INTRODUCTION

Aquatic environment represents an important biotope that should be controlled sensitively as contamination resulting from the industrial, agricultural and anthropogenic activities (Hogstrand et al., 1999 and Basha and Rani, 2003). Heavy metals occur in the environment both as a result of natural processes and as pollutants from human activities (Garcia-Montelongo et al., 1994). The continual loading of metals into our environment creates water pollution problems due to their direct toxic effect on aquatic biota (Bryan, 1971).

Metals are known to inhibit several biochemical and physiological mechanisms vital for fish metabolism (Hogstrand et al., 1999 and Basha and Rani, 2003). Therefore, fish can be considered as an early indicator of environmental risk due to their sensitive responses to environmental fluctuations (Elia et al., 2007**).** So, it is of great importance to know the bioaccumulation potential of heavy metals (Parlak et al., 1999).

At concentrations over the natural levels, heavy metals are serious threats to the aquatic ecosystems due to their high toxicity, non-biodegradable and bioaccumulative properties (Velma and Tchounwou, 2010). Besides, metal pollution may not only lead to disturbance of the integrity of ecosystems but also lead to a challenged human health through the food chain.

Thus, monitoring and prevention of heavy metal contamination have been attracted much attention. The use of fish species is highly recommended for this purpose. Because fish may absorb dissolved heavy metals from the surrounding water that may accumulate in various tissues, organs and even be biomagnified (Abdullah et al., 2007), which makes it much of a significance for the monitoring of the pollution of aquatic ecosystems. In addition, studies which based on the responses of fish species to aquatic metal helps in the better understanding of the potential toxicological mechanism (Zhou et al., 2007).

Heavy metals induced oxidative stress in aquatic animals including fish. The generated reactive oxygen species (ROS) induced by heavy metals is commonly associated with cellular injures because of alterations in DNA, proteins and cellular membranes (Leonard et al., 2004). To counteract the adverse effect of ROS, living organisms have a complex and effective antioxidant defense system including both enzymatic and non-enzymatic mechanisms such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione (GSH) (Kelly et al., 1998). Differences in these antioxidant defenses can be very sensitive in revealing a pro-oxidant condition and have been used as biomarkers of oxidative stress in fish (Ahmad et al., 2006 and Oliveira et al., 2008). Moreover, these biomarkers have been widely used in aquatic ecosystems to assess the exposure and/or effects of pollution on native populations of fish. because of their importance in the tolerance capability of organisms to pollution exposure (Vieira et al., 2008 and Vieira et al., 2009).

Fish and its products are considered as food of excellent nutritional value, providing high quality protein and a wide variety of minerals including phosphorous, calcium, magnesium, sodium and potassium (F.A.O., 2003).

African catfish, *Clarias gariepinus*, *C. gariepinus* was selected in this study as one of the most popular economic catfish species in Egypt (Brewer and Friedman, 1989). Some records showed that Clarias fishery represents about 17% of the annual fish production from all fisheries sectors (Ololade and Oginni, 2010). Moreover, the African catfish were used in fundamental researches and considered as an excellent model for toxicological studies (Mahmoud et al., 2009).

AIM OF THE WORK

The purposes of this work were to:

- Throw lights on the problem of water pollution with heavy metals due to industrial and agricultural drainages.
- Clarify the environmental integrates affecting the study areas in terms of hydrogen ion concentrations, temperature, water hardness, salinity, alkalinity and heavy metals.
- Assess the physiological and biochemical changes in catfish, *Clarias gariepinus* (*C. gariepinus*) which collected from El-Qanater El- Khayriyah as a Reference site, El- Rayah El-Tawfeky as an Industrial polluted site and a canal in Moshtahr village as an Agricultural polluted site during Spring and Summer seasons.
- Screen on the physiological response of some selected organs “liver and muscles” of *C. gariepinus* in terms of determination of tissue enzymes (AST, ALT and ALP), total proteins, (Na^+ and K^+) electrolytes and some oxidative stress indicators.
- Study the histopathological alterations in liver as the organ of detoxification of such heavy metals.

REVIEW OF LITERATURE

1. Water quality parameters:

1.1. Temperature and pH:

Alabaster and Lloyd (1980) stated that water pH below 4.5 to 5.0 considered to be toxic to warm water fishes as low pH affected the productivity of fish and increase the susceptibility of fish to diseases.

Hepher and Pruginin (1981) indicated that higher pH values (9.5) were quite common in productive ponds and can even reach 10-10.2 in ponds receiving organic effluents, with no harmful effects.

Diana et al. (1991) found that pond temperature stratification correlated with air temperature, solar radiation, rainfall and wind velocity.

Robert (1994) stated that the water temperature becomes high during a summer day; the fishes may be reluctant to eat and the food will then be added to biochemical oxygen demand in the culture system and serve as a substrate for the growth of microorganism.

Saleh (1995) found that cold temperature is the major stressing factor affecting cultured tilapias. He added that in cultured fish, *Aeromonas hydrophila* is associated with handling injuries during cold temperature.

Stephen (1995) stated that in temperate climates most problems associated with dissolved oxygen occurs during the warmer summer months, and result from a combination of factors. At higher temperatures, the oxygen holding capacity of water was reduced, and this typically coincides with periods of active growth, high stocking densities and elevate feeding levels. The rapid growth of microorganisms, algae and higher plants during the warmer months also exerts additional oxygen demand. A combination of any or all of these factors may result in critically low dissolved oxygen levels in the tropics and subtropics.

Hisham (1996) concluded that mortality and morbidity rates due to Motile Aeromonas Septicaemia (MAS) increased with increasing temperature.

Ghanem (2006) stated that the average value of water temperature of Lake Qarun was 37.8 ºC during summer. He also recorded that pH values fluctuated between 7.1 and 7.9.

Tayel et al. (2008) indicated that the pH value is reflection to many of the biological and chemical processes occurring in the aquatic environment. Among these are photosynthetic activity of aquatic plant, respiration of aquatic organisms, decomposition of organic matter, precipitation, dissolution, variation in temperature, salinity and oxidation reactions taking place in the environment.

Osman et al. (2010) stated that pH values were in alkaline side (7.8 to 8.4) in the water samples collected from six different sites along the River Nile from Aswan to Rosetta and Damietta branches. They also mentioned that temperature ranged from 22.6° C to 25.2°C.

Osman and Kloas (2010) mentioned that temperature and pH was ranged from 22.7° C to 25.3°C and 7.8 to 8.4, respectively in the water samples collected from different sites along the River Nile from Aswan to Rosetta and Damietta branches.

Carvalho et al. (2012) reported that pH and temperature increased in the Monjolinho River in Brazil.

Oss et al. (2013) recorded that pH and temperature were 7.2 and 22.7ºC respectively in the water of 25 and 50 µg Cu/L after 30 days.

Ali et al. (2013) reported a recognizable depletion in Hydrogen ion concentration (pH) in the water of the River Nile at some locations (e.g. El- Nasria).

1.2. Ammonia:

Thurston et al. (1978) reported that the United States Environmental Protection Agency had established an acceptable level of NH_3 for aquatic organism of 0.016 mg/L.

Eddy (1982) reported that ammonia toxicity was mainly associated with the unionized form and added that the concentration of which was related to the total ammonia content, water pH and temperature.

Cole and Boyed (1986) found that the rate of ammonia production in fish cultures was proportional to the feeding rate.

Piper et al. (1986) reported that the standard analytical methods do not distinguish the two forms of ammonia, and both were lumped as total ammonia. They added that the toxic fraction of ammonia varies with salinity, oxygen concentration and temperature but were determined primarily by pH of the solution.

Stephen (1995) reported that the relative proportion of the two forms of ammonia depended primarily on pH and to a lesser extent on temperature and salinity.

Edward (2000) described that ammonia tended to increase during fall and winter because of the decrease in algal and bacteria metabolism at low temperatures which was considered as the major consumers of ammonia products.

El-Shafai et al. (2004) reported that the toxic levels of unionized ammonia for short term exposure usually lie between 0.6 and 2 mg/l. they decide that two primary processes affect ammonia concentration in ponds, ammonia excretion rate by fish and sediment diffusion that represents one pare of the exogenous ammonia in the pond. They also reported that elevation of ammonia level and reduction of dissolved oxygen concentration during the summer and spring were the major factor responsible for mortality in the sewage - fed fish ponds.

Sukriye et al. (2004) found that ammonia is excreted by plants and animals and produced as a result of the decomposition of organism, the sewage by microorganism, volcanic activity, industrial emission and release of nitrogen fertilizers. The excessive high doses of ammonia can cause reduction in fish growth or even death.

David and Yuen (2006) reported that ammonia is produced in the liver and excreted as NH_3 by diffusion across the gills. Acidification of the water around the fish by CO_2 and acid excretion enhances ammonia excretion and constitutes. Fish have difficulties in excreting ammonia in alkaline water or high concentration of environmental ammonia.

Nicklaus et al. (2007) showed that ammonia is toxic to fish because NH_3 cause hyper- excitability, coma, convulsious and finally death. It also, affects the ionic balance in fish, because NH_4 consubstitute for K^+ in Na^+/K^+ ATPase.

Denise et al. (2008) found that the toxicity of NH_3 to aquatic organism has been attributed mainly to the unionized form (NH_3), while the ionized form ($NH_4{}^+$) is considered less toxic. pH plays an important role in homestasis of aquatic animals. Fish exposed to alkaline water show increase plasma ammonia because asignite proportion of excreted ammonia remains as NH_3 in the water.

Spencer et al. (2008) reported that NH_3 is considered to be more toxic fraction within aquatic environments. It is able to perment tissue. As pH and temperature rise to a higher percentage if NH_3 is present, therefore increasing toxicity.

Osman et al. (2010) stated that ammonia decreased than permissible limits in the water samples collected from six different sites along the River Nile from Aswan to Rosetta and Damietta branches.

Osman and Kloas (2010) reported that ammonia decreased in the water samples collected from different sites along the River Nile from Aswan to Rosetta and Damietta branches.

Carvalho et al. (2012) found that ammonia increased in the Monjolinho River in Brazil.

Oss et al. (2013) recorded that total ammonia concentration was 0.3 mg/L in the water of 25 and 50 µg Cu/L after 30 days.

1.3. Dissolved oxygen and Hardness:

Alabaster and Lloyd (1980) reported that the mature size fish can probably survive dissolved oxygen levels in excess of 3.0 mg/L when other conditions are favorable.

Boyed (1982) used the artificial aeration to improve water quality in pond and also mechanically increase the concentration of dissolved oxygen in fishpond.

Ver and Yvonne (1986) reported that paddlewheel aerator had long been established as a very effective device for adding dissolved oxygen to oxygen-depleted pond water. They added that the non-aerated ponds dissolved oxygen curves had wider DO values and pronounced peak, while the aerated ponds DO curves had a narrower values and non- pronounced peak.

Boyed (1988) mentioned that during the day light hours, photosynthesis in the euotrophic zone released oxygen faster than it is used in respiration and as the photosynthesis stopped at night while the respiration process continued to use oxygen. This pattern of day time production and continuous use of oxygen leaded to daily fluctuation of dissolved oxygen concentration in the euotrophic zone and so the maximum concentration of dissolved oxygen occurred during the afternoon and the minimum concentration at or just after sunrise. He also added that continued exposure to low dissolved oxygen is considered a precursor to bacterial infection in fish.

Diana et al. (1991) reported that daily oxygen stratification was more pronounced with inorganic fertilization probably due to greater water clarity and more even distribution of primary production inorganically fertilized ponds.

Stephen (1995) mentioned that freshwater fishes like tilapias have lower overall requirements for oxygen reflecting their natural habitat in slow moving tropical Rivers, lakes and ponds.

Khalaf-Allah (2001) pointed out that the amount of DO varied from 4 to 6.12 mg/l in Lake Qarun.

Ali (2002) recommended applying the paddlewheel aerator in fish pond during high hours to avoid severe depletion of dissolved oxygen in water due to consumption by fish and aquatic organisms.

Al-Afify (2006) studied the water quality of the River Nile at Damietta branch and reported that the increasing of dissolved oxygen concentration may be attributed to the increase of photosynthesis activity as a result of abundance of phytoplankton.

Osman and Kloas (2010) reported that total hardness was slightly increased in the water samples collected from different sites along the River Nile from Aswan to Rosetta and Damietta branches. The authors also recorded that alkalinity ranged from 96.2 to 124.2 ppm.

Loro et al. (2012) stated that dissolved oxygen and DOC concentration did not vary amongst salinities after exposure to 500 µg/L of Zn for 96h.

Carvalho et al. (2012) reported that dissolved oxygen increased in the Monjolinho River in Brazil.

Ghanem (2011) postulated that water hardness in Lake Qarun was decreased during summer more than spring season. While, salinity was fluctuated between 0.33 during spring and 0.42 during summer

Oss et al. (2013) recorded that oxygen concentration and hardness were 7.9 mg/L and 24.4 mg $CaCO_3$/L in the water of 25 and 50 µg Cu/L after 30 days.

Ali et al. (2013) reported a recognizable depletion in dissolved oxygen (DO) in the water of the River Nile at some locations (e.g. El- Nasria).

1.4. Sources of heavy metals in water:

WHO working group (1972) found that lead content of drinking water may be due to the use of lead pipes or of plastic pipes stabilized with lead compounds.

Liptrot (1974) recorded that lead occurs in nature principally as lead oers containing galena (lead sulphide) usually in association with zinc blend (zinc sulphide).

WHO working group (1977) recorded the major sources of lead in the environment arise from the industrial and technological uses of lead such as application of alkyl lead in fuel additives.

Griggs and Johnson (1978) found that mining and other forms of industrial wastes are responsible for the release of large quantities of heavy metals into the environment such as lead, mercury and copper.

Davis (1984) stated that the main sources of cadmium pollution to the environment are combustion of gasoline, the utilization of cadmium containing pesticides and phosphate fertilizers.

El-Nabawi et al. (1987) found that the industrial and agricultural discharges in Egypt were the primary source of lead poisoning in fish.

Lloyd (1992) recorded that cadmium enters the aquatic environment from many sources, as a result of the use of galvanized pipes or cadmium containing solders in water heaters as well as the effluents from electroplating works and the use of cadmium- rich sewage sludge for agricultural purposes.

1.5. International permissible limits of heavy metals in water and fish:

WHO working group (1984) recorded that the permissible limits of mercury, lead and cadmium in water are 0.001, 0.05 and 0.0005 ppm respectively, as well as the permissible limits of mercury, lead and cadmium in fish flesh are 0.5, 0.6 and 1.0 mg/kg wet weight respectively.

Eu Directive (1998) recorded that the permissible limits of cadmium, lead, and mercury in water intended for human consumption are respectively 5, 10 and 1 mg/L.

FAO (1999) reported that the maximum permissible limits of cadmium, lead, and mercury in drinking water are 0.005, 0.01 and 1.0 ppm, respectively.

Eu-Regulation (2001) recorded that the maximum levels of cadmium, lead, and mercury in fish flesh are 0.2, 0.05 and 0.5mg/kg wet weight.

El-Enany (2004) postulated that the contamination in aquatic animals with heavy metals in Lake Qarun showed higher values than the international permissible one.

Ali and Fishar (2005) proposed that the sullying in sea-going creatures with overwhelming metals in Lake Qarun demonstrated higher qualities than the universal reasonable one.