INTRODUCTION
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INTRODUCTION AND HISTORICAL REVIEW

Increased pollution of water resources has led to the development of more efficient procedures (**Briggs, 1979 and Murakami, 1980**) aimed at the protection of human health and aquatic life. Freshwater pollutants have been monitored mainly by physical and chemical techniques (**Brezonik, 1974 and Hattingh, 1979**). However, these information on unknown hazardous compounds and their potential harmful effects on man and aquatic ecosystems (**Cairns and Gruber, 1979**) are growing. Some of the living organisms show specific responses to hazardous levels of chemicals or mixtures of chemicals. Thus using biological sensors became an easier alternative and increasingly important approach in the prediction and control of water pollution (**Cairns et al., 1977**). Members of the aquatic food chain, such as bacteria (**Kool et al., 1979, Anderson and Abd EL-Ghani, 1980**), algae (**Wong et al., 1979**), protozoa (**Ruthven & Cairns, 1973**), arthropods (**Idoniboye-Obu, 1977 and Marshall, 1979**) and fish (**Price, 1978**) are commonly used as indicators of Pollution.

As the degree of industrialization and technology increases, the volume and complexity of heavy metals which enter water resources also increase. It is already known that relatively low concentrations of certain heavy metals are extremely toxic to aquatic life in general and to fish in particular. The lethal concentrations of many of such metals have been reported (**Lloyd, 1961; Skidmore, 1964; Sprague, 1969; Bryan, 1971, 1976; and Anderson & Abd El-Ghani, 1980**). Little, however, is known concerning the effects of sublethal concentrations on fish. This is in part resulting from being unable to describe such effects quantitatively.

Most information about the effect of environmental pollutants on aquatic animals has been obtained from mortality studies (**Lockwood, 1976**). Often very little is known about damage to different internal organs or about disturbed physiological and biochemical effects of these poisons. Consequently knowledge about the mode of action of toxicants and causes of death in poisoned aquatic animals is often lacking. A better understanding of these mechanisms is necessary to predict the potential harmfulness of various chemicals to the environment.

Various means of investigating sublethal effects of pollutants on aquatic animals are used or are under development. The different approaches include their effects on growth (**Weis & Weis, 1977; Benoit & Holcombe, 1978; Lewis, 1978; Waiwood & Beamish, 1978 and Sayer et al., 1991**), respiratory system (**Gardner & Yevich, 1970; Bilinski & Jonas, 1973 and Voyer et al., 1975**), changes in fecundity (**Wallace & Selman, 1979**) or broad survival (**Linden et al., 1979**), changes in swimming activity (**Ellgaard et al., 1978**) and physiological, biochemical and histopathological alterations (**Baker et al., 1969; Chliamovitch and Kuhn, 1977;**

compounds and their toxic mode of action of fish and other aquatic animals have been reviewed and discussed by **Skidmore (1964)**. Most research concerning zinc toxicity deals with short-term lethal exposures. Some information however, do exist on long-term sublethal effects of zinc on survival, growth and reproduction of fish. A life-cycle exposure of the fathead minnow; Pimephales promelas (Rafinesque) to zinc in hard water was reported by **Brungs (1969)**. The author showed that the most sensitive indicator of zinc toxicity is the significant reduction of egg production at 180 µg Zn/l. In another closely related study **Eaton (1974)** conducted a life cycle exposure of fathead minnows to a mixture of copper, cadmium and zinc in hard water. The author recorded also a significant reduction of egg production, which was attributed to the effects of zinc at 42 µg Zn/l. Both authors, therefore concluded that, sublethal concentrations of zinc affect adult maturation, which directly reduces the number of spawned eggs. Chronic, tests on zinc have been reported also on flagfish; Jardanella floridae, and rainbow trout; Salmo gairdneri (**Singley et al., 1974 and Spehar, 1976**).

Weatherly et al. (1967) are of opinion that zinc contamination of the Molonglo River depresses both the number of species and their abundance and that the absence of crustacians, molluscs and oligochaets in this river water is due to the presence of high levels (lethal and sublethal) of zinc.

Bioaccumulation of mercury in food sources presents a special health hazard to human. Fishes are known by their tendency to accumulate significant amounts of trace metals. **Uthe et al. (1973)** found that mercury burdens in caged rainbow trout (Salmo gairdneri) inhabiting a polluted river increased from 0.05 mg/kg to approximately 0.50 mg/kg within 3 months during summer. On the other hand, laboratory studies have indicated that mercury burden in pike (Esox lucius) is cleared very slowly (half-life up to 780 days) (**Jarvenpaa et al., 1970**).

Industrial and environmental hazards due to the widespread application and abuse of lead have been outlined (**Hammond, 1969; Guinee, 1972 and Hardy et al., 1973**). Lead has been shown to produce an acute haemolytic crisis which results in severe anaemia, haemoglobinuria and stippling of red blood cells in mammals (**Berk et al., 1990; de Bruin, 1971 and Albahary, 1972**). Little is known of the haematological lesions produced by this ubiquitous metal in fish except that chronic lead poisoning damages the erythrocytes of the catfish; Ameiurus nebulosus (**Dawson, 1935**). **Davies & Everhart (1973)** demonstrated that low concentrations of lead in water cause blacking of tails and spinal curvature of rainbow trout; Salmo gairdneri.

At sufficiently high concentrations, heavy metals are toxic for living organisms and so it is of importance to know the critical concentrations and ranges in the environment above which estuarine populations are affected and the commercial species become unsuitable as food.

Pollution of rivers and lakes with heavy metals and their toxic effects on fish species have been of special consideration. **Weis & Weis (1977)** studied the effects of lead, cadmium and mercury on the development of killifish; Fundulus heteroclitus. They concluded that, embryos which developed in lead at concentrations of 1.0 or 10 mg/l were normal in appearance until hatching, at which time they exhibited lordosis. No significant effects of cadmium at concentrations up to 10 mg/l were noticed. When F. heteroclitus embryos were exposed to inorganic mercury at concentrations of 0.03 or 0.1 mg/l at the early blastula stage, the percentage of successful axis formation was reduced leading to cyclopia.

Rodsether et al., (1977) reported that eels (Anguilla anguilla) which were exposed to copper in contaminated fresh water (30-60 mgcu/l) died with signs of vibriosis (Vibrio anguillarum), while eels kept in non-contaminated fresh-water (< 6 mgcu/l) remained healthy. **Benoit & Holcombe (1978)** showed that the life cycle of fathead minnown (Pimephales promelas) was reduced as a result of exposure to various zinc concentrations.**Lewis (1978)** studied the acute toxicity of copper, zinc and manganese as single salts and as mixed salt solutions to juvenile longfin dace; Agosia chrysogaster and the author concluded that, copper-zinc mixtures were more toxic than the copper-manganese mixtures. The toxicity of copper and zinc to the fish was similar, but both were considered more lethal than manganese. **Ellgaard et al. (1978)** declared that sublethal concentrations of cadmium, chromium and zinc hyperactivate locomotor responses of the bluegill; Lepomis macrochirus.

El Deeb et al. (1990) studied the effect of wastes on protein polymorphism of Tilapia nilotica after exposure to 5 different effluents of raw and treated toxic industrial liquid wastes of an Egyptian Plastic and Electricity Company. They concluded that there was a markedly significant decrease of total protein content of fish muscles for all of the exposed groups compared to that value of the control group. **Abd El Moneim et al. (1990)** on their study of the effect of these wastes on amino acids of Oreochromis niloticus concluded that there was a markedly significant decrease of the total protein content and a markedly decrease of the amino acid content of the fish muscle also for all the exposed groups compared to those values of the control group. **Khadre (1990)** in his study on the effect of sublethal concentrations of copper sulphate on gills and 10 blood parameters of the two teleosts : Clarias lazera and Tilapia nilotica pointed out that copper toxicity causes serious disturbances in both gill tissue and blood constituents.

Sayer et al. (1991) studied the embryonic and larval development of brown trout Salmo trutta L. exposed to each of four trace metals (aluminium, copper, lead and zinc) in soft and acid water and they concluded that aluminium reduces embryonic mortality and improves hatching successes. Copper reduces whole body calcium and potasium contents at pH 5.6. Zinc reduces whole body mineral content at pH 5.6.

The world development of technological products and the variability of by-products drainage into water systems is of great concern to aquatic biologists. Even though fish appear to be extremely sensitive indicators of excessive aquatic pollution, little is known on the biological effects of their daily exposure to sublethal doses of thermal effluents, toxic metals, pesticides, fertilizers etc. One of the difficulties in assessing the state of health of natural fish populations is the paucity of reliable references of the normal conditions. Pursuant to this goal, many fish physiologists are of largely considerable interest of studying their haematological aspects because of their valuable diagnostic tool in evaluating normal and disturbed health. Fish haematology continues to offer the potentiality of a valuable tool in establishing normal range values and those under different stresses. Literature in this area are many and have been established for various teleosts (**Barnhart, 1969; Blaxhall, 1972; McCarthy et al., 1973; Panigrahi & Misra, 1978, 1979; Clark et al., 1979; Hilmy et al., 1980; Mazhar et al., 1987; AuBin & Geraci, 1989; Marie, 1990; Khadre, 1990 and Ahmed et al., 1992** among many others). Fishery scientists have lagged behind both human and veterinary medical researchers and toxicologists in the utilization of blood chemical values for the assessment of general fish health.

The small size of most fish used in research delayed fish blood chemistry research due to the small blood volumes available for analysis. New techniques and instrumentation are now being developed to analyse microquantities of blood plasma or serum. Clinical blood analysers are available also, which require from 5 µl to 50 µl per test. These instruments provide rapid sampling, quick results and precision and accuracy that are sufficient for most research purposes.

Blood is the most accessible element of the teleostean fluid system. Consequently, blood variables are commonly used as direct inferential indicators of the functional state of animals. Stressors and pollutants generally produce relatively rapid changes in blood characteristics of fish (**Pickford et al., 1971; Mcleay, 1975; Agrawal & Srivastava, 1976; Larsson et al., 1976; Mazhar et al., 1987; AuBin & Geraci, 1989; Khadre, 1990; Marie, 1991; and Ahmed et al., 1992**). So, the use of haematological parameters as indicators of sublethal effects of a stress can provide information on the physiological responses of fish resulting from changing external or internal environment. This is a result of the close association of the circulatory system with the external environments and with every tissue.

It is the purpose of this research to establish a blood profile for one of the most important economic freshwater fish in Egypt. The Nile teleostian fish; Oreochromis niloticus was choosen for the present investigation. This species is the most abundant fish in the River Nile and its tributaries and is the most popular edible fish in terms of human consumption. Also Oreochromis niloticus is an ubiquitous and prolific fish and its production is of considerable amount with regard to the total fish production in the Egyptian Waters (**El-Zarka, 1956; El-Zarka et al., 1970 and Abd-Elbaky & El-Serafy, 1990**).

In the recent years considerable ecological changes have developed in the River Nile which is considered as the main habitat of Oreochromis niloticus in Egypt. The Nile water has been contaminated by trace metals from mine working and steel factories at Al-Tabeen (South Helwan). These industrial factories drain their by-products directly into the Nile resulting in such contamination. Spectrophotometric analysis of the mixed Nile drainage water at Al-Tabeen using atomic absorption revealed the presence of iron, zinc, manganese and copper in the Nile water.

The present investigation aims to determine some biochemical and haematological aspects of O. niloticus exposed to the same concentrations of iron, zinc, manganese and copper of running Nile water at this area and with other higher-single or combined doses.

The determination of enzymatic activities could be applied to monitor water pollution; establishing "safe" concentrations of these metals if present and an early rapid diagnosis of the state of the fish. Serum enzyme activities reflect sensitively the health condition of fish, so their use in diagnostics has been recommended by a number of authors **(Racicot et al., 1975; Gaudet et al., 1975; Franzmann & Leresche, 1978; Weber et al., 1979; Wieser & Hinterleitner, 1980 and AuBin & Geraci, 1989).**

The aim of the present study is directed also to test the possible variations of protein pattern and enzyme activities of certain enzymes of liver, brain and blood of the fresh water; Oreochromis niloticus raised in water containing salts of iron, zinc, manganese and copper separately or as salt mixtures of these metals. The studied parameters include, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, acid and alkaline phosphatases of liver, brain and blood serum. Further, blood parameters including red blood cell count, haemoglobin and haematocrit were determined for fishes under the forementioned conditions.