RESULTS

The data obtained in the present study are presented in tables (1-30) and figures (1-47) and photographs in order to easily investigate and observe the hazardous effects of metals contaminated diets on ***O.niloticus.***

1- Body indices:-

The data presented in table (1) exhibited the changes in total length, body weight, Fulton condition factor (K), hepatosomatic index (HSI) and gonadosomatic index (GSI) of Nile tilapia (***O. niloticus***) fed on diet contaminated with heavy metals (Cu or Cd or Cu and Cd). The data tabulated in table (2) showed the significance (t-test) of the studied parameters between the average values of control group, and those of treated groups.

The studied tilapia fish exhibited total length ranged from 19.667: 22.00 cm. The total body weight of the control fish recorded average value 138.966 ± 21.956 g (Table, 1). Fishes fed on diet contaminated with Cu exhibited non-significant changes of body weight after 10 and 30 days, where as it was significantly reduced after 20 days. The Cd contaminated diet induces non-significant reduction in fish body weight at all durations.

Regarding the combined effect of Cu and Cd on body weight, it was no significant changes in fish weight was observed after 10 days, whereas it was reduced highly significantly after 20 days, recording percentage difference -37.802% from the control value, on the other hand it was non-significantly reduced after 30 days. (Table, 1).

Fishes of the control group recorded average value 1.613 ± 0.090 % for condition factor (K). The condition factor (K) of fishes fed on Cu-contaminated diet was generally reduced .It was highly significantly decreased after 10 days and very highly significantly reduced after 20 and 30 days (Table, 2).

The cadmium contaminated diet recorded non- significant increase of K value after 10 days, whereas it was very highly significant decreased after 20 and 30 days. The combined effect of Cu and Cd recorded general reduction of K values. It was significantly decreased after 10 days , after 20 and 30 days.

According to the recorded data the hepatosomatic index (HSI %) of the control fish have an average 2.72 ± 0.450 (Table, 1).Hepatosomatic index of fishes fed on diet contaminated with Cu was generally reduced. It showed a highly significantly reduction after 20 and 30 days.

The Cd induced a general reduction of HSI. Fish fed with contaminated diet for 20 and 30 days recorded very highly significantly reduction of HSI compared with those of control group. The combined effect of Cu and Cd induced the same effect.

The gonadosomatic index (GSI) recorded reduction at all tested experiment. The GSI values when compared between fishes of the control group and fishes fed on diet contaminated with Cu were non-significantly decreased after 10 and 20 days, whereas it was very highly significantly reduced after 30 days (Table, 2).

Fishes fed on diet contaminated with cadmium exhibited non-significant reduction of GSI after 10 and 20 days, whereas it was highly significantly decreased after 30 days compared with those of the control group.

Copper and cadmium contaminated diet induced highly significant reduction of GSI after 20 days compared with those of control group.

2-Metal contents:-

Data presented in tables (3&4) symbolize metals content (Cu, Cd, Fe) in fish flesh of Nile tilapia (***O. niloticus***) fed on diets contaminated with Cu or Cd or Cu and Cd and its significance (t-test).

According to these data, the Cu level in flesh of Nile tilapia was very highly significant increased for fishes fed on diet contaminated with Cu after 10 days. There were non-significant changes of Cu content after 20 days, whereas significant decrease was found after 30 days.

The effect of Cd contaminated diet exhibited significant increase of Cu content after 10 days, whereas non-significant decrease was found after 20 days. A highly significant increase was recorded after 30 days, compared with those of control group.

Concerning the combined effect of Cu and Cd on Copper bioaccumulation in tilapia flesh, the data obtained was highly significantly increased after 10 days and significantly increased after 30

days, whereas there was non-significant decrease of Cu accumulation after 20 days, when compared with those of control group.

The Cd bioaccumulation in flesh of ***O. niloticus*** was very highly significant increased due to ingestion of diet contaminated with Cu after 10, 20 and 30 days, compared with those of the control fishes.

Concerning Cd contaminated diet, the obtained data exhibited a drastic increase of Cd content in fish flesh, throughout the experimental durations (Table, 4). The Cd content values showed very highly significant elevation over those reported for fishes fed Cd free diet.

Regarding of the combined effect of Cu and Cd on Cd bioaccumulation in flesh of fishes, the present results elucidated a significantly marked rise of Cd content at all tested durations.

The level of Fe in fish flesh of control group recorded average value 22.854 ± 3.285 ppm (Table, 3). According to the obtained data, there was very highly significant increase of Fe content in flesh of fishes fed on contaminated diet with Cu at all durations.

Cadmium contaminated diet induced a general rise of Fe bioaccumulation in fish muscle (Tables, 3&4). The data exhibited very highly significant increase at all durations compared with those of control group. Similarly the combined effect of Cu and Cd on Fe accumulation in tilapia flesh was observed.

Cytogentic investigations:

The Nile tilapia (***O.niloticus***) have 22 pairs of chromosomes all of which exhibited normal pattern in fishes fed on metals free diet (control) (fig.3).

1-Deletion

Deletion is losing of an acentric fragmentation .It is obvious that there was a very highly significant increase of deletion on fish fed on contaminated diet with copper , fish fed on Cd and also fish fed on Cd &Cu.

2-Chromatid Gap

Gaps occur when the chromatid has unstained area shorter than its diameter or equal it. Chromatid gap in the head kidney cells revealed a highly significant increase in the mean values of fish fed on diet contaminated with Cd and also fish fed on diet contaminated with Cu & Cd (Mix group). On the other hand the differences between the averages values of control and fish fed on diet contaminated with Cu was not significant (Table 6, Figs. 5, 14).

3- Break

Break occurs when the chromosome has unstained area shorter than its diameter or equal to it. The number of chromosomes having chromatid break was also studied in the head kidney cells of ***O.niloticus*** in different contaminated diets. The obtained results showed that there was a very highly significant increase of such chromosomes in the fish which were fed on diet contaminated with Cd and also fish which were fed on diet contaminated with Cu & Cd (Mix group), when compared to the control one. On the other hand, the difference between the average

values of control and fish which were fed on diet contaminated with copper was highly significant (Table **7,** Figs.6, 15).

4-Fragmentation

Fragmentation observed when the chromatid without its centromer. It's apparent that there were a very highly significant increase in the mean values of all treated fish groups than control group (Table 8, Figs.7, 16).

5- End to end association

It was observed when the two chromosomes are attached from one chromatid end. End to end in head kidney cells of ***O. niloticus*** in fish which were elucidated a very highly significant increase in the mean values of fish were fed on diet contaminated with Cu & Cd (Mix group) and also in fish fed on diet contaminated with Cd than control fish. At the same time, in this type of aberration the higher values of abnormalities appear at fish fed on diet contaminated with copper than control (Table 9, Figs.8, 17).

6- Centromeric attenuation

It is a chromosomal break attack the centromeric region, lead to separation of the two chromatid.The current study represented the average number of chromosomes having centromeric attenuation in head kidney cells of ***O. niloticus*** in different contaminated diets. It is clear that, there was a very highly significant increase in the mean values between control fish and fish which were fed on diet contaminated with Cd and fish which were fed on Cd & Cu (Mix group). On the other hand, the difference between the average values of control and fish which

were fed on diet contaminated with Cu was highly significant (Table 10, Figs.9, 18).

7- **Ring**

Ring occurs when choromosomes lack free ends but forms a continous ring. Head kidney cells of ***O. niloticus*** recorded that the number of chromosomes having ring showed a very highly significant increase in fish were fed on diet contaminated with Cd and diet mixed with Cu&Cd. The recorded mean values of ring of fish fed on contaminated diet with copper showed a highly significant increase than control one (Table 11, Figs.10, 19).

8- Stickiness

Stickiness is due to improper folding of chromosome fiber into single chromatids and chromosome fibers are inter- mingled and the chromosomes become attached to each other. Table (12), Figs. (11, 20), symbolize the number of chromosome having stickiness in head kidney cells of ***O. niloticus*** in treated fish. The recorded mean values of stickiness of fish fed on contaminated diet showed a very highly significant increase at both groups of Cd and Mix over that of control values. At the same time, in this type of aberration a significant increase of abnormalities appear at fish fed on diet contaminated with Cu than control.

9- Polyploidy:

When there are more than two sets of chromosomes in an euploid individual or species, the condition is called "polyploidy". Concerned to the polyploidy, it was cleared that at fish fed on contaminated diet with Cu & Cd (mix group) and Cd group the mean values of chromosomal aberration were much

higher than normal. There were highly significant increase in Mix group and Cd group over those of the control. In addition there was no significant difference between control fish and fish fed on diet contaminated with Cu (Table 13, Figs.12, 21).

10-Mitotic index

Mitotic index is a measure for the proliferation status of a cell population. It is defined as the ratio between the number of cells in mitosis and the total number of cells. It is evident that the mean values of the mitotic index of the treated fish (Cu ,Cd &Mix groups) exhibited a very highly significant decrease, than that of the control, on all fish groups that fed on contaminated diet (Table 14, Fig.,22).

Figure (3): Normal metaphase spread chromosomes of head kidney cells of ***Oreochromis niloticus*** ((X 2000)).

Figure (4): Metaphase spread chromosomes of head kidney cells of ***Oreochromis niloticus*** fed on metal contaminated diet showing chromatid deletion ((X 2000)).

Figure (5): Metaphase spread chromosomes of head kidney cells of ***Oreochromis niloticus*** fed on metal contaminated diet showing chromatid gap ((X 2000)).

Figure (6): Metaphase spread chromosomes of head kidney cells of ***Oreochromis niloticus*** fed on metal contaminated diet showing chromatid break ((X 2000)).

Figure (7): Metaphase spread chromosomes of head kidney cells of ***Oreochromis niloticus*** fed on metal contaminated diet showing chromatid fragmentation ((X 2000)).

Figure(8): Metaphase spread chromosomes of head kidney cells of ***Oreochromis niloticus*** fed on metal contaminated diet showing chromatid end to end association ((X 2000)).

Figure(9): Metaphase spread chromosomes of head kidney cells of ***Oreochromis niloticus*** fed on metal contaminated diet showing chromatid centromeric attenuation ((X 2000)).

Figure (10): Metaphase spread chromosomes of head kidney cells of ***Oreochromis niloticus*** fed on metal contaminated diet showing chromatid ring ((X 2000)).

Figure (11): Metaphase spread chromosomes of head kidney cells of ***Oreochromis niloticus*** fed on metal contaminated diet showing chromatid stickiness ((X 2000)).

Figure (12): Metaphase spread chromosomes of head kidney cells of ***Oreochromis niloticus*** fed on metal contaminated diet showing polyploidy ((X 2000)).

٤-Total proteins and total nitrogen content.

The data presented in tables (15 and 16)and figure (23 a) exhibited the effect of diet contaminated with heavy metals on the total protein and total nitrogen content (g/g fresh tissue) in muscle of Nile tilapia ***O.niloticus*** , as well as the significance t-test of the comparison of these data between the control and treated groups.

According to the recorded data, the total protein content in muscle of control fishes recorded average value 0.074 ± 0.005 g/g fresh tissue. The total protein content of fishes fed on diet contaminated with Cu, exhibited highly significant decrease after 10 days, recording percentage difference -13.51% from the value of the control fish. Whereas, it was increased significantly after 20 days and highly significantly after 30 days, being increased over the value of the control group with percentage 13.51 % and 39.19%, respectively (Table, 15).

Regarding the effect of Cd contaminated diet, the data showed that there was very highly significant reduction of total proteins content after 10 days (-9.46 %),then it was increased significantly after 20 and 30 days with percentage 13.51 and 37.84 % respectively.

The combined effect of the Cu and Cd induced non-significant reduction of total proteins content after 10 days, whereas it was very highly significantly increased after 20 and highly significant increased after 30 days, being differed from those reported for control fish with percentage 16.22 % and 64.87 %, respectively, (Tables,15&16).

The data presented in tables (15, 16) and figure (23 b) exhibited also the total nitrogen content in muscle of ***O.niloticus.*** The control fish recorded average value 15.291 ± 2.670 g/g fresh tissue. It is markedly increased due to Cu contaminated diet. The recorded data were found statistically very highly significant at all durations, recording percentage increase 76.03 %, 96.74 % and 134.62% over those of control fish.

A drastic rise of total nitrogen content in fish muscle was recorded due to ingestion of Cd contaminated diet. The data exhibited significant increase after 10 days (35.43 %), very highly significant increase after 20 and 30 days (79.03, 110.36 % , respectively).

Similarly the combined Cu and Cd induce obvious increase of total nitrogen content. The data revealed very highly significant increase at all durations, with percentage increase 69.22, 119.35 and 108.73 %) (Tables, 15&16, figure, 23 b).

5- Total plasma proteins, albumin and globulin content:-

The data presented in tables (17&18) and figure (24 a, b) showed the effect of diet contaminated with Cu or Cd or Cu and Cd on the total plasma proteins content, albumin and globulin of Nile tilapia (***O.niloticus***),as well as the significant t-values between control and experimental groups. According to the recorded data the total plasma protein content in the control fish recorded average value 46.149 ± 1.569 g/l. The Cu enhances its content after 10 and 20 days. It was found statistically very highly significant differed, recorded percentage difference 33.49 % and 20.29 %. Whereas, a highly significant decrease was reported after 30 days (-5.96 %).

In case of fishes fed on diet contaminated with Cd, very highly significant increase in the plasma total proteins content after 10 and 20 days was recorded compared to the control value of proteins. On the other hand, the data recorded non-significant decrease in plasma protein content after 30 days (Tables, 17 & 18, and figure 24 a).

The diet contaminated with Cu and Cd induced very highly significant increase of plasma proteins after 10 days and non-significant increase after 30 days, being 24.04 % and 2.51 % above the value of control. Whereas there was non- significant decrease in its level after 20 days (-1.57 %).

Concerning the total plasma albumin content of fish fed on contaminated diet with Cu, the data exhibited significant decrease after 10 and 30 days, whereas it was increased highly significant after 20 days, compared with those of control group (Tables, 17 & 18, and figure 24 b).

Regarding, fishes fed on diet contaminated with Cd, the data revealed that the level of total plasma albumin compared to the level of control showing a significant increase after 20 days only.

Concerning the combined effect of Cu and Cd on the level values of total plasma albumin, the recorded data exhibited significant decrease after 10 days (-15.75 %) whereas, it was very highly significantly increased after 20 days (35.52 %), compared with those recorded for control fishes.

The total plasma globulin of ***O.niloticus*** fed on Cu contaminated diet showed only a very highly significant increase with a percentage of difference (57.96 %) after 10 days.

Regarding fish fed on Cd contaminated diet, the data showed very highly significant increase after 10 and 20 days.

The Cu and Cd contaminated diet induced a general rise of total plasma globulins. Statistical analysis revealed a very highly significant difference (47.59 %) from the value of the control after 10 days and after 20 days a significant increase was found (7.83%). (Tables 17 & 18 and figure 24 c).

6-Muscle protein electrophoresis:-

6-1-Fractions appearance:-

The percentage appearance of muscle protein fractions of different experimental groups are presented in table (19) and figure (25). The muscle protein of control fish exhibited 9 fractions. Most of these fractions were appeared with a high percentage as the 1st, 2nd, 3rd, 4th, 5th and 6th fractions detected with a percentage of 100%. The 7th fraction appeared with a percentage 75%, whereas the 8th and 9th with low percentage (25%).

The 1st, 2nd, 3rd and 4th fractions were appeared with a percentage of 100% in all different contaminated groups (Resistant proteins).

The Nile tilapia fed on diet contaminated with Cu for 10 days exhibited fractions disappearance the last four, (sensitive proteins) whereas first five fractions appeared in all examined fishes (100%).

In fishes fed on diet contaminated with Cu after 10 and 20 days, most of fractions appeared in all examined fishes. Whereas, four protein fractions (6, 7, 8, &9) were missing for fishes subjected to Cu for 10 days. After 30 days no-fractions were missing but last two ones appeared sparsely.

Related to the fishes fed on diet contaminated with Cd for 10 days, the first five fractions were found in all tested individual, whereas fraction number 9 was missing (sensitive protein). Sarcoplasmic protein fractions (6, 7&8) do not considerably changed from those of control.

Fishes fed on diet contaminated with Cd for 20 days, the muscle proteinogram fractions from 1 to 6 was with absolute appearance high percentage 100%, whereas the 7^{th} fraction was occurred with percentage 62.5% (Not changeable proteins), while the last two fractions was appeared with low percentage (12.5%)(changeable proteins) (Table, 19).

Seven protein fractions from 1 to 7 were fully appeared in all fishes 100%, while the 8^{th} fraction distinguished with a percentage 62.5%. Whereas, the last one was disappeared due to feeding on diet contaminated with Cd for 30 days.

Concerning, ***O.nilotcus*** fed on diet contaminated with mixed metals Cu and Cd for 10 days the last fraction was not detected and fraction number 7 and 8 was sharply reduced, four sarcoplasmic protein fractions were found in all tested samples 100% (Resistant proteins), while fraction number 5 was appeared in 87.5% of tested samples. Sarcoplasmic protein fraction number 6 was found with percentage 62.5%.

Regarding the combined effect of contaminated fed with Cu and Cd for 20 days the fraction from 1 to 4 were appeared absolutely 100%, whereas fractions number 5 and 6 were presented in 87.5% in examined fishes (not changed). The fraction number 7 was found with percentage 62.5%, but fraction number 8 was found in a low percentage 12.5%, while fraction number 9 was not detected, so the last two fractions reflects the metals toxicity.

Related to the mixed effect of contaminated diet with Cu and Cd on Nile tilapia for 30 days, the first six sarcoplasmic protein fractions were not affected (100 % appearance), whereas the 7th and 8th fractions were appeared with a low percentage (50% & 25%). The last fraction was missing due to metal toxic effects.

6.2- Relative mobility

The effect of diet contaminated with Cu or Cd or Cu and Cd on relative mobility of sarcoplasmic protein of ***O. niloticus*** and the significance (t-test) are presented in tables (20 & 21) and figure (26).

Fish fed with Cu contaminated diet for 10 days induced a drastic rise of protein mobility. The significant (t-test)among relative mobilities of muscle protein fractions (1&4) between the fishes of control and those fed on diet contaminated with Cu for 10 days, showed very highly significant acceleration. A highly significant increased mobility was found for the mobility of fraction; 5.

Concerning the fishes fed on diet contaminated with Cu for 20 days, there were non-significant variations for all fractions except the 4th fraction which exhibited significantly accelerated migration on the acrylamide gel.

There were no significant variations in the fraction mobilities between control sarcoplasmic protein and those of fishes fed on Cu contaminated diet for 30 days.

Regarding the tilapia fed on diet contaminated with Cd for 10 days, the relative mobility of the first two fractions changed with

significant difference when comparing relative mobility between this treatment and those of control fish, whereas the difference of the mobility of the 3rd, and 4th fractions were reduced highly significantly from the value of control. The mobility of the 6th fraction was also reduced highly significantly. While the fractions (5, 7& 8) exhibited non-significant differences from those of the control group.

Regarding the effect of diet contaminated with Cd for 20 and 30 days, the relative mobility of sarcoplasmic protein fractions exhibited non-significant differences (Table, 21 and figure, 26).

Regarding the effect of diet contaminated with Cu and Cd exhibited non-significant differences at all durations except in band number 4 and 5 showed significant acceleration than control after 30 days.

٧-Molecular Result

DNA genome has a high molecular weight, so it remained in the wheel of the agarose gel electrophoresis ;(Figure, 28).

The PCR-RFLP products of NADH dehydrogenase gene of all groups (normal and other treated groups) were presented in lanes (1 to 10), respectively, with molecular weight ~1050bp, (Figure, 29).

There are some restriction endonuclease did not differentiate between control group and other treated groups (***Ssp***I and ***Apo***I). The restriction enzyme ***Ssp*I** digested all groups (control ,and other treated groups) into three cuts with lengths (~180 ,~370 and~500 bp; lane;1 to 10 ; Table, 22 and Figures, 30, 39), wherever the restriction endonuclease, ***Apo*I** fragmented all groups into three restrictiction fragments with lengths (~250,~280 and ~520 bp ;Lane 1 to 10; Table, 23 and Figures, 31, 40).

The restriction enzyme ***Eco***NI clustred the ten groups into two clusters. The enzyme cut the normal group into two bands (~290 and ~760 bp; Lane 1) whereas the same endonuclease cut the other groups into four cuts (~50, ~100, ~ 290 and ~610 bp; Lane 2 to 9 ;Table, 24 and Figures, 32, 41).

***Avr*II** restriction endonuclease differentiated the ten individual genes into two groups. The first group, which included the normal and all Copper groups at all durations differentiated into two distinct bands at lengths (~150 and ~900bp ; Lane 1 to 4), whereas the same restriction

enzyme digested the rest into three restricted fragments which have lengths ~130 , ~210 and ~710 bp; (Lane 5 to 10; Table, 25 and Figures, 33,42).

The restriction fragments resulting from the digestion of NADH dehydrogenase gene of the individuals with enzyme ***Bse*RI** are represented in (Table, 26 and Figures, 34, 43).It was noted that, the enzyme digested the gene of all groups to differentiate them into three groups. First group included control and cadmium group at all durations which digested into two distinct fragments with lengths ~30 and ~1020 bp; (Lane 1,5,6and 7) whereas the second group which included copper at all durations separated into four cuts with lengths ~30 , ~270 , ~350 and ~400bp; (Lane 2,3and 4)and third group which included (mix (Cu + Cd) group at all durations) digested into three fragments with lengths ~30 , ~400 and ~620 bp; (Lane 8,9and 10).

By using the enzyme ***Ava*I** NADH dehydrogenase gene of all samples was grouped into three groups .The First group included control , copper after 10 days , Copper after 20 days ,and Cadmium after 10 days the endonuclease digested into two cuts with lengths ~ 150 and ~ 900 bp; (Lane 1,2 and 3) ,when cut the second group (Copper group after 30 days , Mix group (Cu + cd) after 10 days , Mix group (Cu + cd) after 30 days) into three cuts with lengths ~50 , ~150 and ~850 bp; (Lane 4,8and 9) and the third group (Mix (Cu + Cd)after 30 days) into four restriction fragments at lengths (~50 ,~100 ,~150 and ~750 bp; Lane 10). (Table, 27 and Figures, 35, 44).

The ***Ear*I** enzyme restirected the NADH dehydrogenase of the ten groups into three clusters. First cluster included (control group ,

Cadmium groups at all durations) was digested into two cuts at lengths ~330 and ~720 bp; (lane 1 , 5,6 and 7)and the second group (copper group after 20 days , copper group after 30 days , Mix group after 20 days , Mix group after 30 days) into four fragments at lengths ~50 , ~250 ,~330 and ~420 bp; (Lane 3,4 and 9) but the third group (Copper after 10 days , Mix after 10 days) The ***Ear***I restricted gene into three bands at lengths ~50 , ~330 and ~760 bp; (Lane 2 and 8). (Table, 28 and Figures, 36, 45).

The restriction endonuclease ***Pst*I** divided all groups into four clusters .First group included (control , Mix group after 10 days , Mix group after 20 days) was cut into two bands at lengths ~200 and ~850bp ; (Lane 1,8 and 9) and the second group included (copper group after 10 days , Cadmium group after 10 days , Mix after 10 days) was digested NADH dehydrogenase into three fragments at lengths ~200 , ~300 and ~550 bp; (Lane 2,5 and 8) but the third group represented (Copper after 20 days, Copper after 30 days , Cadmium group after 20 days, Cadmium group after 30 days), the enzyme **PstI** cut gene into three bands at lengths ~50 , ~ 200 , ~ 250 and 550 bp; (Lane 3,4,6 and 7). (Table, 29 and Figures, 37, 46).

The restriction fragments which result from the digestion of NADH dehydrogenase with the enzyme ***Ban*I** are presented in (Table, 30 and Figures, 38,47) .In this figure two groups were classified according to bands at different lengths resulted from digestion of ***Ban*I**. First group included (control , copper group after 10 days, Cadmium group after 10 days , Cadmium group after 20 days , Mix group after 10 days , Mix

group after 20 days) was digested into three fragments at lengths ~50 , ~130 and ~870 bp; (Lane 1,2,5,6,8 and 9), whereas the same enzyme cut the rest into four cuts at lengths (~50 , ~130 , ~270 and ~600 bp; Lane 3,4,7, and 10).

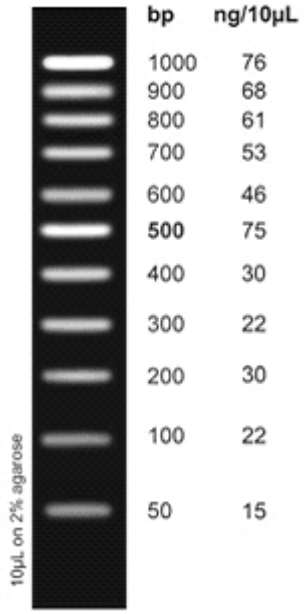
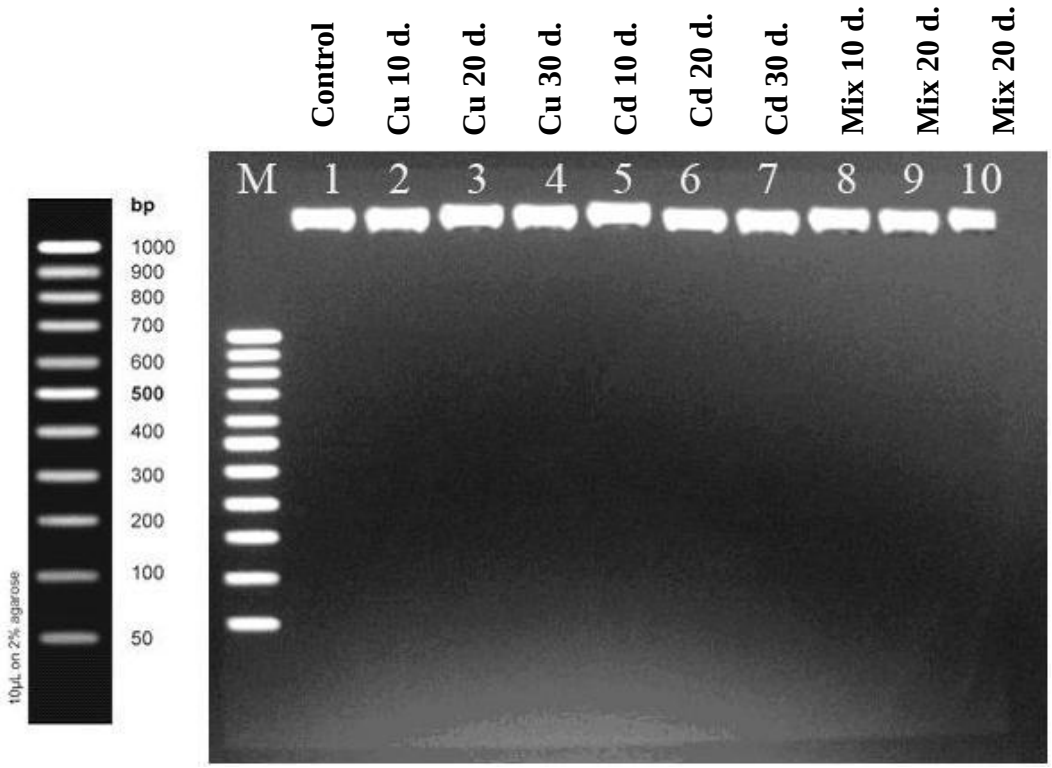


Figure (27): shows DNA marker with 1 Kb.



Figure(28): shows DNA genome from *Oreochromis niloticus*, lane M is 1 Kb DNA ladder.Lane (1-10) represented normal ,Cu after 10 d., Cu after 20 d., Cu after 30 d., Cd after 10 d., Cd after 20 d., Cd after 30d., Cu+Cd after 10 d., Cu+Cd after 20 d., and Cu+Cd after 30d.

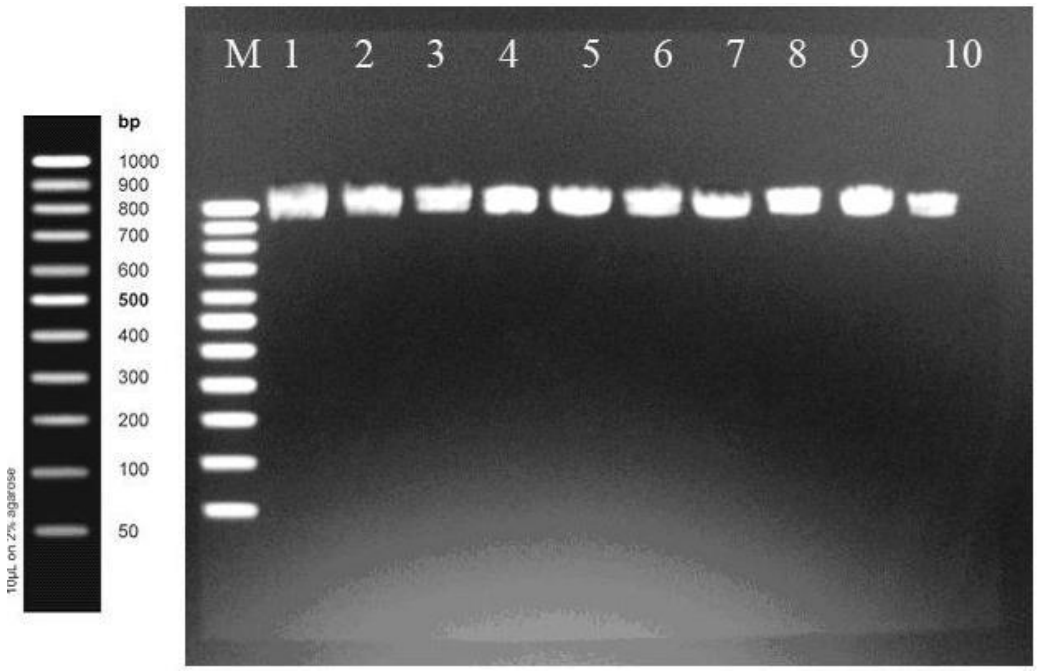


Figure (29): Shows full –segment NADH dehydrogenase gene (~1050 bp) of *Oreochromis niloticus*, lane M is 1 Kb DNA ladder. Lane (1-10) represented normal, Cu after 10 d., Cu after 20 d., Cu after 30 d., Cd after 10 d., Cd after 20 d., Cd after 30d. Cu+Cd after 10 d., Cu+Cd after 20 d., and Cu+Cd after 30d.

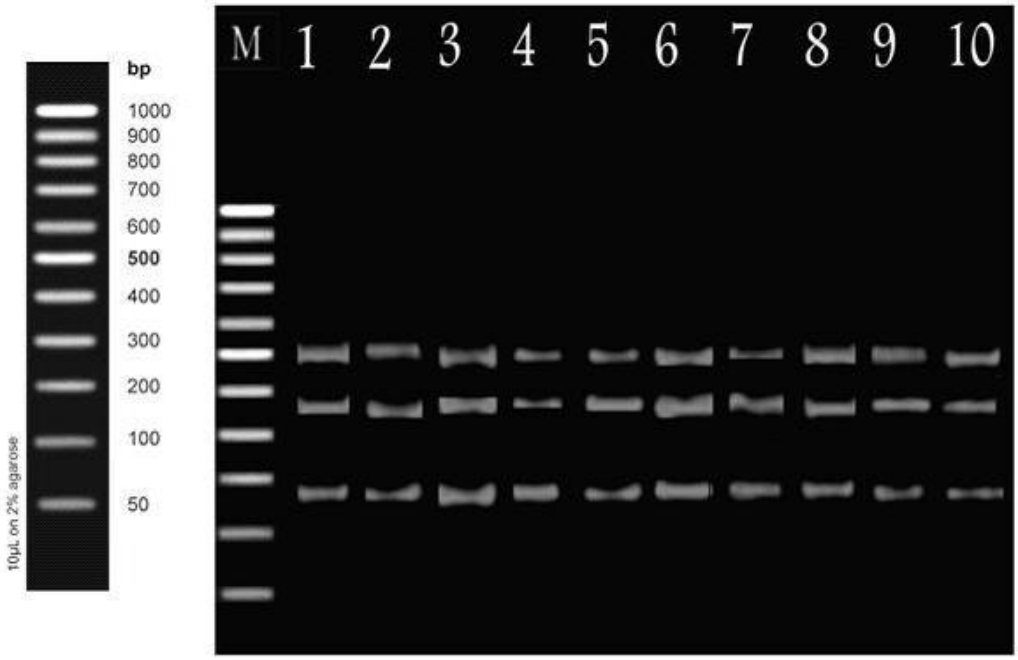


Figure (30): Shows SspI restriction enzyme fragmented all groups into three restricticton fragments with lengths (~180,~370 and ~500 bp ;Lane 1 to 10).

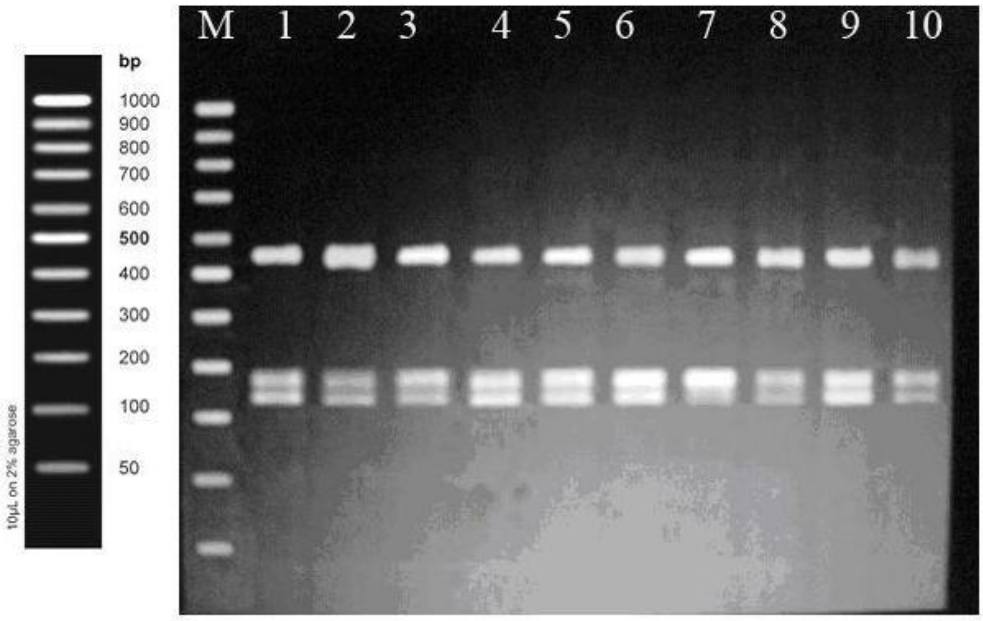


Figure (31): Shows *Apo*I restriction enzyme fragmented all groups into three restricticti̇on fragments with lengths (~250,~280 and ~520 bp ;Lane 1 to 10).

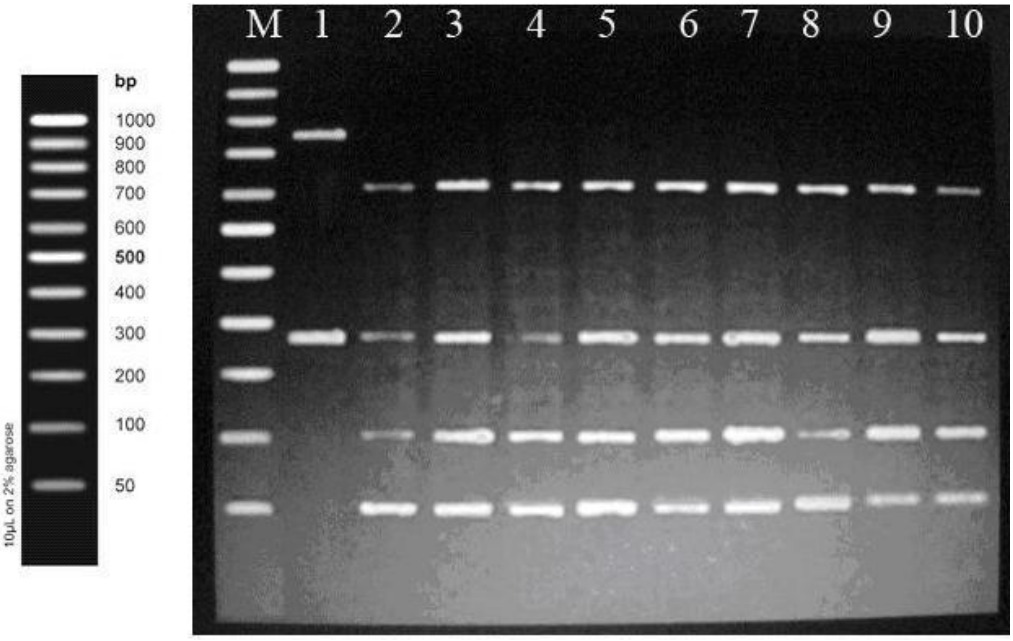


Figure (32): Shows *Eco*NI restriction enzyme clustred the ten groups into two clusters. The enzyme cut the normal group into two bands (~290 and ~760 bp ;Lane 1)where as the same endonuclease cut the other groups into four cuts (~50 , ~100 , ~ 290 and ~610 bp;Lane 2 to 9).

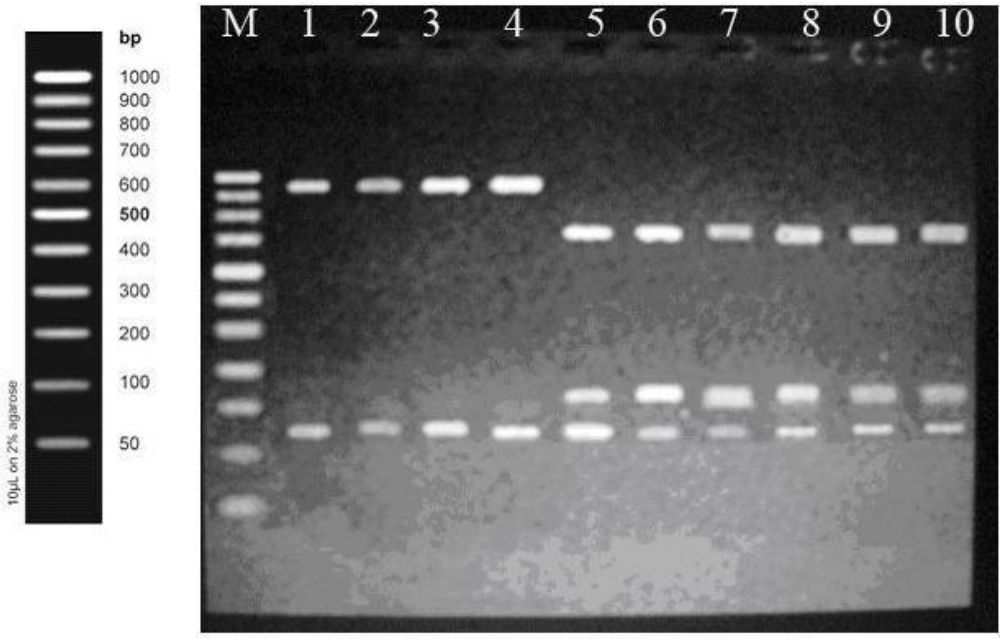


Figure (33): Shows *Avr*II restriction endonuclease differentiated the ten individual genes into two groups. The first group, which included the normal and all Copper groups at all durations differentiated into two distinct bands at lengths (~150 and ~900bp ; Lane 1 to 4), where as the same restriction enzyme digested the rest into three restricted fragments which have lengths (~130 , ~210 and ~710 bp;Lane 5 to 10).

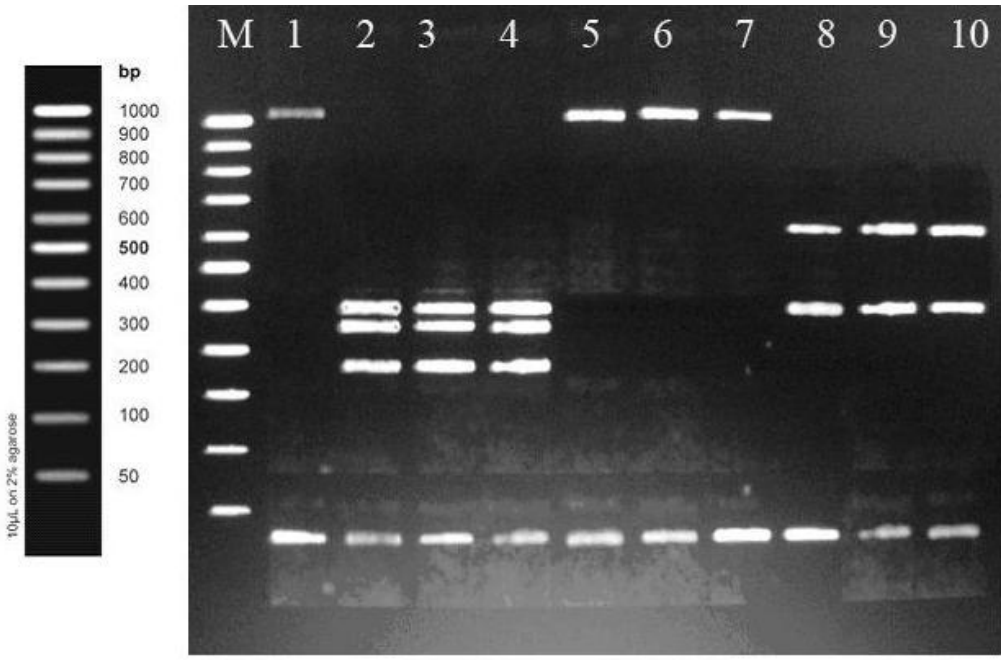


Figure (34): Shows BseRI restriction enzyme digested the gene of all groups to differentiate them into three groups. First group digested into two distinct fragments with lengths (~30 and ~1020 bp; Lane 1,5,6and 7) , the second group separated into four cuts with lengths (~30 , ~270 , ~350 and ~400bp;Lane 2,3and

4) third group digested into three fragments with lengths (~30 , ~400 and ~620 bp; Lane 8,9and 10).

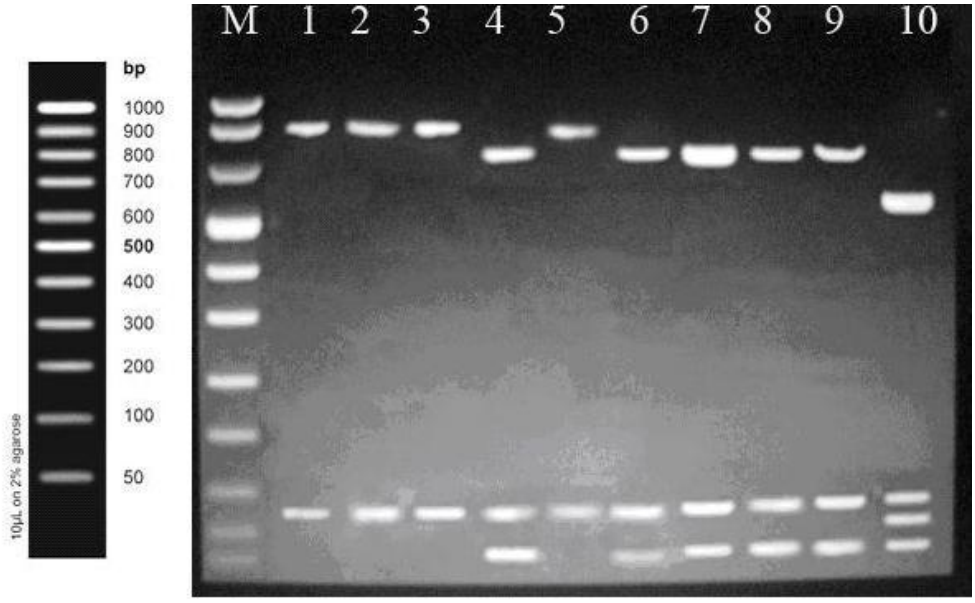


Figure (35): Shows *Ava*I restriction enzyme digested NADH dehydrogenase of all groups into three groups .The First group digested into two cuts with lengths (~ 150 and ~ 900 bp; Lane 1,2 and 3), the second group fragmented into three cuts with lengths (~50 , ~150 and ~850 bp;Lane 4,8and 9) and the third one cut into four restriction fragments at lengths (~50 ,~100 ,~150 and ~750 bp; Lane 10).

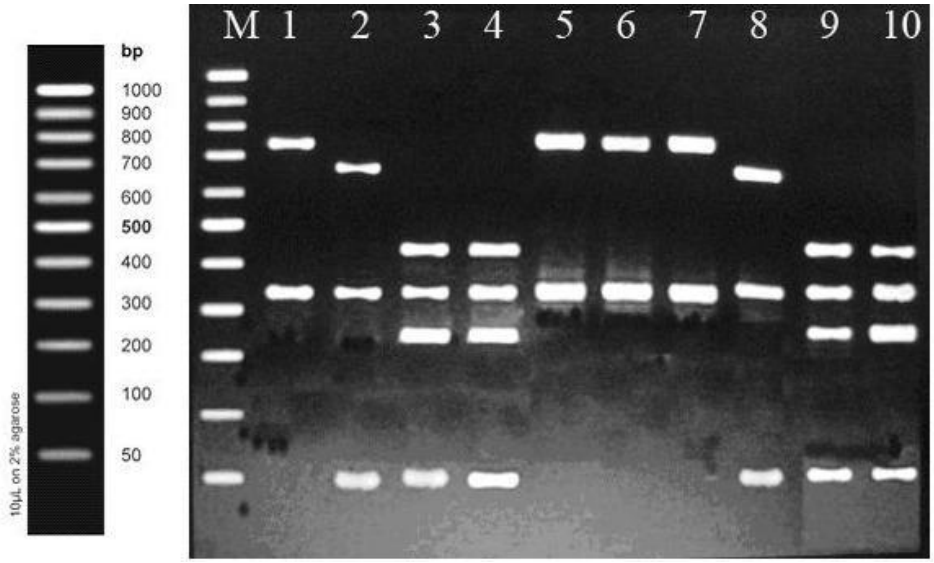


Figure (36): Shows *Ear*I restriction enzyme fragmented the NADH dehydrogenase of the ten groups into three clusters. First cluster two cuts at lengths (~330 and ~720 bp; lane 1 , 5,6 and 7)and the second group into four fragments at lengths (~50 , ~250 ,~330 and ~420 bp; Lane 3,4 and 9) but the third group into three bands at lengths (~50 , ~330 and ~760 bp; Lane 2 and 8).

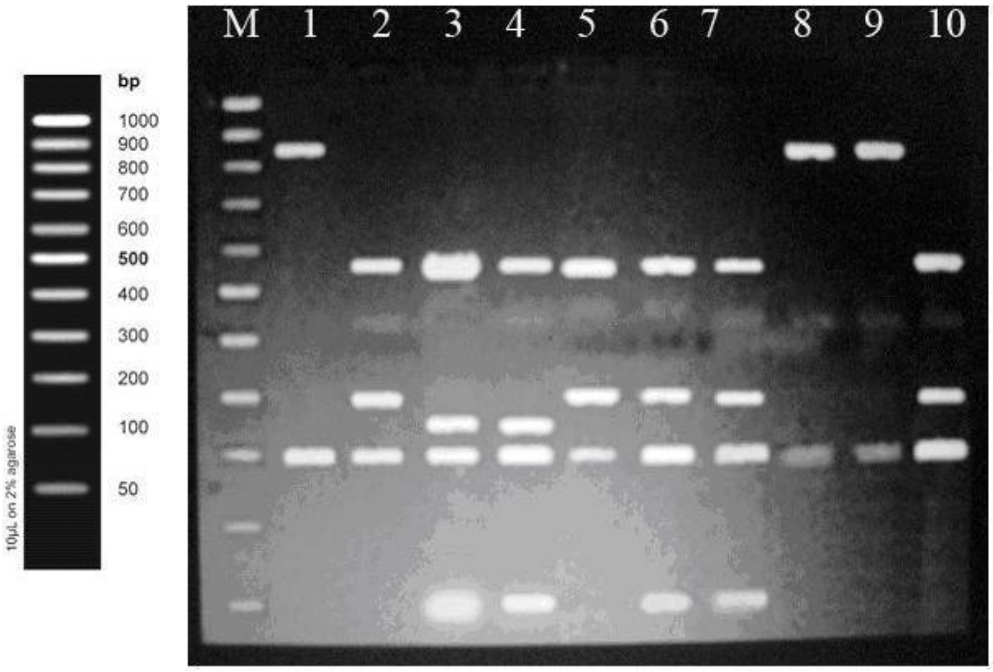


Figure (37):Shows *Pst*I divided all groups into four clusters .First group was cut into two bands at lengths (~200 and ~850 bp ; Lane 1,8 and 9) and the second group was digested NADH dehydrogenase into three fragments at lengths (~200 , ~300 and ~550 bp; Lane 2,5 and 8) but the third group represented (Copper after 20 days, Copper after 30 days , Cadmium group after 20 days, Cadmium group after 30 days), the enzyme PstI cut gene into three bands at lengths (~50 , ~ 200 , ~ 250 and 550 bp; Lane 3,4,6 and 7).

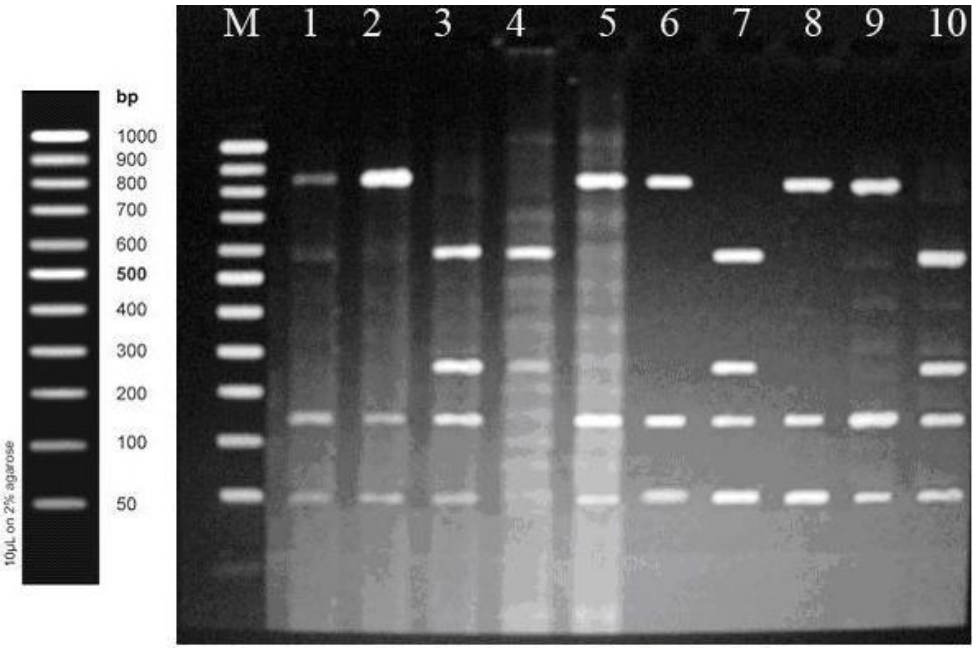


Figure (38):Shows *Ban*I restriction enzyme fragmented all groups into two clusters. First group was digested into three fragments at lengths (~50 , ~130

and ~870 bp;Lane 1,2,5,6,8 and 9), where as the same enzyme cut the rest into four cuts at lengths (~50 , ~130 , ~270 and ~600 bp; Lane 3,4,7, and 10).

Table (22): Shows the lengths of NADH dehydrogenase genes fragments resulted from digestion with *Ssp*I enzyme in the *Oreochromis niloticus* fed on contaminated diet with Cu&Cd.

Groups	Band Number			
	1	2	3	4
Normal	~180	~370	~500	……..
Cu group after 10 days	~180	~370	~500	……..
Cu group after 20 days	~180	~370	~500	……..
Cu group after 30 days	~180	~370	~500	……..
Cd group after 10 days	~180	~370	~500	……..
Cd group after 20 days	~180	~370	~500	……..
Cd group after 30 days	~180	~370	~500	……..
Cu+Cd group after 10 days	~180	~370	~500	……..
Cu+Cd group after 20 days	~180	~370	~500	……..
Cu+Cd group after 30 days	~180	~370	~500	……..

Table (23): Shows the lengths of NADH dehydrogenase genes fragments which restricted with *Apo*I enzyme in the *Oreochromis niloticus* fed on contaminated diet with Cu&Cd.

Groups	Band Number			
	1	2	3	4
Normal	~250	~280	~ 520	……..
Cu group after 10 days	~250	~280	~ 520	……..
Cu group after 20 days	~250	~280	~ 520	……..
Cu group after 30 days	~250	~280	~ 520	……..
Cd group after 10 days	~250	~280	~ 520	……..
Cd group after 20 days	~250	~280	~ 520	……..
Cd group after 30 days	~250	~280	~ 520	……..
Cu+Cd group after 10 days	~250	~280	~ 520	……..
Cu+Cd group after 20 days	~250	~280	~ 520	……..
Cu+Cd group after 30 days	~250	~280	~ 520	……..

Table (24): Shows the lengths of NADH dehydrogenase genes fragments which restricted with *EcoN*I enzyme in the *Oreochromis niloticus* fed on contaminated diet with Cu&Cd.

Groups	Band Number			
	1	2	3	4
Normal	~290	~760	……..	……..
Cu group after 10 days	~50	~100	~290	~610
Cu group after 20 days	~50	~100	~290	~610
Cu group after 30 days	~50	~100	~290	~610
Cd group after 10 days	~50	~100	~290	~610
Cd group after 20 days	~50	~100	~290	~610
Cd group after 30 days	~50	~100	~290	~610
Cu+Cd group after 10 days	~50	~100	~290	~610
Cu+Cd group after 20 days	~50	~100	~290	~610
Cu+Cd group after 30 days	~50	~100	~290	~610

Table (25): Shows the lengths of NADH dehydrogenase genes fragments which restricted with *Avr*II enzyme in the *Oreochromis niloticus* fed on contaminated diet with Cu&Cd.

Groups	Band Number			
	1	2	3	4
Normal	~130	~920	……..	……..
Cu group after 10 days	~130	~920	……..	……..
Cu group after 20 days	~130	~920	……..	……..
Cu group after 30 days	~130	~920	……..	……..
Cd group after 10 days	~130	~210	~710	……..
Cd group after 20 days	~130	~210	~710	……..
Cd group after 30 days	~130	~210	~710	……..
Cu+Cd group after 10 days	~130	~210	~710	……..
Cu+Cd group after 20 days	~130	~210	~710	……..
Cu+Cd group after 30 days	~130	~210	~710	……..

Table (26): Shows the lengths of NADH dehydrogenase genes fragments which restricted with *Bse*RI enzyme in the *Oreochromis niloticus* fed on contaminated diet with Cu&Cd.

Groups	Band Number			
	1	2	3	4
Normal	~30	~1020	……..	……..
Cu group after 10 days	~30	~270	~350	~400
Cu group after 20 days	~30	~270	~350	~400
Cu group after 30 days	~30	~270	~350	~400
Cd group after 10 days	~30	~1020	……..	……..
Cd group after 20 days	~30	~1020	……..	……..
Cd group after 30 days	~30	~1020	……..	……..
Cu+Cd group after 10 days	~30	~400	~ 620	……..
Cu+Cd group after 20 days	~30	~400	~ 620	……..
Cu+Cd group after 30 days	~30	~400	~ 620	……..

Table (27): Shows the lengths of NADH dehydrogenase genes fragments which restricted with *Ava*I enzyme in the *Oreochromis niloticus* fed on contaminated diet with Cu&Cd.

Groups	Band Number			
	1	2	3	4
Normal	~150	~900	……..	……..
Cu group after 10 days	~150	~900	……..	……..
Cu group after 20 days	~150	~900	……..	……..
Cu group after 30 days	~50	~150	~850	……..
Cd group after 10 days	~150	~900	……..	……..
Cd group after 20 days	~50	~150	~850	……..
Cd group after 30 days	~50	~150	~850	……..
Cu+Cd group after 10 days	~50	~150	~850	……..
Cu+Cd group after 20 days	~50	~150	~850	……..
Cu+Cd group after 30 days	~50	~100	~150	~750

Table (28): Shows the lengths of NADH dehydrogenase genes fragments which restricted with *Ear*I enzyme in the *Oreochromis niloticus* fed on contaminated diet with Cu&Cd.

Groups	Band Number			
	1	2	3	4
Normal	~330	~720	……..	……..
Cu group after 10 days	~50	~330	~670	……..
Cu group after 20 days	~50	~250	~330	~420
Cu group after 30 days	~50	~250	~330	~420
Cd group after 10 days	~330	~720	……..	……..
Cd group after 20 days	~330	~720	……..	……..
Cd group after 30 days	~330	~720	……..	……..
Cu+Cd group after 10 days	~50	~330	~670	……..
Cu+Cd group after 20 days	~50	~250	~330	~420
Cu+Cd group after 30 days	~50	~250	~330	~420

Table (29): Shows the lengths of NADH dehydrogenase genes fragments which restricted with *Pst*I enzyme in the *Oreochromis niloticus* fed on contaminated diet with Cu&Cd.

Groups	Band Number			
	1	2	3	4
Normal	~200	~850	……..	……..
Cu group after 10 days	~200	~300	~550	……..
Cu group after 20 days	~50	~200	~250	~550
Cu group after 30 days	~50	~200	~250	~550
Cd group after 10 days	~200	~300	~550	……..
Cd group after 20 days	~50	~200	~250	~550
Cd group after 30 days	~50	~200	~250	~550
Cu+Cd group after 10 days	~200	~850	……..	……..
Cu+Cd group after 20 days	~200	~850	……..	……..
Cu+Cd group after 30 days	~200	~300	~550	……..

Table (30): Shows the lengths of NADH dehydrogenase genes fragments which restricted with *Ban*I enzyme in the *Oreochromis niloticus* fed on contaminated diet with Cu&Cd.

Groups	Band Number			
	1	2	3	4
Normal	~50	~130	~870	……..
Cu group after 10 days	~50	~130	~870	……..
Cu group after 20 days	~50	~130	~270	~600
Cu group after 30 days	~50	~130	~270	~600
Cd group after 10 days	~50	~130	~870	……..
Cd group after 20 days	~50	~130	~870	……..
Cd group after 30 days	~50	~130	~270	~600
Cu+Cd group after 10 days	~50	~130	~870	……..
Cu+Cd group after 20 days	~50	~130	~870	……..
Cu+Cd group after 30 days	~50	~130	~270	~600

Restriction fragments
***Ssp*I**

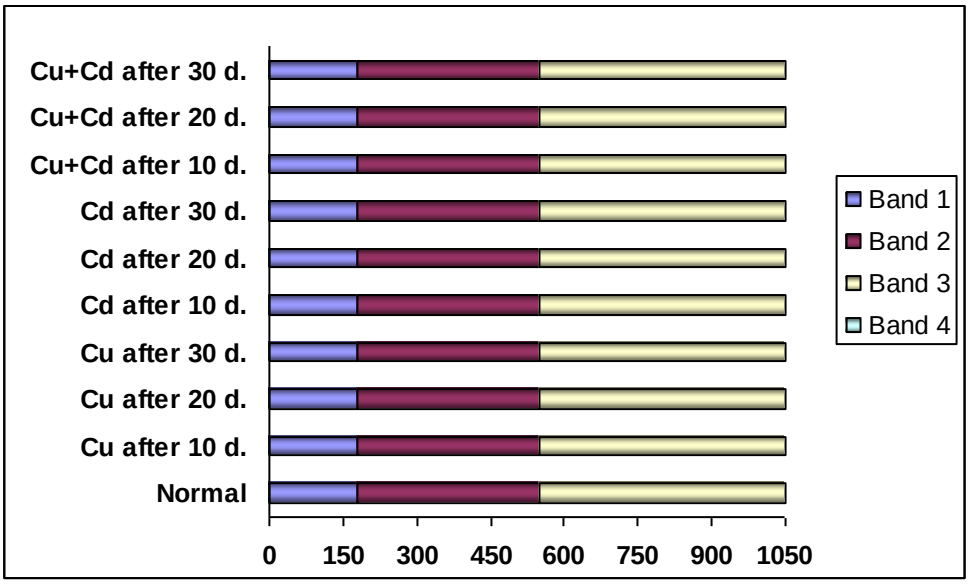


Figure (39): shows that the enzyme *Ssp*I digested all groups into three cuts at the same lengths ~180 ,~370 and~500 bp.

Restriction fragments
***Apo*I**

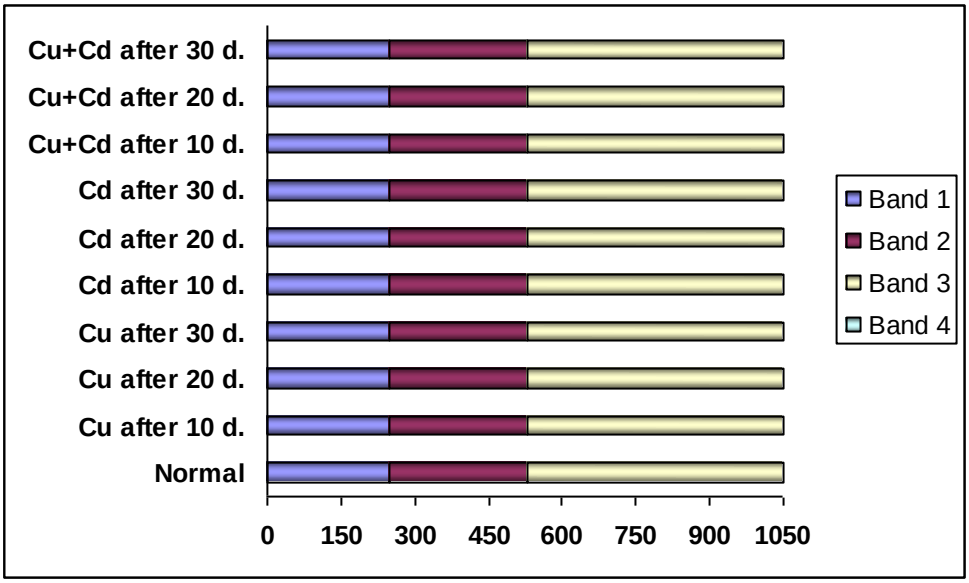


Figure (40): Shows that the enzyme *Apo*I digested all groups into three cuts at the same lengths ~250, ~280 and ~520 bp.

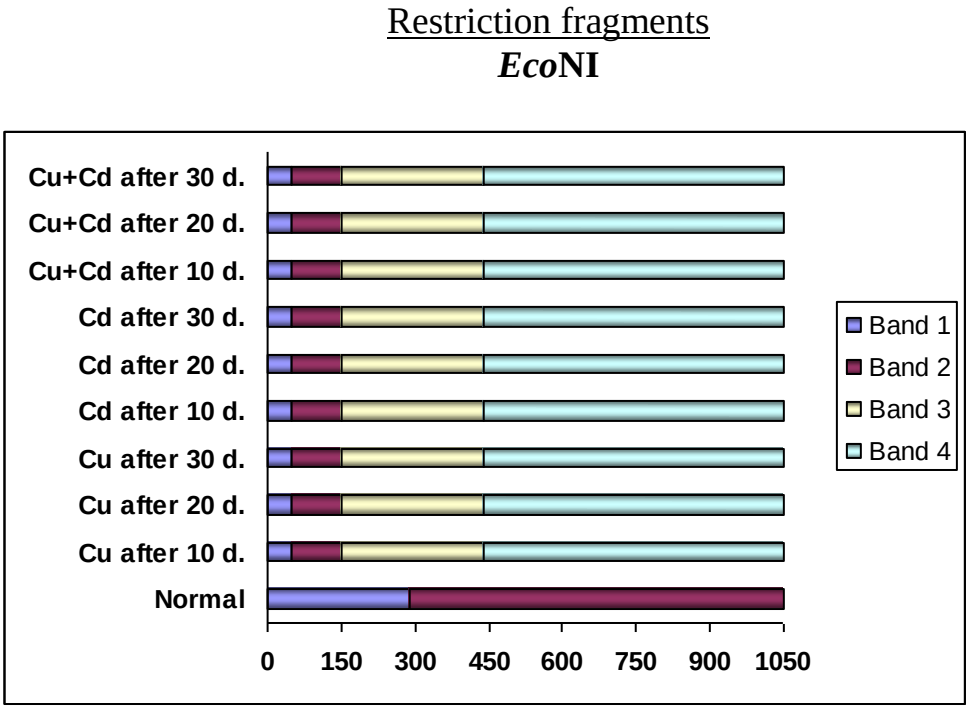


Figure (41): Shows the enzyme *Eco*NI clustered the ten groups into two clusters . The enzyme cut the normal group into two bands (~290 and ~760 bp) where as the same endonuclease cut the other groups into four cuts (~50, ~100, ~ 290 and ~610 bp).

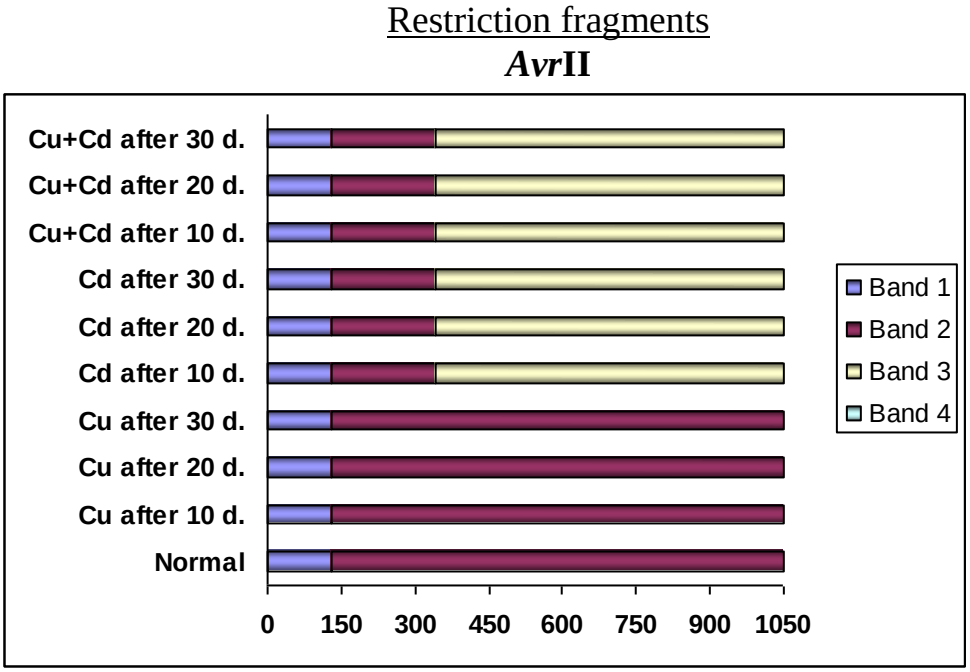


Figure (42): Shows the enzyme *Avr*II differentiated the ten individual genes into two groups. The first group, which included the normal and all Copper groups at all durations differentiated into two distinct bands at lengths (~150 and ~900bp), where as the same restriction enzyme digested the rest into three restricted fragments which have lengths (~130 , ~210 and ~710 bp).

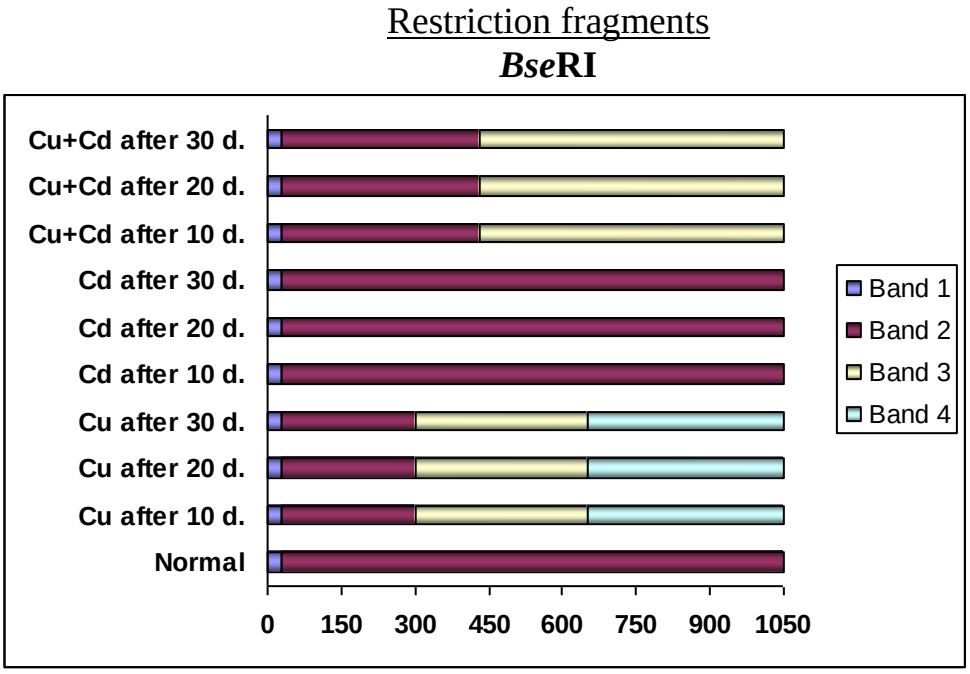
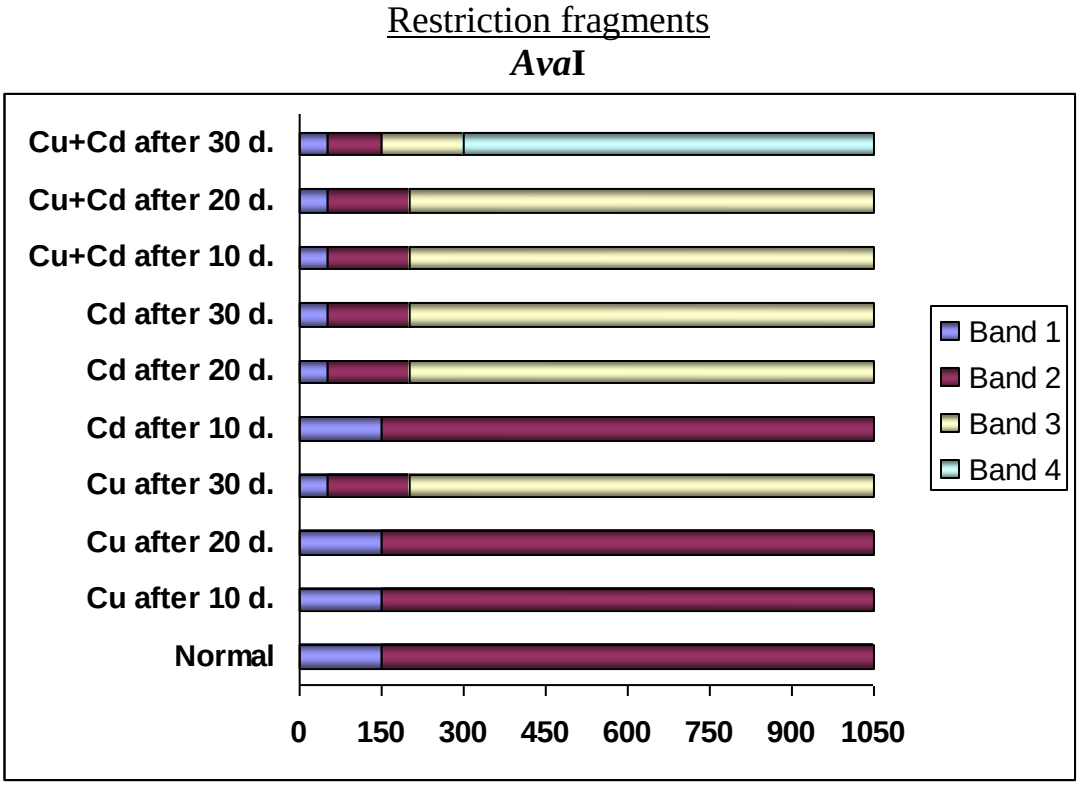


Figure (43): Shows the enzyme *Bse*RI digested the gene of all groups to differentiate them into three groups. First group included control and cadmium group at all durations which digested into two distinct fragments with lengths (~30 and ~1020 bp) where as the second group which included copper at all durations separated into four cuts with lengths(~30 , ~270 , ~350 and ~400bp) and third group which included (Cu + Cd) group at all durations digested into three fragments with lengths (~30 , ~400 and ~620 bp).



Figure(44): Shows the enzyme *Ava*I digested NADH dehydrogenase gene of all samples into three groups .The First group included Normal, Cu after 10 d., Cu

after 20 d.,and Cd after 10 d. the endonuclease digested into two cuts with lengths (~ 150 and ~ 900 bp.) ,when cut the second group Cu after 30d. , Cu + cd after 10 d. , Cu + cd after 30 d. into three cuts with lengths (~50 , ~150 and ~850 bp) and the third group Cu + Cd after 30 d. into four restriction fragments at lengths (~50 ,~100 ,~150 and ~750 bp).

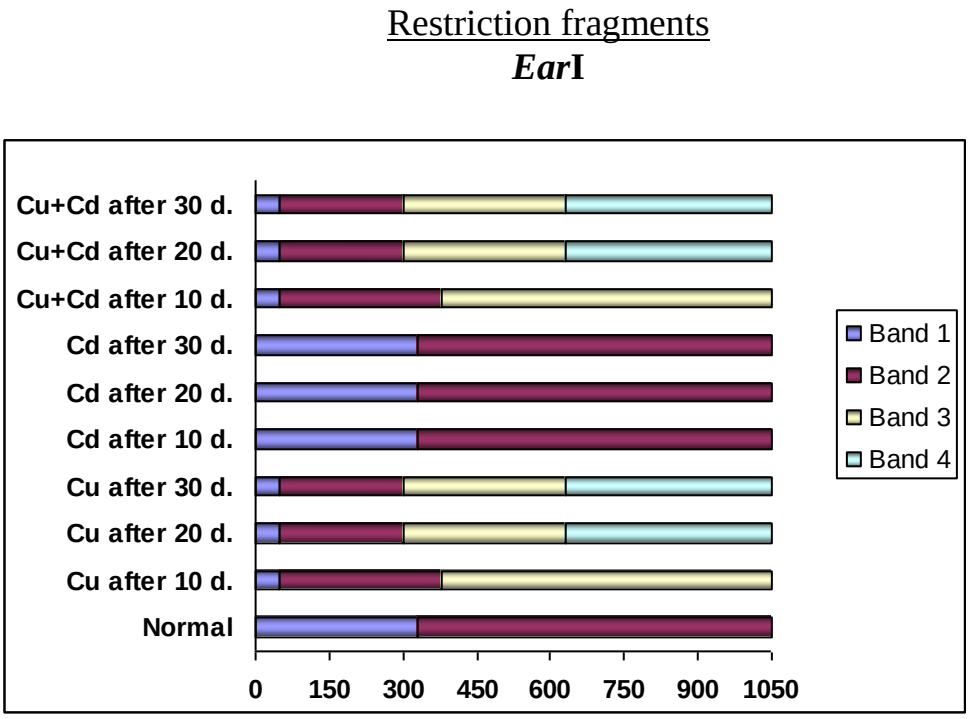


Figure (45): Shows the enzyme *Ear*I restirected the NADH dehydrogenase of the ten groups into three clusters. First cluster included (Normal group , Cadmium groups at all durations) was digested into two cuts at lengths (~330 and ~720 bp) and the second group (copper group after 20 days , copper group after 30 days , Cu+Cd group after 20 days , Cu+Cd group after 30 days) into four fragments at lengths (~50 , ~250 ,~330 and ~420 bp) but the third group (Copper after 10 days , Mix after 10 days) The *Ear*I restricted gene into three bands at lengths (~50 , ~330 and ~760 bp).

Restriction fragments

***Pst*I**

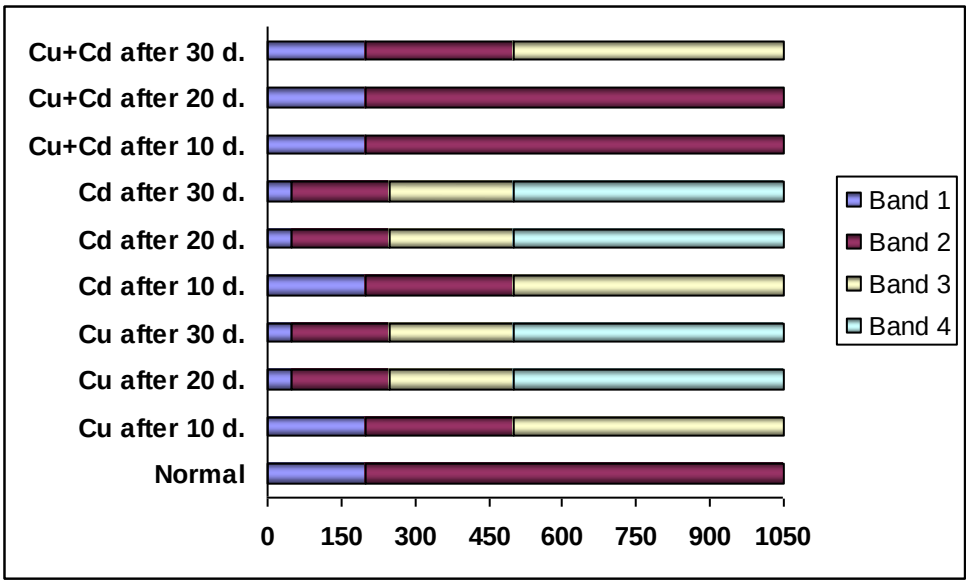


Figure (46): Shows the enzyme *Pst*I divided all groups into four clusters .First group included (Normal , Mix group after 10 days , Mix group after 20 days) was cut into two bands at lengths (~200 and ~850bp) and the second group included (copper group after 10 days , Cadmium group after 10 days , Mix after 10 days) was digested NADH dehydrogenase into three fragments at lengths (~200 , ~300 and ~550 bp) but the third group represented (Copper after 20 days, Copper after 30 days , Cadmium group after 20 days, Cadmium group after 30 days), the enzyme *Pst*I cut gene into three bands at lengths (~50 , ~ 200 , ~ 250 and 550 bp).

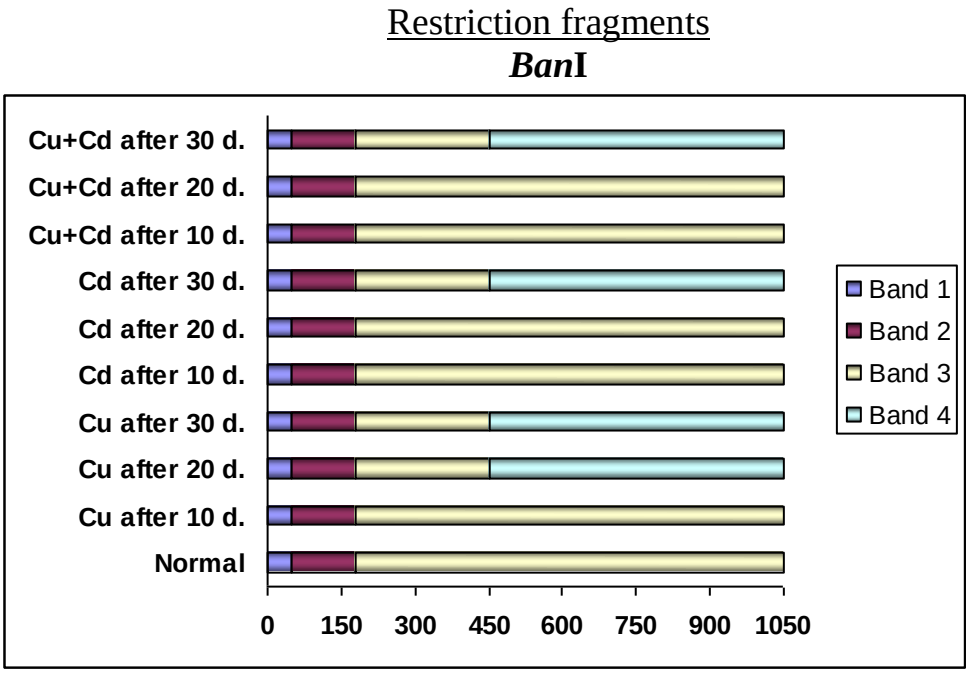


Figure (47): Shows the enzyme *Ban*I clustered all groups into two groups. First group included (Normal, copper group after 10 days, Cadmium group after 10 days , Cadmium group after 20 days , Mix group after 10 days , Mix group after 20 days) was digested into three fragments at lengths (~50 , ~130 and ~870 bp), where as the same enzyme cut the rest into four cuts at lengths (~50 , ~130 , ~270 and ~600 bp).