet on genetic metals by estimating the rate of chromosome aberrations.
- 4- investigate the effect of contaminated diet on Protein electrophoresis of epiaxial muscle.
- 5- Clarify the effect of contaminated diet on NADH dehydrogenase gene using PCR-RFLPs technique.

The study included four grops:

- The first group (Control group) composed of 45 *Oreochromis niloticus* fed on standard diet.
- The second group composed of 45 *Oreochromis niloticus* (Cu group) fed on Cu contaminated diet (2 g/Kg dw diet).
- The third group composed of 45 *Oreochromis niloticus* (Cd group) fed on Cd contaminated diet (10 g/Kg dw diet).
- The fourth group composed of 45 *Oreochromis niloticus* (Cu + Cd group) fed on Cu + Cd contaminated diet 2 g Cu + 10 g Cd /Kg dw diet).

Fishes were reared in well experimental aquaria; the water temperature was kept at 22 ± 1 °C by using thermostat. Throughout the experimental duration (30 days) the fishes were fed to satiation two

times every day. Fish specimens were sacrificed after 10 days, 20 days and 30 days. For each treatment, 5 fishes were used for chromosomal preparation and 8 fishes were used for protein electrophoresis and molecular techniques.



The diet contaminated with Cu or Cd or combination of both induced general reduction of fish weight, comparing with those of the control fish. Also hepatosomatic index (HSI), gonadosomatic index (GSI) and condition factor (K) recorded marked reduction in fishes fed various metals contaminated diets.



The Cu bioaccumulation on flesh of Nile tilapia exhibited significant increase for Cu , Cd, and Cu+Cd groups at all duration. The Cd bioaccumulation in flesh of *Oreochromis niloticus* showed significant increase for Cu , Cd, and Cu+Cd groups at all durations. Also the level of Fe in fish flesh recorded very highly significant increase for fish fed on Cu, Cd , and Cu+ Cd groups at all durations.



The total muscle protein content exhibited significant reduction after 10 days and then significant increase after 20, and 30 days at all treated groups, and also total muscle protein content, total plasma protein, albumin, and globulin decreased significantly, this may be due to that heavy metals react with the cell nucleaoproteins and the nucleic acid and so affect the protein synthesis. The total nitrogen content in fish muscle exhibited generally very highly increase for all treated groups at all durations.

★ Fractionation of soluble sarcoplasmic protein by sodium dodecyl sulphate polyacrylamide gell electrophoresis (SDS-PAGE) was performed for fish. Fed metals contaminated diet as well those fed normal basic diet (control). SDS-PAGE fraction revealed that total numbers of sarcoplasmic protein fractions in control were 9 fractions which decreased in treated groups. Copper, Cd and Cu+Cd contaminated diets cause four, one and fraction missing, respectively.

★ There were a drasic rise of protein mobility of fractions from 1 to 4 on the fish fed Cu contaminated diet after 10 days; it is very highly significant accelerated, regarding those of the control.

★ Concerning fish fed on diet contaminated with Cu after 20 days, there were non significant variations for all fraction except 4th fraction which exhibited significantly accelerated migration on the acrylamide gel. On the other hand there were no significant change on relative mobility on fish fed on diet contaminated with Cu after 30 days.

★ Various chromosomal abberations were observed in the spreads of kidney cells of the different groups, which include deletion, break, gap, centeromeric attenuation, end to end association, ring, polyploidy, and stickiness due to metals contaminated diets.

★ Mutation on DNA can be investigated by comparing the effect of restriction enzymes on NADH dehydrogenase of control and other contaminated group, alterations are visualized as loss and/or gain of bands.



There are some restriction endonuclease did not differentiate between control and other treated group (*SspI* and *ApoI*).*AvrII* restriction endonuclease differentiated the ten individual genes into two groups. The first group included the normal and all copper groups at all durations, the second group included Cd group and Cu+ Cd group at all durations, and this restriction enzyme is useful as biomarker for Cd toxicity.



BseRI restriction enzyme could be used as biomarker for Cu toxicity, as it digested all groups into three group, the first group is control and Cd group at all duration , Second group is Cu group at all duration, and the third group is Cu+ Cd group at all durations. *AvaI* can be used as useful biomarker for Cu toxicity after 10 days.



Thus PCR-RFLP technique indicated mutation increased over time 30 days, mutation of DNA is a time dependant effect that is crucial for long-term exposure.