Introduction and Review of Literature

Importance of Tilapia

Tilapia is the common name for nearly 100 species of cichlid fishes. Tilapias inhabit a variety of fresh and, less commonly, brackish water habitats from shallow streams and ponds through to rivers, lakes, and estuaries. Most tilapias are omnivorous with a preference for soft aquatic vegetation and detritus (**Baker and Jenny, 1988**). For a long time, they were all united in the genus ***Tilapia***, but nowadays, genera ***Oreochromis*** **and** ***Sarotherodon*** are considered distinct.

In Egypt, tilapiane fish represented one of the most common species in the river Nile and numerous lakes. Tilapiane species are extremely recommended as one of successful culturing fish as they primarily exhibit excellent growth rates even on low protein diets. They also tolerate wider ranges of environmental conditions. Moreover, they are highly and widely acceptable as food because of their high delicacy by a huge number of people throughout the world. Consequently special interest has been given to study the biological and environmental conditions related to improve Tilapia production **(Pullin and Meconnel, 1982).**

Tilapiines are also among the easiest and most profitable fish to farm. This is due to their omnivorous diet, mode of reproduction, tolerance of high stocking density, and rapid growth rate. In some regions the fish can be put out in the rice fields when rice is planted, and will have grown to edible size (12–15 cm) when the rice is ready for harvest. One recent estimate for the **(FAO, 2004)** puts annual production of tilapia at about 1.5 million tonnes, a quantity comparable to the annual production of farmed salmon and trout. Unlike salmon,

which rely on high-protein feeds based on fish or meat, commercially important tilapiine species eat a vegetable or cereal based diet.

Tilapias have been raised as food for human consumption for a long time; tilapia farming is believed to have originated some 2,500 years ago. Tilapia have also been transplanted to many countries outside their native range and are now farmed worldwide, **(FAO, 1993)**.

Water Pollution with metals:

The impact of massive chemical pollution on the biochemical functions of fishes has greatly stimulated more research work. Dangerous substances commonly discharged as waste from industrial complexes or agricultural activities. The varieties of pollutants, particularly heavy metals which are known to be toxic to human beings as well as to aquatic organisms, are enormous. Bioaccumulation of heavy metals in fish may critically influence both growth rate and quality of fish **(Hodson *et al.*, 1984 and Haggag *et al.*, 1999).**

The aquatic environment with its water quality is considered the main factor controlling the state of health and disease for both man and animal. Nowadays, the increasing use of the waste chemical and agricultural drainage systems represents the most dangerous chemical pollution. The most important heavy metals from the point of view of water pollution are Zn, Cu, Pb, Cd, Hg, Ni and Cr. Some of these metals (as Cu, Ni, Cr and Zn) are essential trace metals to living organisms, but become toxic at higher

concentrations. Others, such as Pb and Cd have no known biological function but are toxic elements **(Rashed, 2004).**

Source of pollution with metals

Metals have many sources from which they can flow into the water body, these sources are:

1-Natural Sources: Metals are found throughout the earth, in rocks, soil and introduce into the water body through natural processes, weathering and erosion.

2-Industrial Sources: Industrial processes, particularly those concerned with the mining and processing of metal ores, the finishing and plating of metals and the manufacture of metal objects. Metallic compounds which are widely used in other industries as pigments in paint and dye manufacture; in the manufacture of leather, rubber, textiles , paint, paper and chromium factories which are built close to water for shipping.

3-Domestic Wastewater: Domestic wastewater contains substantial quantities of metals. The prevalence of heavy metals in domestic formulations, such as cosmetic or cleansing agents, is frequently overlooked.

4-Agricultural Sources: Agricultural discharge contains residual of pesticides and fertilizers which contains metals.

5-Atmospheric pollution: Acid rains containing trace metals **(Rashed, 2004).**

Fish have been used for many years to indicate whether water is clean or polluted. Fish one of the best biological markers of metals in water.

Nile tilapia ***Oreochromis niloticus*** is one of the aquatic organisms affected by heavy metals, so in many cases, it is used as metal biological marker in toxicological studies in which it is substantiated with sensitivity to toxic effect **(Patin, 1984). Rashed (2001a, b)** studied Co, Cr, Cu, Fe, Mn, Ni, Sr, Pb, Cd and Zn in different tissues of fish (***Oreochromis niloticus***) from Lake Nasser to assess both the bioaccumulation and the lethal level of these metals in fish. Fish samples were collected from two Kohrs in Lake Nasser. This study showed that fish scales exhibited the highest concentrations of Cd, Pb, Co, Cr, Ni and Sr (0.088, 0.95, 0.29, 0.30, 0.25 and 3.21 µg/g DW, respectively). Whereas, whole fish contains the higher concentrations of the studied metals compared to the previous study by **Awadallah *et al.*, (1985)** in the same fish from Nasser Lake, and this mean the increase in metal pollution in Lake water as the results of man activities. This increasing in metal concentration was as the result of increasing pollution loads to the Lake from agricultural wastes, which include chemical pesticide and fertilizers. These agricultural wastes reached the Lake body from the agricultural farms on the beach of the Lake. The source of Pb in the Lake water and fish was resulted from gasoline contains Pb from the fishery boats and tour ships travels from Aswan to Sudan **(Mohamed *et al.*, 1990).**

Adham *et al.*, (1999) used fish as bioindicator for assessing metal pollution in Delta Lakes **(Lake Maryut and Lake Edku).** Lake Edku has 25 sites which have the highest in metal concentrations. Compared to Lakes Maryut and Edku, the Nile water displayed lower levels of metal contamination. **Lillo (1976)** reported that bolti from Lake Mariut contained less Fe content

compared to Nile bolti fish and concluded that the source was from the factories discharge. **El- Nabwi *et al.* (1987)** studied the concentration of Pb in fish, ***Oreochromis niloticus***, from Lake Maryut and found that Pb concentration was 0.42 ppm.

River Nile is the main source for potable water and as the result of man activities in and on the river body it become loaded by metal pollution. Fish in the River Nile was used as biological marker for the River pollution by metals. **Mohamed *et al.* (1990)** used ***O. niloticus*** fish as a biomarker for the Nile water pollution with metals at the discharge. Point of fertilizer factory with the Nile. Ag, Au, Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Sr and Zn were determined in ***O. niloticus*** fish collected from the Nile area at the point of fertilizer discharge to the Nile , south and north this point. The results revealed that fish near the point of the factory discharge possess the highest levels of metals as the result of pollution with metals.

Other study for using fish as biomarker for water pollution with metals was conducted **(Khallaf *et al.*, 1998**). Two species of fresh water fish (***O. niloticus***) caught from River Nile at Hawamdia and Kafer El-Zayat , at North Egypt and also from governmental fish farms (Abbassa and Barseik) were used to detect the presence of industrial wastes especially heavy metals as environmental pollution in the river track and its accumulation in edible fish tissues. The result reveals that heavy metals in different water samples except Cu and Zn were more than the recommended permissible levels. Iron level in Hawamdia and Kafer-El-Zayat tilapia fish recording 63.4 and 54.7 µg/g respectively, which was found higher than its permissible levels, these may be due to the discharge of the adjacent chemical factories that used Fe in their

processing. The Nile cat-fish (***Claris garipinus***) from the same locations (Kafer El-Zayat and Hawamdia) had lower concentration of Cu, Zn, Ni, Cd and Pb than tilapia, while Fe present in higher concentration in tilapia(***O. niloticus***) than in Nile cat-fish (***Claris garipinus***).

Comparing the fish metals from Abbassa and Barseik fish farms, where no pollution, with the same fish spices from Hawamdia and Kafer-El-Zayat River Nile, it seems that fish of farms exhibit lower concentration of Cu, Ni, Zn, Fe and Co than those from River Nile. This indicates that the fish especially Nile tilapia (***O. niloticus***) was highly accumulated the metal from the water, and so susceptible to its toxic effects.

Kalfakakon and Akrida-Demertai (2000) reported that Ca, Mg, Fe, Cu, Zn and Pb exhibited bio-accumulation from water to fish. They demonstrate that metal concentrations in fish are higher than in water, which indicates the bio-accumulation. They study on the transfer of Cd, Pb, Cu and Zn through the trophic chain of Lake Ioannina (Pamvotis, Greece) ecosystem and investigate the environmental pollution from heavy metals on the trophic chain of the lake.

Heavy metals transported into the aquatic environment originate either from natural sources, such as wind borne soil particles, or from sources result of heavy industry and the burning of industrial or domestic waste. On average, man made emissions of elements such as, Cd, Cu, Ni, Zn, and Pb greatly exceed the biogenic inputs of these elements **(Nriagu, 1989).**

Heavy metals pollution is being introduced into aquatic environment through geologic weathering mining effluents, domestic, agriculture and industrial effluents. Metal input from rural areas and atmospheric sources which are being discharged into surface water **(Forstner and Wittmann, 1979).**

Exposure to heavy metals even in low concentrations affects survival of fish and other aquatic organisms. Also, metals have been reported as a major cause of anemia **(El-Ezaby, 1994),** and to induce changes in physiological functions that reduce the ability to survive **(Abdel-Hameid, 1994).**

Heavy metals, contrary to most pollutants, are not biodegradable and undergo global exobiological cycling in which natural waters are then main pathways **(Norberg, 1984).** Thus, heavy metals have a great ecological significance due to their accumulative toxic behavior **(Purves, 1985; Schmitt *et al*., 2007).**

Cd as an environmental health problem

The importance of Cd as an industrial and environmental pollutant has become increasingly apparent in recent years; it is currently ranks number 7 in the United States. **(ATSDR, 2003).** During the past century, Cd and its compounds have been used extensively in the electroplating industries, and in the manufacturing of batteries, dyes, paints and plastics. Large amounts of Cd have also been released into the environment through the burning of refuse materials that contain Cd and through the use of Cd-contaminated sludge and phosphate salts as fertilizers **(Elinder, 1986; Page *et al*., 1986 and**

ATSDR, 1999). Tobacco contains significant amounts of Cd and smoking is one of the primary sources of Cd exposure in the general population **(Ostergaard, 1977; Ellis *et al.*, 1979; Scherer and Barkemeyer, 1983 ; Satarug and Moore, 2004).**

Cadmium has been found in varying concentrations in air, food, soil and water. Drinking water containing cadmium over 10 ppm can be rejected according to United States public health service because of the harmful health effect to the consumers **(Flick *et al.*, 1971).** While emissions from industrial activities not directly associated with cadmium. However, some industrial activities may be of importance. Among these are combustion processes involving coal, oil, wood and paper. Moreover, a cadmium concentration in coal has been reported up to 50 ppm ***(*Schroeder and Balassa, 1961).**

The industrial demand for cadmium has been increasing body burdens of the metal in man ***(*Elinder and Kajellstorm, 1977*),*** because cadmium is widely used as a coating material, so it is used in paint pigments and plastic industries on extensive scale. It is applied in nickel cadmium batteries, and as fungicide. The presence of cadmium in phosphate fertilizers constitutes a very diffuse source of cadmium contamination. Cadmium contents in fertiilzers vary from 1-2 ppm for tertiary calcium phosphate to 50-170 ppm for superphosphate **(Vandegrift *et aL.*, 1971*;* Booth and McDonald 1982).**

Cadmium salts are considered significant water pollutant not only because of their direct toxicity in water in the 0.01 mg/L range but also due to their ability to be concentrated and incorporated into the food chain by aquatic organisms and plants. Studies have been shown that fresh water fish can concentrate cadmium to levels 10 to

1000 times higher than the cadmium concentration of ambient water **(Fleischer *et al.*, 1974).**

Cadmium possesses problems for both occupational health and community health. Prolonged exposures to cadmium even at low levels, whether through food and water in occupationally unexposed population or through contaminated in specific occupations, produces manifestation of chronic toxicity **(Nordberg, 1976).** The chronic toxicity of cadmium is a result of its cumulative effect **(Commission of the European Communities, 1978).**

Cadmium is recognized as one of the most harzardous environmental pollutants and is toxic to many living organisms. Experimental and environmental exposure to cadmium has been reported to cause disease in human and other animals **(Friberg *et al.*, 1986).**

Clark (1989) reported that fish from areas known to be contaminated contain higher concentrations of copper than those from uncontaminated, copper do not generally accumulative in food chains.

Abo-Salem *et al.* (1992) found high levels of Pb, Hg, and Cd (0.45 ± 0.18, 0.190 ± 0.58 and 0.144 ± 14.3 ppm, respectively) in water of industrial locality at Benha city. The copper and zinc are not detected in the same locality.

Regardless of the route or pattern of exposure, essentially all Cd that reaches the systemic circulation is bound to proteins and other materials in blood (**Nordberg *et al.*, 1986; Zalups and Ahmad, 2003; Barbier *et al.*, 2005; Bridges and Zalups, 2005).**

The circulating Cd may either be tightly bound to specific metal binding proteins such as metallothionein (**Webb, 1986),** or may be loosely associated with other materials, such as albumin, amino acids or sulfhydryl compounds such as glutathione or cysteine.

Because of the high affinity of Cd for metallothionein Cd that is bound to metallothionein (Cd–metallothionein) is not available for uptake by most tissues, although the Cd– metallothionein complex can be taken up by specific tissues such as the epithelium of the proximal tubule **(Foulkes, 1978; Squibb *et al.*, 1984)** where it may contribute to the nephrotoxic effects of Cd (**Cherian *et al.*, 1976; Kotsonis and Klaassen,1978; Dudley *et al.*, 1985; Dorian *et al*., 1992**).

By contrast, the interaction of Cd with most other materials in the blood is of a lower affinity (**Fuhr and Rabenstein, 1973; Rabenstein, 1989; Trisak *et al.*, 1990**). Consequently, Cd that is associated with these materials can dissociate and bind to other target molecules on the cell surface and, in some cases, enter the cell **(Bruggeman *et al*., 1992; Foulkes and Blanck,1999; Foulkes, 2000; Barbier *et al.*, 2005),** although there is also evidence for the direct uptake of Cd–thiol conjugates in some tissues (**Foulkes, 2000; Zalups and Ahmad, 2003; Bridges and Zalups, 2005**).

Hepatotoxicity

With low level oral or respiratory exposure, Cd is initially distributed to the liver, where it can bind to glutathione and/or induce the synthesis of the Cd-binding protein metallothionein, which are both thought to serve as intracellular lines of defense against Cd toxicity. Under these conditions, the liver is usually not injured. By contrast,

with the acute, higher levels of exposure that are often used in animal studies, the liver rapidly accumulates high levels of Cd that overcome these defense mechanisms and the liver becomes one of the primary sites of injury(**Chan and Cherian, 1992).**

The general sequences of inflammatory events that lead to parenchymal cell death following Cd exposure have been reviewed by **Rikans and Yamano (2000)** and **Kuester *et al.* (2002)**.

Many studies have described the acute toxic effects of Cd, but very few have addressed the mechanism of toxicity at the molecular level. During the last decade, Cd has been shown to induce *in vivo* apoptosis **(Habeebu *et al.*, 1998; Harstad and Klaassen, 2002; Tzirogiannis *et al.*, 2003)** and *in vitro* **(Hart *et al*., 1999; Achanzar *et al.*, 2000; Kim *et al*., 2000; Li *et al.*, 2000; Kondoh *et al.*, 2002; Jimi *et al.*, 2004)** at various concentrations. Therefore, Cd-mediated toxicity is thought to involve, at least in part, the induction of apoptosis.

In recent years, the molecular mechanisms responsible for apoptosis have been elucidated (**Robertson and Orrenius, 2000).**

At low concentrations, Cd stimulates DNA synthesis and cell proliferation **(Beyersmann and Hechtenberg, 1997).** This stimulatory effect could be related to the induction of transcription of specific genes, as well as interference with the mito-gen-activated protein kinase (MAPK) signaling pathways which regulate proliferation.

Cadmium (Cd) is one of the most deleterious heavy metal pollutants in aquatic systems. It leads to a variety of severe damage,

such as anemia and emphysema **(Nriagu *et al.*, 1998; Peraja *et al.*, 1998**). Various evidences indicate the toxicity of Cd may be associated with oxidative damage for the production of reactive oxygen species (ROS) (**Bagchi *et al.*, 2000; Shi *et al.*, 2005**).

Supplied by glucose-6-phosphate dehydrogenase (G6PDH) **(Venugopal *et al.*, 1997)** or exported from the cell, like some GSH conjugates, via multi-drug resistance associated with proteins **(Keppler *et al.*, 1997**).

Fish require copper and zinc as micronutrients (**Watanabe *et al.*, 1997)** and can obtain these metals from either water or their diet (**Handy, 1996; Wood, 2001**). The mechanisms of waterborne Cu and Zn uptake and toxicity to fish gills are beginning to be well understood (**Wood, 2001),** however, the uptake and toxicity of diet-borne metals in fish is not as well characterized. Toxic concentrations of dietborne Cu and Zn have been described for some commonly tested species rainbow trout (***Oncorhynchus mykiss*)**, channel catfish (***Ictalurus punctatus***) and common carp (***Cyprinus carpio*)** using laboratory-prepared diets supplemented with metal salts (e.g. $CuSO_4$, $CuNO_3$, $ZnSO_4$), however, much of these data is contradictory **(Lanno *et al.*, 1985b; Julshamn *et al.*, 1988; Handy, 1993).**

Paripatananont and Lovell (1995a, 1997) have shown that when dietborne metal concentrations are low, metals chelated to methio-nine or 'proteinate' complexes can be more readily absorbed by fish than inorganic forms. Metals may be bound to chitin, metallothionein, or complexed in insoluble granules (**Bryan, 1976**) and the bioavailability of dietborne metals in these forms is unknown.

Relative Cu concentrations in the intestine and gills may be a useful tool to determine the route of Cu exposure in a polluted

environment **(Handy, 1992a, b, 1996).**Also this way may not be completely reliable, especially when trying to characterize episodic exposures, because Cu concentrations in most internal organs of rainbow trout was decreased within 12 d after a dietborne exposure (**Handy, 1992).**

When rainbow trout were simultaneously exposed to waterborne and dietborne Cu, the relative importance of Cu uptake from each route depended on the concentrations present in each medium (**Miller *et al.*, 1993)**. As waterborne Cu concentrations increased from 13 to 127 mg Kg^{-1}, uptake across the gills increased relative to intestinal uptake and contributed up to 37% of the hepatic Cu burden, even in the presence of a high dietborne Cu concentration (684 mg Cu Kg^{-1} dry diet). **Miller *et al.* (1993)** pointed out that water-borne Cu accounted for almost a third of the hepatic Cu even though dietborne Cu concentrations were three-fold higher.

Copper sulfate is often used as an algaecide in commercial and recreational fish ponds to control growth of phytoplankton and filamentous algae and to control certain fish disease (**Boyd, 1990; Tucker and Robinson, 1990). Boyd (1990)** stated that the concentrations of copper sulfate used for phytoplankton control are seldom directly toxic to fish, but do kill large numbers of invertebrate food organisms such as rotifers, cladocerans and copepods. However, above a specific concentration, copper is toxic to fish including such cultured species as salmonids, cyprinids and catfish **(Wurts and Perschbacher, 1994).**

Nile tilapia is a native fish species of Egypt that has become popular species worldwide mainly as a valuable fish, easy to breed and grow in a variety of aquaculture systems **(El-Sayed, 2006).** However, it

is an omnivorous fish consuming detritus, phytoplankton and zooplankton **(Abdelghany, 1993; Abdel-Tawwab, 2000; Abdel-Tawwab and El-Marakby, 2004).** Thus, treating plankton with copper compounds may lead to copper bioaccumulation reaching a toxic level in fish.

The impact of copper on the aquatic environment is complex and depends on the physicochemical characteristics of water **(Laurén and Mcdonald, 1986; Erickson *et al.*, 1996; Mazon and Fernandes, 1999; Tao *et al.*, 1999; Takasusuki *et al.*, 2004**). Therefore, recommendations for the safe use of copper sulfate have been based on hardness (**Sawyer *et al*., 1989; Perschbacher and Wurts, 1999)**, total alkalinity (**Boyd, 1990; Reardon and Harrell, 1990; Perschbacher and Wurts, 1999**), and pH (**Masuda and Boyd, 1993**) of the water.

Metallothionein (MT) is a low molecular weight cysteine-rich intracellular metal binding protein to function in regulation of essential metals such as copper and zinc, and in detoxification of non-essential metal ions such as cadmium and lead. Although a variety of agents have been shown to induce the synthesis of MTs, metal ions are the most potent and common inducer **(Price, *et al.*, 1985).** The MT gene expression in tilapia tissues have been used as biomarker of exposure to metal contaminations in polluted waters **(Chan, 1995; Zhou, *et al*, 1998; Wu *et al.*, 1999;Wong, *et al.*, 2000).**

Lam *et al.*, (1998) reported that tilapia is a copper-resistant species (24 h-LC_{50} value is 2.82 ppm) relative to carp, which is sensitive to copper ions (24 h-LC50 value is 200 ppb), and significant MT mRNA induction was found in gills and liver of tilapia exposed to Cu^{2+} and Zn^{2+}. The induction of MT mRNA expression was demonstrated in liver of tilapia treated with Zn^{2+} and its cDNA from was cloned by Reverse Transcription (RT)-Polymerase Chain Reaction

(PCR) method using a fish MT oligonucleotide probe derived from the conserved N-terminal amino acid sequence of fish MTs **(Chan, 1994).** **Andrew *et al.*, 2004** indicated that Tilapia MT mRNA levels in gills and liver are sensitive biomarker of exposure to various metal ions.

Chromosomal abnormalities:

Several studies have linked increases in cytogenetic abnormalities in fish and shellfish to polluted environments. This was done largely through laboratory bioassays of polluted water sample in nature **(Alink *et al.*, 1980; Hose *et al.*, 1987 and Metcalf, 1988).**

Karyotypes of cichlid species, especially tilapia, have been described but generally are found to be very similar, consisting of 44 chromosomes (22 chromosome pairs). The first and second pairs are conspicuous size, especially the first one which is larger (thought to be the marker chromosome**).** The other twenty pairs are small with short arms and gradually decrease in size **(Thompson, 1979 ; 1981; Aral and Koike, 1980; McAndrew and Majumdar, 1984 and Crosetti, *et al,* 1988).**

Karyological studies of teleost fishes can contribute significantly to the solution of many problems in areas of research ranging from taxonomy, systematic or genetics to phylogenetics, or environmental toxicology ***(Al-Sabti, 1985)***.

Evans (1983) postulated that the actions of chemical mutagens in inducing chromosome damage stems not only from the possibility that the presence of a chemical mutagen in the environment could result in an increased incidence of cancer, but also, from the fact that

exposure to these agents may result in an increased incidence of transmitted genetic disease.

Obe et al. (1982) concluded that, DNA is the primary target for the induction of chromosomal damage. Also, **Evans (1977)** , reported that the chromosomal damage arises as a consequence of mis-repair or mis-replication of the damaged DNA.

Many water borne pollutants have cytogenetic properties which in fish cause enhanced frequency of chromosomal aberrations **(Alink *et aL*., 1980; landlot and Kocan, 1983 and Al-Sabti *et aL*., 1984).**

Mahmoud (2006) indicated the effect of industrial water pollution on genetic structure of ***Tilapia zillii.***

Al-Sabti *(1985)* distinguished the following chromosomal aberrations: (1) structural chromosomal abnormalities which included single chromosome breaks with fragments, chromatid gaps, ring chromosome formation and dicentric chromosomes, (2) Aneuploidy loss of chromosomes, (3) Non-specified metaphases, the morphology of chromosomes was not well defined. Since many types of DNA damage are caused by mutagens present in water which induce alterations in chromosomes, therefore the measurement of chromosomal abnormalities offers an acceptable parameter for monitoring mutagenic substances in water.

Moreover, chromosomal abnormalities selectively count only the primary DNA lesions that are repaired by the machinery of the cell ***(*Evans, 1977*).*** So chromosomal damage (chromosomal aberrations) can be used as an indicator of DNA damage.

Irrespective of the nature of the primary lesions induced by chromosome breaking, the ultimate lesions responsible for aberration formation are DNA-strand breaks (**Natarajan and Obe, 1978).**

Badr and El-Dib (1978*)* reported that water pollution increases the frequencies of the different types of chromosomal aberrations in the three species of tilapia taken from regions of the Nile and a delta lake (Lake Maruit). These places differed in the amounts of industrial wastes being drained into them. They concluded that, the higher degree of pollution, the more pronounced is the effect.

Different cells of the same organism and different individuals of the same species have as a rule the same number of chromosomes, except the gametes cells have only half as many chromosomes as somatic cells. Furthermore, homologous chromosomes are, as a rule uniform in the number and order of genes they carry. But the number, size and organization of chromosomes vary from Organism to another. The chromosomes of organisms evolve by changes not only in their number and size but also in their organization, segments may change their location within a chromosome or move from one chromosome to another. Changes in the number, size and organization of chromosomes are known as chromosomal mutations, abnormalities or aberrations **(Ayala and Kiger, 1984).**

Types of chromosomal abnormalities:

The types of chromosomal abnormalities can be classified into structural and numerical:

I-Structural aberrations (abnormalities):

Changes in chromosomal structure may involve the whole chromosomes or individual genes, both of which have the same underlying basis of alterations in the linear ordering of DNA nucleotide sequences ***(*Avers, 1980).**

1- Deficiencies or deletions:

Deficiencies and deletion are used as synonyms, because in either case an acentric fragment is lost. A spindle fiber can not attach to an acentric fragment during mitosis or meiosis and guide it to one of the poles. If the DNA in the chromatid that is lost is crucial for viability, sterile gametes or non-functional somatic cells may result. Most deficiencies, however, are lethal when homozygous, and these may involve no more loss of chromatin than in the example of white locus ***(Avers,* 1980).** A loss of a large segment of a chromosome may result in a dominant lethal effect even if a normal homologue is present with it in the heterozygote. If the dominant effect of **a** deletion is not lethal, it may result in an abnormal phenotype. Chromosomal deficiencies arise through breaks caused by one or more agents, including radiation, viruses and chemicals **(Avers, 1980).**

2-Duplications:

Duplications are repeats of chromatin segments within a chromosome. Chromosomes containing duplication therefore possess more than the normal amount of DNA. There are several possible mechanisms for generating duplicate DNA segments in

chromosomes. Unequal crossing over between homologous chromatids may yield one chromosome with duplication and its homologue with a corresponding deletion, duplications and deletions may also be generated by the breakage, fusion-bridge cycle **(Wagner *et al.*, 1980 and Avers, 1980).** The duplication of structural gene without the corresponding duplication of its regulator may lead to a large array of new phenotypes including lethality **(Avers, 1980). Pai (1985)** stated that duplication occurs when a small portion of a chromosome can be doubled or replicated several times, and the various repeats are situated next to each other along the length of the chromosome.

3-Inversions:

Inversions result when two breaks occur within a chromosome and the broken segment is rejoined to the original chromosome in a reversed order **(Pai, 1985).**

4-Translocations:

A translocation is the result of a transfer of a segment of one chromosome to another non homologous chromosome. If the transfer involves only the attachment of part of one chromosome to an intact non homologous one, this is called a simple translocation, if non homologous chromosomes change segments; this is called a reciprocal translocation **(Avers, 1980).**

5-Isochromosomes

Isochromosomes are believed to have arisen by a transverse rather than a longitudinal splitting of the centromere of a replicated chromosome, the two halves of sister chromatids become the two

arms of the isochromosome. Isochromosomes have been seen in karyotypes of many species of plants and animals **(Pai, 1985).**

6-End to end association:

It was observed when two chromosomes are attached from one chromatid end probably due to chromatid exchanges **(Hondt *et aL*., 1981).**

7-Gaps and Breaks:

Gaps occur when the chromatid has unstained area shorter than its diameter or equal to it. This is due to the local loss or despiralization of both DNA and chromosomal basic proteins. This loss can occur on a chromatid or on both chromatids in the same locus and does not represent real discontinuities in the chromosome **(Stoian and Paicu, 1975*).*** Break occurs when the chromosome has unstained area larger than gaps. Biologists divided the cell life cycle into stages, which are referred to as the cell cycle which consists of M = Mitosis (division), G_1= Prereplication gap. If cells are exposed to radiation or to mutagenic agents during S phase, a break could appear in both haves of the replicated chromosome. If replication is completed at the time of exposure to mutagen, only one chromatid may be involved ***(*Pai, 1985).**

When a chromosome breaks, the two broken ends usually undergo restitution or healing, but this does not always happen, and the result can be the formation of (1) deficiencies, (2) duplications, (3) inversions, or (4) translocation. The first three of these ordinarily involve a single chromosome, while the fourth involves two or more chromosomes **(Pai, 1985).**

8-Centromeric attenuation:

It is a chromosomal break attack the centromeric region, lead to separation of the two chromatid **(Darrance *et al.*, 1975).**

9-Centric fusion:

Centric fusion and centromeric interactions leading to the formation of star-shaped or x-shaped configurations **(Gautam and Kapoor, 1991).**

10-Ring chromosome and intrachromosomal vacuole:

These signs of damage were discovered by **Mitus and Colcman (1970).** In cultured leucocytes from patients treated with a large does of chloramphenical. As their name implies, ring chromosomes lack free ends but forms a continuous ring. Such chromosomes tend to be unstable, as they are liable for breakage and reunion during mitosis and meiosis ***(*Avers, 1980).**

11-Chromatid Fragments:

Chromatid fragment in which the single chromatid is observed without its centromere. It is produced as a result of chromosomal changes in the structure accompanying the process of deletion.

II- Numerical aberrations:

Numerical aberrations of chromosomes may involve the entire set, a condition called euploidy, when only individual chromosomes are involved; this is called aneuploidy **(Pai, 1985).**

1-Polyploidy:

When there are more than two sets of chromosomes in an euploid individual or species, the condition is called "polyploidy" and the individual or species is "polyploidy". The symbol (n) has

been adopted to designate the haploid number of chromosomes, so that 2n, 3n, 4n, indicate diploid, triploid and tetraploid numbers, respectively **(Ford, 1974; Avers, 1980).**

2-Aneuploidy:

Aneuploidy has at least one more or one less chromosome than the dipioid number, but they do not have multiples of chromosome sets. If there is one extra copy of a chromosome in a dipioid, the individual is trisomic and the condition itself is called trisomy (three copies or chromosomes of one kind), the chromosome constitution would be shown as (2n+l). If there are two extra copies of a particular chromosome in the dipioid, it is tetrasomic and shown as (2n + 2). When there is one less than a complete set of chromosome in a diploid, it is called a monosomic and the conditionis called monosomy, it is (2n -1).Aneuploids usually arise because of non-disjunction of homologous chromosomes at meiosis, or by non-disjunction of sister chromatids at mitosis. The failure to disjoin or separate accurately can occur at any nuclear division, and its consequences vary according to the division in which the event occurs and the time of occurrence. Non-disjunction at meiosis gives rise to gametes with one more or one less chromosome than usual. If such gametes are viable and fuse to produce a zygote, the zygote will be trisomic or monosomic for the non-disjoined chromosomes **(Avers, 1980).**

3- Haploidy:

It is also termed as monoploid, in such case the organism have one set of chromosomes or one genome (n) in the nuclei of their body cells. It is distinguished from the so-called "polyploids". In the

haploid set, the chromosomes are non-homologous and lack any homologue; therefore they are found as univalents **(Pai, 1985).**

4- Chromosome stickiness*:*

Which is now interpreted as due to improper folding of chromosome fiber into single chromatids and chromosome fibers are inter- mingled and the chromosomes become attached to each other **(Brogger, 1974).**

Effects of pollution on chromosome:

Cytogenetic methods are probably the most sensitive and efficient ways to detect the effects of genotoxins. Water pollution is becoming a major problem threatening fish production all over the world. The fisheries of fresh and salt water are source of great importance, involving the interest and welfare of a very large number of people. That they are now seriously threatened by different pollutant agent **(Tait, 1972).**

Chromosomal abnormalities selectivity counts only the primary DNA lesions that are repaired by the machinery of the cell **(Evans, 1977).** So chromosomal damage (chromosomal aberrations) can be used as an indicator of DNA damage. Irrespective of the nature of the primary lesions induced by chromosome breaking, the ultimate lesions responsible for aberration formation are DNA-strand breaks **(Natarajan and Obe, 1978).**

Karima and Halima (2002) reported that water pollution (Agricultural and Industrial waste water) have a significant effects on *Clarias lazera* and *Oreochromis niloticus* which appeared as

chromosomal aberrations, breaks, deletions and centromeric attenuation in somatic cells and also there was a significant increase in germ cells that appeared as x-y univalent.

Al-Sabti (1985) measured the cytogenetic changes by observing the frequency of chromosomal aberrations in the tissues of gills and kidney of rainbow trout exposed to five pollutants and he reported that, the frequency of aberrations were raised compared to the control. **Manna and Mukherjee (1986)** found a higher frequency of chromosomal abnormalities in ***Oreochromis mossambicus*** treated with malathion and mercuric chloride than that of untreated fish, indicating its genotoxic effects. The same effects have been found in treating ***O. mossambicus*** with inorganic weedcide, sodium arsenate, (**Manna** & **Mukheriee 1989).**

Manna and Sadhukhan (1992) reported that, the chromosomal aberrations were more pronounced in *Oreochromis mossambicus* subjected to Aldrex; an insecticide used for the control of soil insects. The recorded Chromosomal aberrations were chromatid breaks, constriction, gap, fragment, polyploids, and stickiness as gross types. The difference between the number of affected metaphases and the aberrations was statistically significant.

Rishi and Grewal (1995) found that, chromosome aberration test was applied for an organophosphorus insecticide, dichlorvos using ***Channa punctatus.*** The aberrations observed were chromatid gaps, centromeric gaps, precocious separation of chromatids and

polyploidy, which were found to be significantly higher as compared to that of the control.

The occurrence of cytogenetic damage in fish exposed to pollutants was demonstrated by an enhanced frequency of micronuclei in peripheral blood erythrocytes. This chromosomal alteration was most evident after 7 days of exposure (**De Flora *et al*., 1993). Mattar *et al. (*1992*)** examined structural aberrations of the chromosomes in the kidney cells of grass carp(***Ctenopharygodon idella***)treated by sevin and they found that the frequency of aberrations chromatid gaps, chromatid breaks, or chromatid deletions, fragmentation and stickiness were significantly higher than that of control after 15 days of exposure. **Krishna and Gupta (2001)** observed that Aflatoxin B_1 is a potential clastogenic agent causing damage to chromosomal complements. The common abnormalities encountered were fragmented chromosomes, a centric chromosomes and ring. **Bovero *et al.* (2002*)*** studying the effect of two geographically distant populations of ***chironomus riparius* (*Syn. thummi*)** from two environmentally polluted sites (*Santena, Italy and Varna, Bulgaria)* show numerous somatic and inherited chromosomal aberrations (Inversions, deletions and deficiencies).

Karima and Iman (2002) recorded that the exposure of *Clarias gariepinus* to $ZnCI_2$ and $CdCl_2$ resulted increased in the frequency of chromosomal aberration compared to the control level and also $ZnCI_2$ was more effective on catfish metaphases than $CdCl_2$. Combination of salts reduced the structural chromosomal aberration percentages compared to that obtained by each salt alone. Meanwhile, such combination increases the numerical aberrations.

Mathew and Jahageerdar (2003) recorded that the percentage of total metaphase spreads with chromosomal aberrations increased with the increasing concentration levels after exposure of different

doses (0.19, 0.038 and 0.075 ppm) of mercuric nitrate, these aberrations included breaks, fragments, dicentric and ring type chromosomes.

krishna and Gupta (2002) found that exposure of fingerlings *labeo rohita* to copper as (0.5, 1.5, 3.0 ppm), cadmium (0.1, 0.2, 0.4 ppm), zinc (1.0, 2.0, 4.0 ppm) and Aflatoxin for different duration resulted a highly significant increase in the chromosomal abnormalities such as breakage in chromosomal arms, a centric chromosomes, ring chromosomes and abnormally small and thick chromosomes.

Electrophoretic study:

Proteins are large molecules varying in molecular weight from 1 to 1000 kDa and containing a linear sequence of amino acids covalently linked by peptide bonds **(Harrison and Levitt, 1987).** Proteins are present in all body fluids, but it is the proteins circulating in the blood are readily accessible and can be analyzed directly **(Fountoulakis *et al.*, 1998).**

Analysis of proteins for clinical purposes began in the middle of the 19th century; various fractionation techniques have been designed to separate and identifying proteins **(Joliff, 1992).** But the modem understanding of the protein composition of serum and plasma was derived from the electrophoretic techniques **(McPherson, 1996).**

The word "electrophoresis" derives from Greek and means carried by electricity. It was initially used to describe the behavior of electrically charged colloidal particles in an electric field. The migration of true solutes was originally referred to as "lonophoresis". Eventually,

electrophoresis became the recognized term for the migration of all kinds of particles under the influence of an electric field **(Laas, 1998).** Polyacrylamide is apolymer which can be poured as a liquid. After pouring, and with the appropriate added catalysts, it sets to a clear flexible, solid support in which electrophoresis of proteins can be carried out **(Luzio and Thompson, 1990).** Polyacrylamide gels are formed by co-polymerization of acrylamide monomer,CH=CH-CO-NH2 in the presence of smaller amounts of N,N,-methylene bisacrylamide which is used as across- linking agent, a catalyst, ammonium persu;lfate and accelerator, tetramethylenediamine (TEMED) which decompose the persulfate ion to give a free radical. The mechanism of gel formation is free radical vinyl addition **(Andrews, 1986).**

The proteins in polyacrylamide gel electrophoresis (PAGE) migrate in response to the electrical field through the pores in the gel matrix. The porosity of gels is easily adjusted by changing the composition of acrylamide prior to polymerization, where pore size decreases with higher acrylamide concentrations. The composition of gel pore size and protein charge and shape determines the migration rate of the protein **(Gallagher and Leonard, 1987).**

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) can be carried in either continuous or discontinuous buffer systems **(Jenkins and Guerm, 1997).** Continuous buffer systems are often chosen for use with small pore size gels. In contrast discontinuous buffer systems utilize different buffer ions, at different pH values which permit stacking or sample concentration and provide superior resolution.

Molecular weight determination of polypeptides using SDS-polyacrylamide gel eleclrophoresis (SDS-PAGE) was first introduced, empirically by **Shapiro *et al*. (1967),** as a rapid and simple tool. It was also confirmed and extended by **Weber and Osborn (1969).**Those

authors showed that the relative mobilities of the polypeptides were related to their molecular weights.

Laemmli (1970) added SDS to the Tris-glycine/Tris-HCl system developed by **Ornstein (1964)** and Davis (1964) creating the "Laemmli system" for SDS electrophoresis which gave further improvement in resolution. The most popular and widely used electrophoretic method until now is the SDS-PAGE system of Laemmli. Because of the procedure is straight forward and highly reproducible and results can be obtained within a few hours using only a few micrograms of materials **(Jenkins and Guerin, 1997).**

Laemmli system is a discontinuous slab gel system consisting of stacking (upper) gel and separating or resolving (lower) gel. The sample first passes through a stacking gel, which has large pores. So it allows the protein to move freely and concentrate under the effect of the electric field into a sharp band. Then protein enters the main separating gel which has a smaller pore size, a higher salt concentration, and higher pH compared to the stacking ones **(Scopes and Smith, 1998).** The use of high quality reagents is a prerequisite for reproducible high resolution gels **(Margulies and Tiffany, 1984).**

Gels between 10% and 20% acrylamide are used in SDS-PAGE, where the smaller pore size introduces a sieving effect that contributes to the separation of proteins according to their size **(Wilson and Walker, 1996).**

In **1965, Meyer and Lamberts** used a solvent system of methanol-acetic acid-water (5:1:5) for coomassie dye to visualize proteins in polyacrylamide gel.

Shapiro, *et al.*, (1967) first observed that in an SDS-buffer system, the mobility of a protein was related to its molecular weight,

specifically they reported that log molecular weight was inversely related to mobility of protein.

Fractionation of proteins by polyacrylamide gel electrophoresis in SDS containing buffer is considered as the universally applied high resolution technique to investigate the composition of sample mixtures **(Hames and Rickwood, 1986).** Also, SDS-PAGE is an excellent method to identify and monitor proteins during purification and to assess the homogeneity of purified fractions **(Laemmli, 1970).**

Rothe and Maurer (1986) mentioned that SDS-PAGE used in wide aspects that due to many reasons:

1) It separates proteins strictly according to a single molecular parameter and molecular size.

2) The resolution is generally very high.

3) The technique is inexpensive and easy to set up and perform.

4) The separation is fast.

5) The method is applicable to most separation problems, as SDS soluibilizes most existing proteins.

Electrophoretic study of the blood proteins of a fish, *Notropis cyprinindae* resulted in the identification of the slowest migrating fraction as a globulin fraction **(Menzel, 1970)**. Moreover, previous studies on blood serum proteins have shown that under conditions of stress **(Bouk, 1972)** or heavy metal exposure **(Rai 1987 and Dutta *et al.*, 1992)** the number of protein fractions either increased or decreased.

Richmonds, (1989) induce changes in serum protein of blue gill sunfish *Lepomis macrochirus* by the exposure to malathion. However, **Dutta et al., (1992)** observed that changes in serum protein, in the relative position, height and area and a new fraction of protein was noticed after 48 h exposure. Also, **Munshi *et al.*, (1999)** found that the

high concentration of malathion (4 mg/L) exposed to Indian cat fish induced more alterations in serum proteins.

Richmonds and Dutta (1989) and Dutta *et al.*, (1992) observed necrosis in the gills of blue gills, *Lepomis macrochirus* and Indian cat fish, *Heteropneustes fossilis* exposed to malathion in position, height and area observed in the sera of exposed fish may be due to the possible changes in the amount of different proteins caused by the necrosis of the cellular components.

Serum total protein and globulin tended to decrease Significantly with the increase of atrazine level and exposure time in tilapia while total protein and globulin decreased significantly in *Chrysichthyes auratus.* Although serum albumin did not differ significantly in tilapia groups and it was significantly decreased in *Chrysichthyes auratus* groups **(Hussein *et al.*, 1996)**. However, **Khalaf-Allah (1998)** reported that a general decrease in the mean total protein, albumin and globulin values in serum samples collected from ***O. niloticus*** fish exposed to diazinon and malathion as compared to controls. The results of electrophoretic pattern of serum protein revealed a decrease in the gamma globulin fraction. The mean globulin fractions were lower in the groups exposed to malathion and diazinon as compared with control group.

El-Serafy et al., (2007) used the SDS-PAGE as a tool to differentiate tilapia species in the river Nile.

Boone and vijayan(2002) showed that heat shock protein (hsc70) of rainbow trout cultured cells exposed to metals (Cd&Cu), exhibited high expression**. Feng *et al.*, (2003),** found that rainbow trout exposed to copper exhibited high expression of heat shock protein in

cultured hepatocyte. Further more, **Roy and Bhattacharya (2006)**, studied the heat shock protein 70 of ***channa puncatatus*** exposed to Arsenic(pollution), they found positive corolletion of heat shock protein exprestion and the degree of metal concentration.

Zhu *et al.*, (2006) used brain proteins polyacrylamide gel electrophoresis (PAGE) of ***Paralicthys Olivaceus*** as a biomarkers to cadmium pollution levels in sea water.

Atli *et al*., (2006) indicated the effect of heavy metals on catalse activity of ***O. niloticus***, and emphasized that catalase may be considered as a sensitive bioindicator of the antioxidant defense system.

It is known that dehydrogenase enzyme is used to remove hydrogen from its substrate, which is used in the cytochrome (hydrogen carrier) system in respiration to produce a net gain of ATP. Also, it reversibly catalyses the oxidation of NADH (Nicotinamide Adenine Dinucleotide, reduced form to NAD (Nicotinamide Adenine Dinucleotide) and reduced acceptor (**Lehninger, 1970).**

The feasibility of mitochondrial DNA(mtDNA) based approaches in solving proplems of identification of tilapia species and their hybrids, isolated from River Nile by using the PCR-RFLPs analysis of mitochondrial NADH dehydrogenase gene(mtND2) **(Awwad, 2002** & **El-Serafy *et al*, 2007)**.

NADH is a powerful antioxidant and has a strong defense against the damage of free radicals. The oxidative form of NADH plays a central part in DNA repair, as the repair enzyme uses an oxidized form of NADH as a substrate. This process causes a depletion of NADH and ATP simultaneously **(Lemire *et al*., 2008).**

ATP depletion is believed to be one of the most critical factors leading to necrosis or apoptosis. The higher levels of NADH the better DNA repair mechanism works. In addition, it prevents cells from ATP depletion, and may stimulate important metabolic pathways such as glycolysis and the hexose monophosphate shunt. NADH plays an active role and has a positive, impact on the body's network of defense systems and enhances the body's immune system in fighting disease **(Sauberlich, 1987).**

NADH is directly involved in the metabolic burst as the rate of energy being released. The metabolic burst appears to be one of the initial steps leading to the cell's destruction of a foreign invaders, and harmful organisms. Neutrophils contain NADH-oxidase, which generates superoxide in the phagosomes which destroy foreign organisms. The killing mechanism associated with phagocytosis is fueled by NADH and NADPH which are internally derived from an increased activity of the hexose monophosphate shunt. NADH is therefore directly involved in the cellular immune defensive system. Coenzyme NADH stimulates the production of many different brain neurotransmitters, including dopamine, norepinephrine or noradrenaline, and serotonin **(Alberts,1983).**

Reduced nicotinamide adenine dinucleotide phosphate (NADPH) and NADH-tetrazolium reductase (NADH-TR) are enzymes which have the property of transferring electrons from the reduced nicotinamide adenine dinucleotide phosphate (NADPH) or the reduced nicotinamide adenine dinucleotide (NADH) to various electron acceptors. Early research showed that selenium and copper affect various organelles in the hepatocytes, such as lysosmoes **(Kapalj-Klobucar *et al.*, 1996).** Also, **Couture and Kumar (2003)** suggested that mitochondrial enzymes are targets for inhibition by

copper. Therefore, alterations in the activity of NADPH- and NADH-TR following intoxication by selenium or copper could serve as indicators of these two elements' impacts on mitochondrial populations in the liver. It is well known that the liver is the prime organ in the accumulation of copper and selenium **(Pandey *et al*., 2001; Fan *et al*., 2002).**

Namely, copper is ubiquitously present in elevated concentrations in freshwaters as a result of agriculture use (as fungicide in viticulture, etc.) and industrial processes **(Mazon *et al*., 2002)** such as mining and smelting activities **(Couture and Kumar. 2003).**

According to **Rothe *et al*.,(1999) and Khanna and Porter (2001)** NADPH- and NADH-TR activity is bound to the mitochondria, therefore the marked granules represent mitochondria. The diffuse distribution of NADH-TR in the cytoplasm is in agreement with the fact by which NADH-TR activity is represented in the endoplasmic reticulum in addition to the mitochondria **(Malik *et al*., 2000).**

It is well known that NADH-TR marks activity of complex in the respiratory chain of the mitochondria(**Malik *et al*., 2000**) in which NADH denotes electrons in order to generate energy(ATP). According to **(Abdel-Tawab et al., 2007)** it is possible that selenium and copper induce certain biochemical processes that require energy and thus indirectly affect the oxidative metabolism of the hepatocytes. Such process could include the defence reaction in which these elements are transformed into less toxic compounds or excretory products. With copper intoxication, these intoxication processes can relate to binding copper to the apothioneins, the synthesis of which requires energy. It is

well known that small concentrations of copper induce the synthesis of metallothioneins (**Viarengo and Nott, 1993; Park *et al*., 2001).**

Selenium and copper can act pro-oxidatively **(Hartikainen *et al*., 2000)** forming free oxygen radicals, which can incite the peroxidation of lipids, thus damaging membranes. Mitochondrial membranes are particularly sensitive to the peroxidation of lipids **(Shamberrger,1986).** Selenium and copper can damage mitochondrial membranes, thus affecting the functioning of NADH-TR and disrupting intracellular oxidation processes. This agrees with the inhibition of mitochondrial respiration demonstrated during long-term exposure to low concentrations of cadmium and lead **(Meyer *et al*., 1991).**

Chromium reactivity is apparent from its interaction in cell-free systems with glutathione, NADH and hydrogen peroxide to form hydroxyl radicals (**Shi and Dalal 1989; Aiyar *et al*. 1991).** The enhanced production of O^{2-} anions and hydroxyl(OH) radicals has been demonstrated to be a cause of chromium-induced oxidative stress, DNA damage, and apoptotic cell death in cultured human chronic myelogenous leukaemic K562 cells and promyelocytic leukaemic HL-60 cells **(Bagchi *et al*. 2000).**

The generation of $O2^{-}$ radicals by the mitochondrial electron transport chain is an uninterrupted physiological phenomenon.Heavy metals may interact with different sites in the respiratory chain to keep certain electron transport components in reduced form to serve as an electron source for $O2^{-}$ **(Halliwell and Gutteridge, 1984).**

The divalent metal cations (Cd, Zn, Co, Ni, Mn and Pb) have been shown to bind to mitochondrial membranes, causing inhibitory effects on electron transport and oxidative phosphorylation **(Bittell *et al.*, 1974; Kesseler and Brand 1995).**

Inhibition of respiration in the presence of excess metal ions and its increase under mild metal stress is reported to occur in animal and plant mitochondria **(Lösch & Köhl 1999).** *In vitro* studies with Pb, Cd, Zn, Co and Ni on corn mitochondria **(Bittell *et al*. 1974)** and with Cd on potato tuber mitochondria **(Kesseler and Brand 1995)** showed inhibition of succinate and NADH oxidation and uncoupling of phosphorylation from the respiratory electron transport.

Generation of $O2^{-}$ radicals in the course of mitochondrial electron transport is a normal physiological phenomenon **(Scandalios 1993).** There are two separate sites of O2– production in the mitochondrial electron transport chain; the flavoprotein, NADH dehydrogenase, and the ubiquinone-cytochrome *b* segment **(Rich and Bonner 1978;Turrens *et al.*, 1985).**

In aerobic organisms, the mitochondria are the main supplier of energy and are the site of the tricarboxylic acid (TCA) cycle, a metabolic network involved in the generation of reducing factors that power of ATP production **(Costello *et al.*, 1997; Fernie *et al.*, 2004; Manev *et al.*, 1997)**. According to **Lemire *et al.*, (2008),** the ability of Zn to inhibit numerous mitochondrial enzymes perturb ATP production in human liver cells.

The aim of the present study is, firstly, throwing head lights over the proplem of contaminated diet with Copper and Cadmium.

Secondly, clarify the effect of contaminated diet on genetic processes of Nile tilapia **(*Oreochromis niloticus*)** by estimating the rate of chromosome aberrations in somatic cells. The Third goal is to investigate the effect of contaminated diet on total Protein content in muscle and plasma albumin and globulin. The fourth goal investigate the effect of contaminated diet on electrophoreteic pattern of epiaxial muscle. The last objective is to clarify the effect of contaminated diet on NADH dehydrogenase gene using PCR-RFLPs technique.