

## **Heavy Metals at Kafr Elsheikh Governorate and the use of algae in fish cultured**

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### **Abstract**

The aim of this review is to compile some dispersed literature published about heavy metals affect fish especially that cultured in fresh water in Egypt (Kafr Elsheikh Governorate) such as Iron, Zinc, Copper, Manganese, Cadmium and Lead (Fe, Zn, Cu, Mn, Cd and Pb) in relation to the effect of using some microalgae (*Cyanobacteria*) in order to help in prevention of heavy metals adverse effect or at least to ameliorates it's adverse effect on fish. In Kafr Elsheikh Governorate the agricultural drainage water is considered as one of the most important water sources for fish farms in addition to industrial drainage water especially from Kitchener canal which collects El Gharbia governorate agricultural, industrial drainage water and sewage wastewater and sewage drainage water of Kafr Elsheikh city and industrial drainage water of spinning factories of Kafr Elsheikh. So drainage water is therefore contaminated with salts, agricultural chemicals (pesticides and heavy metals) and other pollutants as pathogens from domestic

**Keywords:** *heavy metals, microalgae, fish, antioxidant.*

### **The Egypt's northern Delta Lakes:**

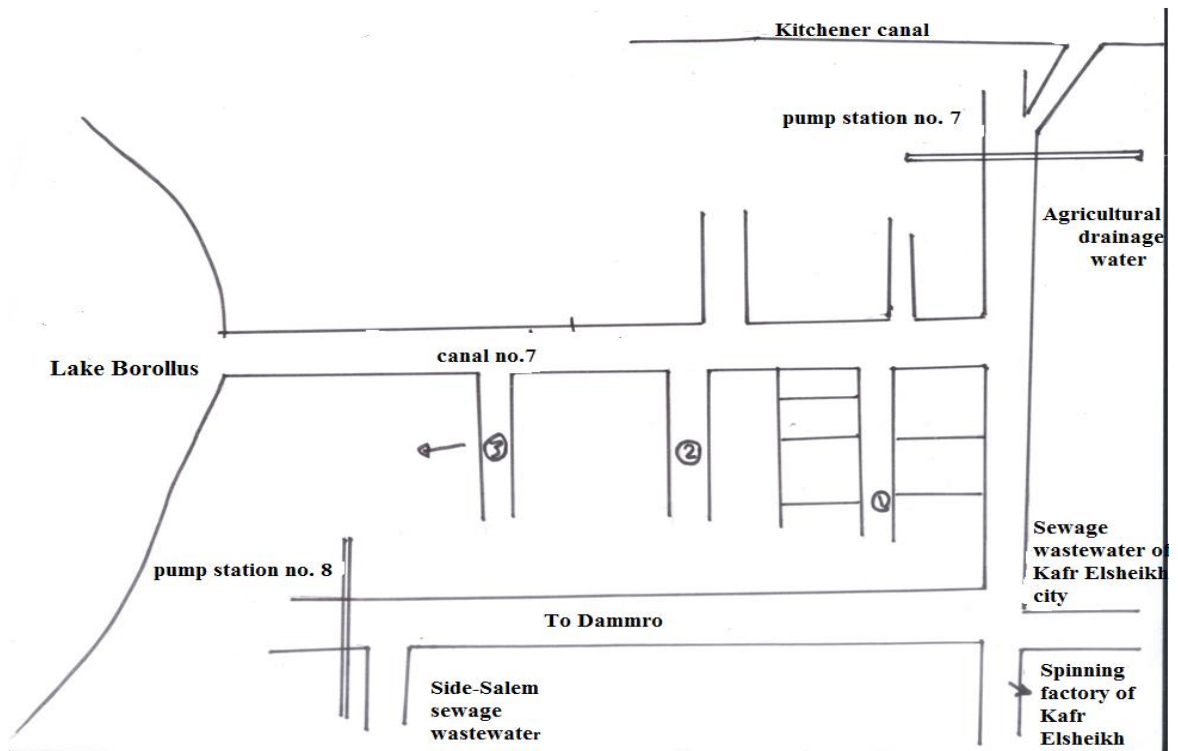
The aquatic environment with its water quality is considered the main factor controlling the state of health and disease in both cultured and wild fishes. Pollution of the aquatic environment by inorganic and organic chemicals is a major factors posing serious threat to the survival of aquatic organisms including fish (**Saeed and Shaker 2008**). The Egypt's northern Delta Lakes include Edku Lake, Borollus Lake, Manzala Lake, and Mariut Lake. These lakes are situated on the Mediterranean Coast of the Delta and cover about 6% of the non-desert surface area of Egypt and contributed less than 12.22% of the country's total fish production (**GAFRD, 2006**), so in Egypt, the production of fish coming from aquaculture which represents about 65% of the total fish production sources (**GAFRD, 2009**). All the metals attained their maximum values at Lake Manzala. Lake Borollus, ranked second in accumulation of metals, while Lake Edku was the less polluted one. This may be attributed to the increased cover of the aquatic and higher plants which absorb metals from water and sediments (**Saeed and Shaker 2008**).

### **The agricultural drainage water:**

The agricultural drainage water is considered as one of the most important water sources for fish farms in Egypt, due to shortage of water. On the other hand, its water contains fertilizers, pesticides and effluents of industrial activities, in addition to sewage effluents, supplying the water bodies and sediment with huge quantities of inorganic anions and heavy metals (**Saeed & Mohammed, 2012**). Also the most extremely northern part of Nile Delta suffers from the shortage of water for irrigation purposes and reuse the waste water (sewage, industrial and agricultural drainage) (**Elsanfawy et al., 2005**). The farmers of this region obligate to reuse the waste water in planting especially in summer seasons where rice is planting, and concentrations of all studied heavy metals in sewage water were higher than the permissible limits of FAO (1985) (Fe ' Pb ' Ni ' Mn ' Cu ' Cd). Drainage water is therefore contaminated with salts, agricultural chemicals (pesticides and heavy metals) and other pollutants as pathogens from domestic sewage and industrial discharge (**Gad 2005**). Changes in the chemical composition of the water are followed by significant changes in structure of their biota (**Marneff et al., 1996**).

#### **Bioaccumulation of the heavy metals in fish body:**

Bioaccumulation of the studied heavy metals (Cu, Pb, Zn and Mn) in gills, liver, kidneys and muscles of the Nile tilapia; *Oreochromis niloticus* collected from the eastern and western sectors of the Borollus lake was much higher than that of fish collected from El-Boughaz inlet (**Zaghloul et al., 2007**). Industrial waste discharges affect greatly on fish that harbor in borollus lake which in turn effects on human due to consume this fish leads to accumulation of heavy metals in human body threaten thier life (**Ahmed 2013**). Fish living in polluted waters tend to accumulate heavy metals in their tissues. Generally, accumulation depends on metal concentration, time of exposure, way of metal uptake, environmental conditions (water temperature, PH, hardness, salinity) and intrinsic factors such as fish age and feeding habits. Various metals show different affinity to fish tissues. Most of these metals accumulate mainly in liver, kidney and gills. Fish muscles, compared to the other tissues; usually contain the lowest levels of metals. Accumulation of heavy metals in various organs of fish may cause structural lesions and functional disturbances (**Barbara and Malgorzata 2006**). The gills, liver and kidneys are commonly the primary target organs for pollution. Histopathological lesions and increase in size were reported in various fish exposed to heavy metals (**Alazemi et al., 1996**).



**Diagram for fish farms and its drainage water in Kafr Elsheikh Governorate**



**Kitchener canal**



**Kitchener canal**



**Pump station no. 7**

### **Heavy metals :**

Heavy metals have long been recognized as major pollutants of the aquatic environment. When it is present in high concentrations in the milieu, it causes serious impairment in metabolic, hematological, biochemical and structural systems and consequently on mass and quality of the field product (**Hegazi et al., 2014**). The term heavy metal refers to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations. Examples of heavy metals include mercury (Hg), cadmium (Cd), arsenic (AS), chromium (Cr), thallium (Ti) and lead (Pb). Heavy metals are natural components of the earth' crust. They can't be degraded or destroyed. Heavy metals (HM) are natural trace components of the aquatic environment, but their levels have been increased due to industrial wastes, geochemical structure, agricultural and mining activities (**Sprocati et al., 2006, Zeitoun and Mehana 2014**). Fe, Mn, Cd and Pb (in Manzala Lake) and Mn, Pb in Borollus Lake recorded levels above the international permissible limits in water (**Saeed and Shaker 2008**).

### **Effect of heavy metals on fish immune response:**

Lead, mercury and cadmium have inhibitory effect on phagocyte activity of fish macrophages and so having an inhibitory effect on cell mediated immune response. The effect of these metals on humoral immune response revealed also that these metals having inhibitory effect on humoral immune functions which is manifested by low levels of antibodies and high mortality rates in fish exposed to these metals than in the control fish after experimental infection by *Pseudomonas fluorescens*. Immune response by these metals provides opportunities for the entry of pathogens and developing of many diseases in fish (**Omima 2010**). Heavy metals are surrounded with great care and special importance due to their highly toxic effects on fish as they affect survivability, growth and reproduction (**Gill and Pant 1985 and Omima 2010**).

There was highly significant ( $P < 0.01$ ) difference in WBCs of fish collected from the different studied sites along Borollus lake. Moreover, fish collected from the western and eastern sites of the lake had a significant increase in WBCs count, than that of fish collected from El-Boughaz opening. Leukocytosis reported in the present study may be attributed to increased leukocyte mobilization to protect the body against infections in metals-damaged tissue (**Zaghloul et al., 2007**). The increase in WBCs of fish was suggested to indicate alteration in defense mechanism against the action of the highly toxic and the bioaccumulated heavy metals in fish tissues as previously reported by **Haggag et al. (1999); Zaghloul (2001); Mazon et al.,(2002) and Zaghloul et al. (2005)**.



Figures (1), (2), (3) and (4) showing *O. niloticus* naturally exposed to Cadmium suffering from emaciation (1), scoliosis (2), degeneration in ovaries (3) enlarged pale spleen and spotted liver with inflammatory patches (4) (Eissa et al., 2011)

#### **Effect of heavy metals on antioxidant enzymes:**

Heavy metals are known to reduce the activities of antioxidant enzymes (e.g. superoxide dismutase, Catalase) (Ahmed 2013). Heavy metal redox cycling and interaction with organic pollutants contribute to oxidative stress from aquatic pollution. Oxidative stress resulting from reactive oxygen species (ROS) has been identified as a causative agent in a number of pathologies in fish (Iqbal et al. 2005). Where oxidative stress occurs as a consequence of excessive production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and it is ameliorated by endogenous antioxidant enzyme activity and exogenous dietary antioxidants (Sugino 2007).

Both of redox-active and redox-inactive metals may cause an increase in production of ROS such as hydroxyl radical ( $\text{HO}^\cdot$ ), superoxide radical ( $\text{O}_2^{\cdot-}$ ) or hydrogen peroxide ( $\text{H}_2\text{O}_2$ ). Enhanced generation of ROS can overwhelm cells intrinsic antioxidant defenses, and result in a condition known as oxidative stress. Cells under oxidative stress display various dysfunctions due to lesions caused by ROS to lipids, proteins and DNA (Ercal et al. 2001). Oxidative stress due to the toxic effect of pollutants is usually indicated by increased levels of products of oxidative damage (LPO) as represented by MDA (Malonaldehyde) and subsequent increase in defense

enzymes (GSH, SOD activity and CAT ase) in response to the stress (*Doherty et al. 2010*) or decrease due to overwhelming effect of the pollutants (*Olagoke, 2008*). Nitric oxide is a highly versatile and unique ubiquitous signaling molecule, and is known to play diverse physiological functions including those of adaptation to various stresses. Nitric oxide is produced in the body by the oxidation of L arginine through a catalytic action of NO synthases in the presence of molecular oxygen and NADPH (*Griffith and Stuehr 1995*) which is known to provide some protection to the cell against the oxidative stress (*Vile et al. 1995*). The demonstrated LPO levels indicated by increase in MDA were parallel to metal bioaccumulation evaluated the levels of heavy metals and certain biomarkers of oxidative stress as surrogate bio-indicators of aquatic pollution in *Clarias gariepinus* (*Faramobi et al. 2007*). Glutathione reductase reduces glutathione disulfide to GSH, thereby supporting the antioxidant defense system. Glutathione reductase has a disulfide bond in its active site, but lead interferes with the disulfide bond and inhibits the enzyme. This inhibition prevents the reduction of glutathione disulfide, making cells more susceptible to oxidative damage (*Othman and El Missiry 1998*). Other antioxidant enzymes, which remove peroxides and superoxide radicals as CAT'ase and SOD activity, are also potential targets for lead. Because lead is known to inhibit heme synthesis, and since CAT'ase is a heme-containing enzyme, it causes CAT'ase activity to decrease (*Ercal et al. 2001*). Antioxidants present in the diet can delay lipid peroxidation by inhibiting the initiation or propagation phase of oxidizing chain reactions by scavenging free radical (*Sherwin 1990*). The role of various antioxidants in the protection against heavy metal poisoning has been documented in many studies. **Mourente et al. (2002) and Senug et al. (2007)** reported that antioxidants significantly prevented fish from heavy metal toxicity.

#### **Effect of Cyanobacteria Microalgae on heavy metals:**

Cyanobacteria from Microalgae or microscopic algae grow in either marine or freshwater systems. They are primary producers in the oceans that convert water and carbon dioxide to biomass and oxygen in the presence of sunlight (**Chisti 2008**). Algal biomass and algal –derived compounds have a very wide range of potential applications, from animal feed aquaculture to human nutrition and health products (**Hua-Bin et al., 2007**). Some algae are considered as rich source of natural antioxidants. The extracts from selected green, brown and red algae have been reported to demonstrate antioxidant activity by a variety of in vitro methodologies. The above evidence suggests a potential for protective effects of microalgae against lipid peroxidation and oxidative stress (**Geetha et al., 2010**).

*Cyanobacteria* are photosynthetic prokaryotes that capture sunlight for energy using chlorophylla and various accessory pigments. They are common in lakes, ponds, springs, wetlands, streams, and rivers, and they play a major role in the nitrogen, carbon, and oxygen dynamics of many aquatic environments. They are referred to in literature by various names, chief among which are Cyanophyta, Myxophyta,

*Cyanochloronta*, *Cyanobacteria*, blue-green algae, blue-green bacteria (Vincent 2009). Microalgae to be very effective biosorbents, as they possess a large surface area and high binding affinity (Roy et al., 1993). Cell wall of these microalgae consists of polysaccharides, proteins and lipids having lots of negative groups which are the dominant binding sites of toxic metal cations (Vonshak, 1997).

*Cyanobacteria* as *Spirulina*, *Chlorella* and *Anabeana* where *Spirulina* is nature's richest whole food source of antioxidants where it contains a spectrum of practically every natural antioxidant known, including the antioxidant vitamins B<sub>1</sub>, B<sub>5</sub>, and B<sub>6</sub>, the minerals zinc, manganese and copper, the amino acid methionine and the super antioxidants B-carotene, vitamin E and the trace element selenium (Kaur et al. 2012). *Spirulina* contains phycocyanin (14%) chlorophyll (1%) and carotenoids (0.37%) pigments. B-carotene of *Spirulina* maintains the mucous membrane firmly and thereby entry of toxic element into the body is prevented. Chlorophyll of *Spirulina* acts as a cleansing and detoxifying phytonutrient against the toxic substances (Henrikson 1994). Also Hegazi et al., (2014) said that, *spirulina* treated groups significantly decreased lead residue in bone, muscle and liver. *Spirulina platensis* was effective in removing Hg from water. Hg concentration in water was 69.880±0.156 µg L<sup>-1</sup> and it decreased significantly (P< 0.05). The addition of dried *Spirulina platensis* improves the haematological parameters (RBCs, Hb and Hct) and ameliorates the toxic effect of Hg which indicating the capability of *Spirulina platensis* to chelate Hg from the media (Kaoud et al., 2012). Fish survived in water exposed to Pb (0.01±0.001), Cd (0.01±0.001), Hg (0.001±0.001) and a mixture of Pb +Cd + Hg, and used hyacinth and *chlorella* as feed additives reducing the pollutant effect of this heavy metals in water, and decreasing their negative impact and residual effect on the exposed fish (Shaker et al., 2008). There is no any paper about *Anabeana* microalgae in relation to heavy metals but the present paper about its effect on fish immunity where *anabeana* improve immune response of Nile tilapia fish and resistance against *Aeromonas hydrophila* infection (Sabreen et al., 2013). So we advice more further study needed to proof the results and determine the best inclusion rate of *cyanobacteria* in fish diets to decrease heavy metal toxicity or the best culture microalgae at inlet water of the farm especially in presence of Cyanobacteria Research Laboratory and The Field Drainage Dept., Sakha Agricultural Research Station - Kafr Elsheikh.

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