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EFFECT OF AQUATIC POLLUTION ON PHYSIOLOGICAL ASPECTS AND REPRODUCTION OF FISH

(REVIEW)

Adel Mohamed Essa Shalaby

Hatchery And Fish Physiology Department, Central Laboratory For Aquaculture Research, Agriculture Research Center

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Abstrac

Many industrial and agricultural chemicals including heavy metals and pesticides are presented in the environment and have adverse effects on metabolism and the reproductive function in fish. Many studies were conducted to assess the toxicity of these chemicals against fish physiology and reproduction. The present article aimed to provide a bibliography of publication that describes the effects of aquatic pollution (organic and inorganic chemicals) on serum haematology, biochemistry and reproduction status of teleost fish. Also, this article explored the relation between exposure of pollutant (water concentration and fish tissue burdens of pollutants) with special references to

1-The ecological measure (growth and movement)

2- The cell function

3- The physiological and endocrine status of fish (plasma hormones levels, intermediary metabolism, histopathology and reproductive status

4- The toxicity of these chemicals against fish reproduction.

The goal of this article is also to develop and increase our knowledge of the impact of endocrine disruption compounds (EDCs) in the aquatic environment on fish reproduction. Specifically, we propose to 1) investigate novel mechanisms of endocrine disruption in fish including impacts on retinoid status and thyroid hormone homeostasis 2) to detect and characterize ligands in complex effluents that interact with fish hormone receptors (estrogen, androgen) and 3) to investigate the mechanisms underlying the reproductive abnormalities observed in fish exposed to complex effluents, including alterations in gonadal steroid production, incidences of masculinization/ feminization and altered ovarian follicular development. The use of these methods along with tools developed previously with CNTC funding will increase our ability to screen complex effluents for endocrine-active compounds and enhance understanding of biochemical mechanisms by which endocrine disrupting compounds (EDCs) may alter the growth and reproduction of fish.

Key words: Fish, chemical contamination (heavy metals, estrogenic pesticides), blood biochemistry, haematology and reproduction

INTRODUCTION AND REVIEW OF LITERATURE

Pollutants that originate from agriculture such as pesticides (insecticides, herbicides and fungicides) and heavy metals or metals ions from industrial wastes are some time found at higher concentration in fresh water ecosystem. Most of the xenobiotics are potential immunomodulators and could impair fish health by altering their immunocom petence (Dunier, 1994)

With the increase of industrialization, pollution by heavy metals have become a serious problem in Egypt. Heavy metals such as copper, zinc, cadmium, lead and mercury are presented in relatively high amounts in different waste effluents of the textile industrial wastes at Alexandria (Saad *et al.* 1982). Industrial and agriculture discharges are considered the primiray source of pesticides and metal – poisoning of fish in Egypt (El-Nabawi *et al*., 1987). The wastes of fertilizer factory and National Companies of Metallic Industry at Abou- Zaable and Kaha area drain in Ismalia canal and El-Qualyobia, El-Raizi, respectively (Mazroh, 1988).

Due to the pollution increasing concentrations of various chemical compounds have increased in aquatic environment. The toxic effects of pollutants may affect behavior, cellular metabolism, endocrine regulations and reproduction of aquatic organisms [Rurangwa *et al*., 1998]. Disrupted reproduction may result in limited propagation of species and eventually can lead to their extinction.

Currently there are numerous governmental, research centers and universities sponsored programs to identify suborganismal bioindicators or biomarkers of chemical intoxications, diseases or stress on fish. One of such set of indicators that have been used to asses the fish health and could potentially provide both spatial and temporal comparisons of populations are the measurement of clinical blood chemistry and haematology (Hugget *et al*., 1992). In human and veterinary medicine, the relationships between specific tissue lesions or infection and change serum parameters such as enzymes, metabolites, electrolytes..ect is used to ascertain and confirm tissue dysfunction or damage.

The present article was meant to review and discuss the impact of pollution on serum chemistry, haematology and reproduction status of teleost fish. Therefore, the present article mainly is a general overview about the effect of pollution as endocrine disruptors (EDCs) as well as some biochemical and hematological changes which take parts as an indicator for metabolite mobilization which in turn affect of the fish production.

Chapter 1

1. Water pollution definition , type and resources

Pollution may be defined as the introduction, by man into the environment of substances or energy liable that cause hazards to human health, harm to living resources and ecological system, damage to structure or amenity or interference with legitimate uses of the environment (Abel, 1998). Pollution also defined as a large set of adverse effects upon water bodies such as lakes, rivers, oceans, and groundwater caused by human activities. Although natural phenomena such as volcanoes, algae blooms, storms and earthquakes also cause major changes in water quality and the ecological status of water, these are not deemed to be pollution. Water is only called polluted when it is not able to be used for what one wants (Pink, 2006). Pollutants in water include a wide spectrum of chemicals, pathogens, and physical chemistry or sensory changes. Many of the chemical substances are toxic. Pathogens can obviously produce waterborne diseases in either human or animal hosts. Alterations of water's quality include acidity, temperature, and eutrophication. Eutrophication is the fertilization of surface water by nutrients that were previously scarce. Even many of the municipal water supplies in developed countries can present health risks. Water pollution is a major problem in the global context. It has been suggested that it is leading worldwide cause of deaths and diseases (Pink, 2006 and West, 2006) and that it accounts for the deaths of more than 14,000 people daily (West, 2006).

Water pollution is one of the environmental and public health problems in Egypt and the Middle East region (Anwar, 2003). In Egypt, pollution is generally associated with heavy industrialization and dense population and is one of the ecological problems of the River Nile system (Ali and Soltan, 1996). The River Nile is the principal freshwater resource for the Egypt, meeting nearly all demands for drinking water, irrigation and industry (Mohamed *et al*., 1998). The pollution of the River Nile resulted from many source, such as accidental spillage and deficiencies in the treatment of chemical wastes, discharge of industrial or sewerage effluents, domestic wastewater, the disposal of untreated sewage and gasoline from fishery boats (Handy, 1994; Ali and Soltan, 1996; Mohamed *et al*., 1998). Furthermore, the agricultural activities have introduced several polluting substances such as organic matter, chemical fertilizer and insecticides into the River Nile and the drainage systems.

2. Sources of water pollution: Some of the principal sources of water pollution are:

*Geology of aquifers from which groundwater is abstracted

*Industrial discharge of chemical wastes and byproducts

*Discharge of poorly-treated or untreated sewage

*Discharge of contaminated and/or heated water used for industrial processes

*Surface runoff containing pesticides or fertilizers or spilled petroleum products

*Acid rain caused by industrial discharge of sulfur dioxide (by burning high-sulfur fossil fuels)

*Excess nutrients added (eutrophication) by runoff containing detergents or fertilizers

*Underground storage tank leakage, leading to soil contamination, thence aquifer contamination

*Inappropriate disposal of various solid wastes and littering on a localized scale.

Fig (1): Showed accumulation of litter and organic debris in Salford Quays, a section of the Manchester Ship Canal in Greater Manchester, UK. (Lord, 1998.)

3.Contaminants

Contaminants may include organic and inorganic substances.

A-Some organic water pollutants are:

- Insecticides and herbicides, a huge range of organohalide and other chemicals.

- Bacteria often from sewage or livestock operations.

- Food processing waste, including pathogens.

- VOCs (Volatile organic compounds) such as industrial solvents from improper storage.

B- Some inorganic water pollutants include:

- Heavy metals including acid mine drainage.

- Acidity caused by industrial discharges (especially sulfur dioxide from power plants).
- Chemical waste as industrial by products.
- Fertilizers in runoff from agriculture including nitrates and phosphates.

4. Type of pollution:

There are many types of pollution which affect both terrestrial and aquatic ecosystems and have negative effects on vertebrate life. The main types being:

A. Heavy Metals

B. Pesticides.

C. Sewage and other effluents.

D. hydrocarbons..

A. Heavy metal pollution:

Heavy metals are the important source of pollution for aquatic habitats. In spite of their natural occurrence in the aquatic ecosystem, heavy metals represent a major problematic environmental issue of increasing concern (Gill *et al.,* 1990; Hunaiti and Soud, 2000) and their monitoring has received worldwide significant attention in the field and under laboratory conditions (Gilli *et al.,* 2000; Almeida *et al.,* 2001; Rashed, 2001; Adham, 2002; Almeida *et al.,* 2002; Pandey *et al.,* 2003; Das *et al.,* 2004 and Abdel- Tawwobe *et al.,* 2007). The presence of heavy metals in different kind of food constitutes serious health hazards, depending on their relative levels. Lead for example causes renal failure and liver damage (Emmerson, 1973). Some other metals cause poor reproductive capacity, hypertension, tumors and hepatic dysfunction (Mansour and Sidky, 2002). The level of heavy metals in the water and sediment of some parts of the River Nile is higher than the tolerance levels or limits set recommended by the Egyptian General Authority for Standards and Quality Control (Anwar, 2003).

B. Pesticides

A variety of chemicals are being introduced into the environment and those used in agriculture can gain access into ponds. Some pesticides are extremely toxic to fish and others are of low toxicity. Most pesticides used today have a low toxicity to fish, and most persistent insecticides have now been banned from use. Many of the currently used insecticides are short-lived, especially when exposed to water and are usually broken down and non-toxic by the time they get into ponds. Problems can, however, occur when someone carelessly sprays a pond while spraying a field, or when heavy rains wash pesticide-loaded silt into a pond immediately following application on a nearby field. Washing out a spray tank and equipment in a pond can also cause fish mortalities. It is difficult to establish, with certainty that a fish mortality was related to chemical use. Pesticides may affect food organisms; they may alter fish reproduction, or they may be added stress, causing decreased resistance to low oxygen levels and diseases (Louis *et al.*, 1996).

Agricultural runoff can carry sediment, nutrients and pesticides to surface waters.
USDA Soil Conservation Service

Types of Pesticides

Pesticides are categorized according to their target use. The three major groups of pesticides are herbicides (weed control), insecticides (insect control) and fungicides (disease control). Nematicides are pesticides used to control soil, leaf, and stem-dwelling nematodes (round worms). An acaricide is a pesticide that controls mites and ticks (Louis *et al*., 1996).

Herbicides

Herbicides are the most commonly used pesticides in the world. They are widely applied to agricultural crops, gardens and lawns. Herbicides often are directly applied to lakes and ponds to control nuisance growths of algae (colonial, filamentous and single cells), submersed water grasses (coontail, milfoil, naiad, pondweed), floating water plants (water lily, spatterdock, water hyacinth, duckweed), and emergent water plants (cattails, rushes, reeds). Herbicides are rarely poisonous to non target organisms. Dense growths of algae and rooted waterweeds can interfere with swimming, fishing, and boating. They also can discolor water impart unpleasant taste and odors to water supplies also degrade property values and water quality. Herbicides generally are less toxic to fish and aquatic life than insecticides. Many are short-lived and do not accumulate in the environment. However, some are highly toxic to aquatic animals and should be avoided or used with extreme caution near water ways and aquatic environments. Of the approximately 200 herbicides registered by the EPA for use in the United States, only about 10 are labeled for use in aquatic systems. The five herbicides most commonly used in ponds and lakes include copper sulfate, fluridone, glyphosate, zx, and diquat as well as copper sulphate.

Insecticides

Types of agricultural insecticides:

1- Organochlorine insecticides (OCs). There were synthetic chemical and were also very toxic to fish and wildlife. e.g DDT (dichlorodiphenyl-trichloroethane), aldrin, toxaphene, dieldrin, mirex and heptachlor.

2- Organophosphates (Ops) and carbamates insecticides (CBs). Many OP and CB insecticides are extremely hazardous to fish and wildlife. OP and CB insecticides are water soluble and metabolized quickly. They generally have short persistence (half-lives of days to months) and their residues do not pose long-term problems for aquatic animals. The CB insecticide carbofuran is extremely toxic to wildlife and fish

3- Biological insecticides (BIs) are highly toxic to aquatic animals, they seldom cause fish kills because: (1) They are strongly absorbed to bottom mud (2) They are short lived and usually last only days (3) They rapidly decompose in 1 to 10 days when exposed to sunlight and (4) They usually are applied at lower rates compared to the other insecticides (Louis *et al*., 1996).

Fungicides

Fungicides, like herbicides, generally are not as highly toxic to fish and aquatic animals as insecticides. However, some fungicides have been banned due to their adverse effects on the environment. Fungicides containing mercury were discontinued for home and agricultural use. Mercurial fungicides accumulated in the environment and concentrated up the food chain, causing fish kills. Some currently-registered fungicides are extremely toxic to aquatic organisms. Some fungicides are poisonous to beneficial soil invertebrates. Their use should be avoided or carefully managed near aquatic systems (Louis *et al*., 1996).

C. Sewage and other effluents:

One of the main problems and threats to vertebrates is nutrient loading which causes eutrophication, caused by sewage discharge, organic waste discharge by industries and run off of fertilizers from agricultural lands. The increase in nutrients such as carbon nitrogen and primarily phosphorous, causes water eutrophication. This increase further causes algal blooms of cyanobacteria, which increase the dead organic matter accumulation, which uses up the oxygen supply. The reduction of oxygen kills the invertebrates and fish which require high oxygenated waters. Some algae are containing neurotoxins which have devastating effects on mortality rates in fish farms. For example, chrysochromolins polylepis. Other examples where the toxic effect of din flagellate had occurred in 1997, in the Mediterranean, where endangered Monk seals have been dying. Dead seals have been found with more than 20 saxitoxins (neurotoxins). Another example was occurred in 1987, where 14 Humpback whales were killed by the similar din flagellate neurotoxins. (Freedman, 1995)

Another disturbing trend caused by effluents is hormone disruption in certain vertebrates. These are caused by certain compounds in the effluents. Hormones are chemical messengers, controlling many functions, such as growth, metabolism, reproduction and the function of many organs. The hormones that are being disrupted the most, are the ones which control sexual characteristics. There are many chemicals which are now occurring in the aquatic environment, which are blocking or mimicking the action of hormones especially the female sex hormones (oestrogen). The presence of these hormone during the development of fish, birds, amphibians and reptiles can alter the sex to show female characteristics, even when the organism is genetically male. The effects of hormone disruption were brought to attention in the 1980s when male roach of the River Lee in North London, showied signs of part feminization. Feminization of fish was occurred directly below sewage and industrial out flow effluents. The feminized fish produce vitellogenin which is used in the production of nutritious yolk for eggs. In many male fish, the level of vitellogenin is similar to that found in female fish. Hormone disruption has been witnessed in other animals. Male alligators and turtles in Lake Toptaka, in Florida, were being born with abnormally small penises and mis-shapen testicles. A number of chemicals have been known to have this oestrogenic effect. Feminisation of fish in the UK waters was probably down to ethinyl oestradiol i.e. the artificial oestrogen in the female contraceptive pill, which is found in most sewage effluent. Although the excreted form is inactive, it can be changed back into an active form by bacteria in the sewage. Other oestrogenic chemical is Phthalates, which is used as a plasticizer (Lord, 1998).

Fig (3): Showed raw sewage and industrial waste flows into the U.S. from Mexico as the New River passes from Mexicali, Baja California to Calexico, California. (Lord, 1998).

D. hydrocarbon pollution:

Oil pollution can be caused by any spillage of crude oil or its refined products. However, the largest and most damaging pollution events usually involve spills of petroleum or heavy bunker fuel from disabled tankers or drilling platforms at sea, barges or ships on major inland waterways or from blow outs of wells or broken pipelines on land. Crud oil is the major energy source for the world industries and it is also the starting product of most synthetic organic product (i.e, plastic). It is mixture of many different organic compounds (Ramamurthy and Sreenivasan, 1983).

Chapter 2:

2. 1. Toxicity of water pollution on fishes

The effects of pollutants on the whole organism are considered under three main headings, namely neurophysiological, behavioral and reproductive effects. These effects can often be inter-related: neurological changes can affect behavior; changes in behavior can affect reproduction and so on. These effects eventually can be either acute or chronic.

1- Acute toxicity occurs rapidly, are clearly defined, often fatal and rarely reversible. Acute tests are designed to evaluate the effects of pesticides or heavy metal on survival following exposures for a short period of their lifespan. Animals used in these tests are normally exposed for 24-, 48-, 72-, or 96 hours in order to estimate acute toxicity

2- Chronic effects develop after long exposure to low doses or long after exposure and may ultimately cause death. A poison is lethal when it causes death, or sufficient to cause it, by direct action, and it is sublethal when the poison is below the level that directly causes death.

3- Chronic toxicity tests evaluate effects over a significant portion ($1/10^{th}$ of lifetime or longer) of the organism's life span. These tests often evaluate sublethal effects on reproduction, growth and behavior as well as mortality. Relative to acute effects, chronic effects may occur following exposures to lower concentrations of the pesticide. This chronic toxicity information is not always readily available because of the considerable expense associated with testing (Chris, 2006).

From the lowest value obtained for the 48h LC50, determined on the basis of a test substance or product is classified into one of the following toxicity categories:

0- Substances of almost no toxicity: substances in which the 48h LC50 is higher than 10 000 mg per litre.

1- Substances of very low toxicity: substances in which the 48h LC50 is between 1 000 and 10 000 mg per litre.

2- Substances of low toxicity: substances in which the 48h LC50 is between 100 and 1 000 mg per litre.

3- Substances of medium toxicity: substances in which the 48h LC50 is between 10 and 100 mg per litre.

4- Substances of high toxicity: substances in which the 48h LC50 is between 1 and 10 mg per litre.

5- Substances of very high toxicity: substances in which the 48h LC50 is between 0.1 to 1 mg per litre.

6- Substances of extreme toxicity: substances in which the 48h LC50 is less than 0.1 mg per litre.

The lethal concentration (LC50) is defined as that concentration which causes death to the half test fish within a specified period of time (Frequently 96-h.)

Pervious work on the toxicity of some pollution for many fish. Sesha and Balaparameswara (1996) found that the 96-h LC50 of *Lebeo rohta* to the chromium was 40 mg/l. Shalaby (1997) showed that acute toxicity (LC50) of copper , cadmium and zinc for common carp (*Cyprinus carpio*) were 3.8, 35 and 43 ppm respectively. Koyama and Kakuno (2004) investigated the toxicityof heavy fuel oil on marine fish (*Pargus major*) and found that the mean lethal concentration of the water accommodated fractions was 325 ug/l. Shalaby *et al.* (2005) found that the 96-h LC50 of Nile tilapia *(Oreochromis niloticus*)to Saturn (thiobencarb)and glyphosate (round up) herbicides were to be 1.725 and 6 ppm respectively. Also, Shalaby *et al.* (2007) and Giron *et al*. (2007) found that the 96-h LC50 of Nile tilapia (*Oreochromis niloticus*) for the butataf (herbicide) and diazinon (organophosphorus pesticides ,OPs) were 3.85 and 7.830ppm respectively.

2.2. Fish are exposure:

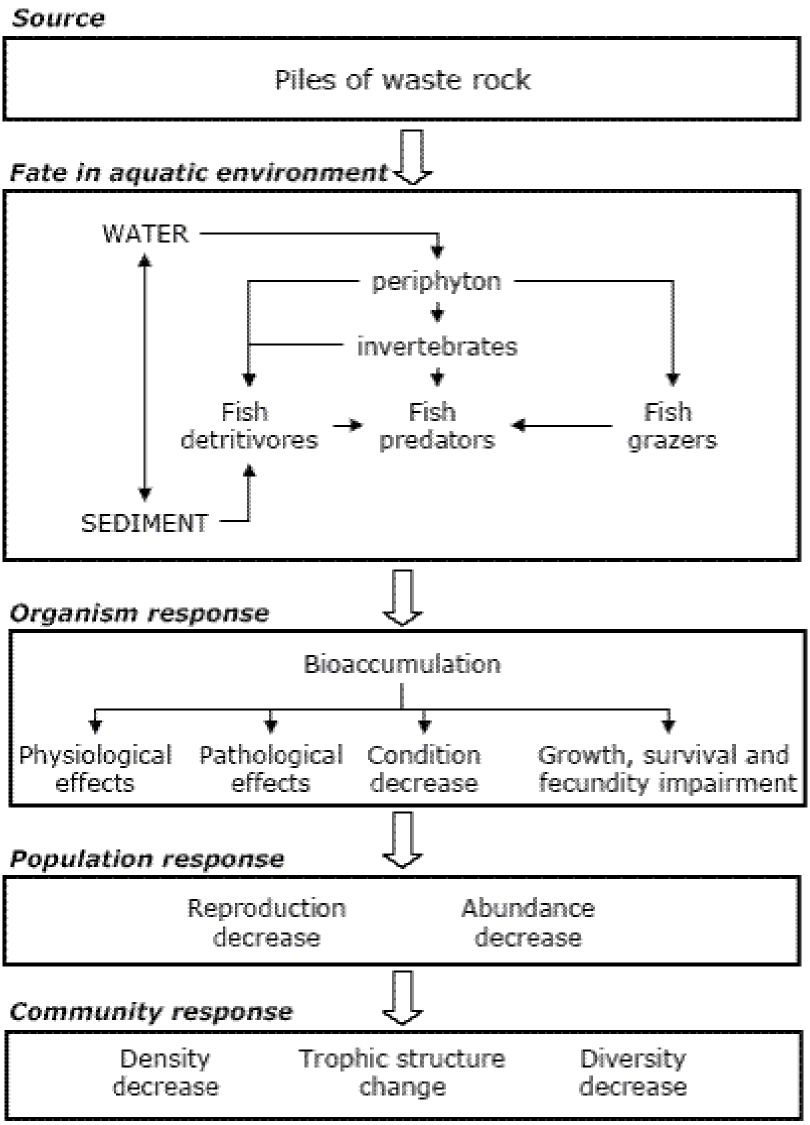
Fish and aquatic animals are exposed to pesticides in three primary ways: (1) Dermally direct absorption through the skin by swimming in pesticide-contaminated waters, (2) Breathing, by direct uptake of pesticides through the gills during respiration and (3) Orally, by drinking pesticide-contaminated water or feeding on pesticide-contaminated prey. Poisoning by consuming another animal that has been poisoned by a pesticide is termed "secondary poisoning" For example, fish feeding on dying insects poisoned by insecticides may themselves be killed if the insects they consume contain large quantities of pesticides or their toxic byproducts.

2.3. Physiological responses to hypoxia in fish:

Hypoxia is defined A deficiency of oxygen reaching the tissues of the body. At the individual level, animals exposed to hypoxic conditions attempt to maintain oxygen delivery to the tissues in the face of reduced levels in the environment. Fish increase gill ventilation and gill diffusing capacity to enhance the transport of oxygen across the gills into the blood. Heart rate is reduced but stroke volume is increased and the changing pattern of blood flow through the gills increases gill diffusing capacity for oxygen (Randall, 1982). Decreased red blood cell phosphate levels result in an increase in haemoglobin oxygen affinity and this also facilitates oxygen uptake at the gills (Val and de Almeida-Val 1995). Blood erythrocyte levels are increased initially due to release from the spleen and then subsequently due to erythropoiesis in response to the hormone, erythropoietin (EPO), produced by the kidney (Kakuta and Randall, unpublished observations). Anaerobic metabolism increases during hypoxia (Randall, 1982). There is an up-regulation of anaerobic enzymes, increased glucose transport and utilization of liver glycogen. The magnitude of the glycogen stores are an important determinant of hypoxic survival. There is a down-regulation of energy expenditure coupled to the up-regulation of anaerobic metabolic pathways. Fish respond to aquatic hypoxia by initially increasing activity in an attempt to leave the area, escaping from hypoxic areas, which are often patchy in nature. Fish decrease swimming activity during prolonged hypoxia and tend to move to colder waters (Schurmann and Steffensen, 1994). This move to colder temperatures reduces energy metabolism and is associated with an increase in the oxygen content of water. Fish exhibit reduced food intake during hypoxia (Zhou *et al.,* 2001) and this also decreases energy expenditure. Zebrafish (*Danio rerio*) stopped feeding about six hours after the onset of hypoxia. Protein synthesis was reduced by 40% during anoxia in carp (Smith *et al.*, 1996 and 1999) the liver showed a much larger reduction (85%) than the muscle (40%). Protein synthesis in the brain was maintained at the normal low rates seen under normoxic conditions.

Fig (5): Showed many species, including fish, are sensitive to low oxygen levels and die as a result. (USDA Forest Service).

Chapter 3: Physiological aspects:



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3.1. Contribution of physiological work to fisheries:

The physiology studies are of great significant with regard to survival, growth and propagation of fish. These include a variety of processes with special reference to respiration, circulation, digestion, excretion, osmo-regulation, coordination, growth, maturation and reproduction. The environmental factors as water quality, flow – characteristics, and biological factors as well as the genetic map, sexes organ, body composition, stage of life cycle, food and feeding habits, metabolism, growth, maturation and behavior of fish alone or in a group of species significantly affect the physiological responses. Water pollution and environmental changes directly and indirectly affect the fish physiology and consequently their survival, growth and reproduction. The physiological response may be characterized as (i) Whole animal responses (ii) Tissue responses and (iii) Cellular responses. The former includes in responses of all systems or a single system/organ expressed by the whole animal whereas the latter concerns physiological responses at the level of the unite of body or the cell, which basically including enzymatic and or hormone related changes. The physiological responses of enzymatic/ hormones can affect the fish drastically in its performance, survival, growth and reproduction (Singh, 2000).

3.2. Fish haematology:

Haematology is defined as the study of blood and the blood forming tissues. Blood is composed of liquid plasma and the blood cell (erythrocyte and leukocyte, some times called formed elements). Serum is the fluid left after blood has clotted it has essentially the same chemical makeup as plasma, except for the absence of some of the clotting factors. Many works included the chemistry of plasma as part of haematology (Folmar, 1993). Blood volume represents about of 2-4 % of body weight, compared with the value of 5-8 % for other vertebrate. Blood parameters may also be affected by other victors such as age sex, diet species, and time of year, water temperature and osmotic disruption. A haematology profile measures the numbers of red and white blood cells together with their physical characteristics. Table (1) gives the main parameters measured together with the type of health information they can provide.

Table (1): showed commonly measured haematology parameters and an outline of their clinical significance from Bossart and Dierauf (1990).

PARAMETER	CLINICAL SIGNIFICANCE
Total red blood cell count (RBC)	Decreased in anaemia and poor nutrition Increased in dehydration
Haematocrit or packed cell volume (PCV)	Decreased in anaemia and poor nutrition Increased in dehydration
Haemoglobin concentration (Hb)	Decreased in anaemia and malabsorbtion
Mean corpuscular volume (MCV)	May increase or decrease in different types of anaemia
Mean corpuscular haemoglobin concentration (MCHC)	May increase or decrease in different types of anaemia
Blood smear	To identify if immature or abnormal RBCs are present as found in different types of anaemia
Total white blood cell count (WBC)	Elevated by stress and bacterial infection
Neutrophils	Elevated by stress and bacterial infection Decreased by overwhelming bacterial infections and toxaemia
Lymphocytes	Increased in viral infections Decreased by stress
Monocytes	Increased in chronic inflammatory conditions
Eosinophils	Increased in parasite infections Decreased by stress
Basophils	Rarely seen, significance unclear
Bands or immature neutrophils	Increased in infections

3.2.1. Fish blood cell and their measurement:

Blood cells:

The blood cells of fish are produced in the hematopoietic tissue which located primarily in the spleen and kidney (Fange, 1992). In contrast to mammals, there is no bone marrow in fish nor do they lymph nodes blood is the most accessible of teleostean fluid system. Consequently, blood variables are commonly used as direct indicators of the functional state of animal. Stressors and pollutants generally produce relative rapid changes in blood characteristics of fish (Ahmed *et al* ., 1992 and Shalaby, 1997). So the use of haematological parameters as indicators of sublethal effects of a stress can provide information on the physiological response of fish resulting from changes of external and internal environment.

Haematological parameters

Erythrocyte count (Red blood cells, RBCs): RBCs of fish are ovulated with a flattened ellipsoid shape and are similar in size with leucocyte and in contrast to mammals they not contain nuclei. The cells are usually of about 11x9 um in size. The erythrocytes contain heamoglobin which enables the blood to hold a far greater amount of oxygen.

Leucocyte: these cells can form up to 10 % of blood cell population with lymphocyte by far the most abundant. Leukocyte can be recognized into: monocyte , thrombocyte, eosinophils and basophils.

Haematocrite value (Hv) is present the ratio of RBCs volume to total blood volume.

Haemoglobin concentration (Hb) :whole blood is analyzed after the cells have been hemolyzed releasing haemoaglobin into solution.

3.3. Effect of chemical contamination on haematological alternation:

The value of the classical blood variables (erythrocyte count, hemoglobin, hematocrit) and their derivatives (mean erythrocytic volume, and mean erythrocytic hemoglobin content) as indicators of fish health is assessed from the viewpoints of measurement precision and the distortion of information content by sampling procedures and storage conditions. So also is the concept of hematological norms and their validity in relation to natural fish populations. Few of the assumptions underlying the use of haematological data for health assessment purposes can be regarded as other than marginally valid. Of the primary indices, erythrocyte numbers and hemoglobin, if corrected for nonviable cells and accompanied by information on isomorph abundances, appear to be the more useful indicators of O_2 carrying capacity. Hematocrit and hematocrit-dependent Wintrobe (1978) indices emerge as suspect. Higher-order indices describing red cell anisocytosis and yielding an "erythron profile" provide additional and more sensitive means for assessing blood status (Houston, 1997). Exposure the fish to chemical pollutants or environmental hypoxia can induce either increase or decrease in the different heamatological measures; the trend is almost the same in all three traditional measures that produce anemia and then consider the opposite physiological response.

(i) Effect of heavy metals on haematological parameter.

Heavy metals due to their bio – accumulative and non-biodegradable properties constitute a core group of aquatic pollutants. Chromium, particularly enters the aquatic media through effluents discharged from tanneries, textile, electroplating, metal finishing, mining, dyeing, printing, ceramic, photographic and pharmaceutical industries etc, changes haematology of fishes in response to stress agents or indicator of stress. The reduction of blood parameters (erythrocyte count , Hb concentration and Hct values) in tilapia exposed to sublethal levels of mercury, cadmium and chromium might be due to the destruction of mature RBCs and the inhibition of erythrocyte production due to reduction of haemtosynthesis that affected by pollution (Shalaby, 2001, 2007 and Shalaby *et al .,* 2006). Zaghlou *et al*. (2007) reported that freshwater catfish exposed to sublethal levels of nitrite for 30 days had shown a significant decrease in RBCs, WBCs, Hb and Hct. The reduction of these parameters in Nile tilapia, *O. niloticus* at sublethal levels of cadmium might be due to the destruction of mature RBCs and the inhibition of erythrocyte production due to the reduction of haemosynthesis that affected by pollutants (Wintrobe, 1978). Also, cadmium has been reported to cause anemia of common carp (*Cyprinus carpio*) and Nile tilapia (*O. niloticus)* at low and high concentration (Shalaby, 1997 and Shalaby , 2007). The mechanism involved in both fish is reported largely due to a reduction in absorption of iron from the gut for synthesis of haemoglobin, or may be cause an abnormality of large number of malformed erythrocyte (Houston *et al* .,1993). Chronic exposure of the cyprinid to lead (Pb) for up to 60 at 47 ug/l results in sever reduction (12 to 31%) in RBCS, Hb and Hv and MCV (Tewari *et al.*, 1987). Lead has shown to cause anemia in mammals by inhibiting of Hb synthesis and shorting the lifespan of circulating erythrocyte (Hermberg, 1976). Lead also inhibits the enzymes ALA-D which is required in the early stages of Hb synthesis in haemopoietic tissue.

(ii) **Effect of pesticides on haematological parameter.**

Any changes in haematology of fish in response to stress agents are indicator of this stress. The organophosphorus pesticides or any organic phosphate compounds lowered the affinity of blood to the O_2 because of the combination of the hemoglobin with these organophosphates causing hypoxia (Chanutin and Cornish, 1967 and Benssch *et al*., 1968). The erythrocyte count, Hb concentration and Hv values in tilapia exposed to Saturn or gyphosate were reduced than control resulting hypochromic anemia (Sancho *et al.*, 2000, Shalaby et *al.*, 2005). The decreas in the RBCs, Hb and Hct in tilapia may be due to the elimination of the RBCs from circulation as results of butataf induce extravasations of blood (Mousa, 2004 and Shalaby *et al.*, 2007). Also , the changes in heamatological parameters (RBCs, Hb and Hct) of the common carp were decreased significantly after exposure to hinosan and sevin pecticides (Shalaby and Rezkalla, 2000).

(iii**) Effect of crud oil on haematological parameter.**

Studies have shown that when the water quality is affected by toxicants, any physiological changes will be reflected in the values of one or more of the haematological parameters (Van Vuren, 1986). Thus, water quality is one of the major factors, responsible for individual variations in fish haematology. The decrease in MCV after short-term exposure to crud oil coupled with low haemoglobin content indicates that the red blood cells have shrunk, either due to hypoxia or microcytic anaemia. At this stage, microcytosis may be due to the decrease in the haematocrit during exposure. Changes in blood parameter of *O. niloticus* and *Spaus aureatus* exposed to sublethal dose of crud oil may be due to direct erythrocyte injuries which is the first and most important sign in poisoning of fish (Lynda, 2005). Most studies reported sever reduction in erythrocyte, haemolglobin and haematocrit in fish after chronic exposure to organic and inorganic toxicant.

(iv) Pulp mile effluent (cause anemia).

Long term exposure (25 days) to the effluent from pulp mills has been found to cause anemia in salmon (McLeay, 1973). The composition of this effluent is extremely variable between different mills and various times at a single one (Davis, 1976). There are several toxic components in the effluent; one of whish is dehydroabietic acid, one of the naturally occurring resin acid extracted from softwood tree.

3.4. Biochemical indicator.

Biochemical and physiological indicators such as enzymes, could be used (as biomarkers) to identify possible environmental contaminations before the health of aquatic organisms is seriously affected (Barnhoorn and van Vuren, 2004). Such a biochemical approach has been advocated to provide an early warning of potentially damaging changes in stressed fish (Casillas *et al.*, 1983). In toxicological studies of acute exposure, changes in concentrations and activities of some enzymes may reflect cell damage in specific organs (Heath, 1996).

Of all the blood chemistry measurements, the plasma enzymes have the greatest variability. Much of that variability is directly related to the method of analysis (manual vs, automated) the assay condition and the skill of the person conducting the assays. There is also substantial variability, particularly in the older literature, in the unite used to report enzyme activity. In this review, only that publication with the results reported in international Units per liter (IU/L) were considered. Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Acid phosphatase (ACP) and Lactate dehydrogenase (LDH)

Lead enters aquatic systems from urban, mining and agricultural runoff, atmospheric precipitation, plating process, the use of phosphate fertilizers and gasoline containing lead that leaks from fishery boats and a variety of natural sources, including erosion and volcanic emissions (Handy, 1994). Recent studies reported high lead contamination in Egypt in the range of 2000 mg/l in soil and 400 mg/l in river sediments (Elsokkary *et al.*, 1995), and in food such as fish, spices and milk products at mean concentrations above the permissible limits proposed by FAO (Dogheim *et al.*, 2004).

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes are frequently used to diagnose the sublethal damage to certain organs specially the liver (Benedeczky *et al.*, 1984). Mukhopadhyay, *et al.*, (1982) reported that there is an intimate relation between serum transaminases levels and liver integrity and mentioned that AST and ALT levels increased due to releasing from the damage liver cells due to the effect of any pollution. Shalaby (1997, 2001 and 2007) showed that AST , ALT and ACP activity increased significantly in plasma of Nile tilapia exposed to sub lethal dose of Cd. The activity of AST and ALT enzymes in blood may also be used as a stress indicator. The significant changes in activities of these enzymes in blood plasma indicate tissue impairment caused by stress (Svoboda, 2001). In addition, the increase of plasma AST and ALT may be attributed to the hepatocellular damage or cellular degradation by this heavy metal, perhaps in liver, heart or muscle (Yamawaki *et al.,* 1986).

The decrease in AST enzyme activity in serum and the liver in all concentration of saturn and glyphosate treated fish might be due to the decrease in the membrane permeability or the inhibition of the enzymes synthesis following the complete damage in the liver cells as previously mentioned by Mousa (1996 & 2004) and Shalaby (2000) and Shalaby *et al*. (2005). Also, Shalaby *et al.* (2007) found similar results in plasma AST and ALT of Nile tilapia after exposure to butataf herbicide for 30 days. This indicates a great disturbance in the liver function due to the effect of the toxicant.

On the other hand, the ALT activities in serum, liver and muscle increased during the time course of endogenous cortisol elevation induced by saturn , glyphosate and carbofuran pesticides (Shalaby *et al* ., 2005 and Marzouk *et al.,* 2006).

The cytoplasmic enzyme LDH is widely used as marker of organ or tissue lesions in toxicology and in clinical chemistry. It has been used for demonstrating tissue damage in fish (Das *et al.,* 2004). In most cases of tissue damage, whether due to a disease or a toxic compound, the activity of LDH was reported to be significantly affected (Singh and Sharma, 1998). LDH is a source of the oxidized coenzyme during the period of transient anaerobiosis or a reduced form of such coenzyme during aerobiosis (Coppes, 1992). Therefore, LDH has been also used as indicative of hypoxic conditions in the organism (Das *et al.*, 2004). Different metals (Pb, Zn, Hg) seem to influence the activity of LDH by reduction (Gill *et al.*, 1990, Salah- El-Deen *et al*., 2000, Osman, *et al.*, 2007). G6PDH with LDH play an important role during glycolysis and they have a direct effect on the development of fish (Shaklee *et al.*, 1974). Otherwise, Chandrasekara and Pathiratne (2007) indicate that brain AChE of Nile tilapia is a promising biomarker for assessment of anticholinesterase pesticide contaminations in water. However, acetylcholinesterase was decrease significantly in Nile tilapia after exposure to chlorpyrifos and carbosulfan for 14 days. Glucose-6-phosphate dehydrogenase (G6PDH), lactate dehydrogenase (LDH) and pyruvate kinase (PK) are used as a biomarker of pollution-induced carcinogenesis in fish. The (G6PDH) activity increased and LDH activity decreased with increasing of lead concentration (Osman *et al.*, 2007) in as indicated in tables (2 &3) due to the effect of lead nitrate.

Table Effect of different concentrations of lead nitrate on the activity of G6PDH (mean ± SE) during early developmental stages of the African catfish *Clarias gariepinus*. Values are in units per gramme protein

Embryonic stages*	Control	100 μg/l	300 μg/l	500 μg/l
30 h-PFS (pre-hatching)	189.2 ± 5.0 (158.1–195.0) a (A)	227.2 ± 6.8 (221.5–234.7) a (B)	149.6 ± 3.2 (145.4–152.0) a (C)	133.0 ± 14.4 (118.9–145.4) ab (C)
48 h-PFS**	81.8 ± 1.7 (79.3–82.6) b (A)	95.9 ± 11.5 (85.9–105.7) b (B)	104.1 ± 1.9 (102.4–105.7) b (BC)	109.9 ± 5.6 (102.4–115.7) a (C)
96 h-PFS	34.7 ± 1.9 (33.0–36.4) c (A)	55.4 ± 1.6 (52.8–56.2) c (AB)	67.8 ± 14.4 (59.5–89.2) c (B)	106.6 ± 23.3 (76.0–132.2) a (C)
144 h-PFS	37.2 ± 3.2 (33.0–39.7) cd (A)	52.9 ± 4.7 (46.2–56.2) c (AB)	69.4 ± 2.7 (66.1–72.7) c (B)	147.9 ± 18.2 (132.2–165.3) b (C)
168 h-PFS	47.9 ± 11.0 (33.0–56.2) d (A)	61.1 ± 9.5 (52.9–69.4) c (A)	104.1±7.9 (95.9–112.4) b (B)	162.8±1.7 (161.9–165.3) b (C)

* Embryonic stages showing similar lower case letters are insignificant within the treatment at 0.05 levels (vertical comparison). Treatments showing similar capital letters are insignificant within the embryonic stages at 0.05 levels (horizontal comparison)

** The hatching process started 40 h after fertilisation

Table Effect of different concentrations of lead nitrate on the activity of LDH (mean ± SE) during early developmental stages of the African catfish *Clarias gariepinus*. Values are in units per gramme protein

Embryonic stages*	Control	100 μg/l	300 μg/l	500 μg/l
30 h-PFS (pre-hatching)	229.7 ± 19.2 (211.5–247.9) a (A)	295.0 ± 40.7 (234.7–323.9) a (B)	242.1 ± 7.3 (234.7–251.2) a (A)	244.6 ± 14.0 (228.1–257.8) a (A)
48 h-PFS**	129.7 ± 4.9 (125.6–135.5) b (A)	114.9 ± 14.6 (99.2–129.9) b (AB)	95.8 ± 2.7 (92.5–99.2) b (BC)	90.1 ± 11.9 (72.7–99.2) b (C)
96 h-PFS	44.6 ± 3.3 (42.9–49.6) c (A)	41.3± 1.9 (39.7–42.9) c (AB)	36.4 ± 2.7 (33.1–39.7) c (B)	35.5 ± 3.1 (33.1–39.1) c (B)
144 h-PFS	90.1 ± 7.8 (79.3–95.8) d (A)	65.3 ± 3.2 (62.8–69.4) c (B)	62.0 ± 3.2 (59.5–66.1) d (BC)	47.1 ± 12.8 (33.1–59.5) c (C)
168 h-PFS	166.1 ± 28.9 (132.2–191.7) e (A)	122.3 ± 9.7 (112.2–135.5) b (B)	109.1 ± 14.0 (95.9–128.9) b (BC)	81.8 ± 12.5 (72.7–99.2) b (C)

* Embryonic stages showing similar lower case letters are insignificant within the treatment at 0.05 levels (vertical comparison). Treatments showing similar capital letters are insignificant within the embryonic stages at 0.05 levels (horizontal comparison)

** The hatching process started 40 h after fertilisation

Table (4): The most commonly measured blood chemistry parameters and an indication of their clinical significance Cornell (1983).

PARAMETER	CLINICAL SIGNIFICANCE
Liver associated enzymes	
Aspartate aminotransferase (AST)	Elevations in liver and muscle damage
Alanine amino transferase (ALT)	Elevations in liver and muscle damage
Gamma glutamyl transpeptidase (GGT)	Elevation in liver diseases
Alkaline phosphatase (ALP)	Elevation in liver diseases and during normal growth
Bilirubin:	
Direct	Elevations in obstructive liver disease
Indirect	Elevation in haemolysis and pre-hepatic disease
Creatine kinase (CK)	Elevated with cardiac or skeletal muscle damage - e.g. handling or transport
Blood urea nitrogen (BUN)	Elevated with kidney disease, may be reduced with liver damage
Creatinine	Elevated in kidney disease
Lactic dehydrogenase (LDH)	Increased in general cell damage
Glucose	Increased in stress and diabetes mellitus
Triglyceride	Deceased with prolonged fasting
Cholesterol	Increased in hypothyroidism and diabetes mellitus, decreased with malabsorbtion and starvation
Total Protein	Increased in dehydration, decreased in starvation and malabsorbtion
Albumin	Increased in dehydration, decreased in malnutrition and liver or kidney disease
Globulin	Increased in some immune system diseases
Electrolytes: Sodium, Potassium, Chloride, Phosphorus	Electrolyte levels give an indication of the operation of a variety of homeostatic mechanisms in the body.

3.5. The metabolic indicators:

Hypersecretion of adrenalin and cortisol are considered primary stress responses. These effects trigger a broad suite of biochemical and physiological alterations called secondary stress responses. Metabolic effects include hyperglycemia, depletion of tissue glycogen reserves, catabolism of muscle protein, and altered blood levels of protein, cholesterol and free fatty acids (Wendelaar Bonga, 1997).

The influence of toxicant on total protein content of fish has also been taken into consideration in evaluating response of the animal against stressors . The quantitative determination of the total protein in plasma, muscle and liver reflects the liver capacity of protein synthesis and denotes the osmolarity of the blood and the renal impairments. So, it is of a valuable factor in the diagnosis of toxicity in fish. Most studies reported significantly decreased in total plasma, muscle and liver protein and the effect was dose dependent. This decrease might have been attributed to several pathological processes including plasma dissolution, renal damage and protein elimination in the urine, a decrease in liver protein synthesis, alteration in hepatic blood flow and/ or hemorrhage into the peritoneal cavity and intestine (Mousa & Khattab, 2003 and Shalaby, 2004). The decrease in plasma and tissue protein may occurred due to the increase of protein breakdown as a result of stress stimulated corticosteroid hormones to supply amino acids for gluconeogenesis to provide glucose to compensate the increase of energy demands under stressful conditions. Also Joseph (2007) showed that the plasma protein in teleost, *Mystus vittatus,* was highly decreased significantly after chronic exposure to metasystox and sevin pesticides.

The blood glucose measurements are known to be sensitive indicator of environmental stress in fish. The blood glucose of several species of normal unstressed fish falls in the range of 20- 80 mg/ 100 ml however under severe stress a transient (temporary) elevation of up to

300-400 mg/l was observed (Thomas *et al*., 1981). Saturn, Glyphosate, Endosulfan pesticide induced hyperglycemia in Nile tilapia and common carp exposed to sublethal dose of pesticides (Chandrasekar & Jayabalan, 1993 and Shalaby *et al.*, 2005). This increase may be due to the decrease in serum insulin required to control the glucose level either through the reduction in its rate of production or the effectiveness of the available insulin which in turn could be alter the rate and degree of digestion, absorption and utilization of glucose due to the exposure to the pesticides . The rapid rise of glucose results from glycogenolysis (release of glycogen reserves in muscle and liver) initiated by catecholamines, while sustained elevation of serum glucose are maintained by cortisol stimulating gluconeogenesis (protein catabolism). Hyperglycemia associated with lowered lipids and proteins in *Prochilodus lineatus* fish was noticed after exposure to sub lethal lead concentration (Martinez *et al.,* 2004). Also, Sweilum (2006) found that the glycogen, protein and lipid in muscle of Nile tilapia gradually decreased with the increased dimethoate or malathion pesticide concentrations.

Total lipids in plasma increased significantly in fish exposed to Cadmium (Shalaby, 2007). The same author (2001) reported that the absorption of excess heavy metals disturbed the metabolism of lipid.

Chapter 4: Fish reproduction:

4.i. Introduction:

Due to increasing pollution, concentrations of various chemical compounds have increased in aquatic environment. The toxic effects of pollutants may affect fish behavior, cellular metabolism, endocrine regulations and reproduction of aquatic organisms (Rurangwa *et al*., 1998). Fish reproduction is sensitive to environmental pollutants via modulation of endocrine function. Recent field and laboratory studies showed that certain compounds mimic estradiol, producing unexpected effects in feral and laboratory fishes (Jobling & Sumpter 1993 and

Ashfield, *et al*., 1988). In several studies, male fish exposed to sewage effluent or estrogenic pesticides produced the yolk-precursor protein, vitellogenin (Denison et *al.,* 1981 and White *et al.,* 1994). In addition, fishes living below effluent from paper mills processing pine trees were shown to have modulated endocrine function [Van der Kraak *et al.,* 1992 and LeBlanc *et al.,* 1997). Wu et al. (2003) reported negative effects of hypoxia on reproduction of the common carp (*Cyprinus carpio*). They found that gonad development was reduced when fish were exposed to hypoxia for 8 weeks. There was a significant reduction in the number of spermatocytes and spermatids, lowered incidents of mitosis, decreased lobular diameter of testes and reduced sperm motility in male carp.

4.ii. Fish reproduce:

In most fish, spawning (egg laying) and egg fertilization are external, completed in the water. Male and female fish simultaneously release sperm (milt) and eggs (roe) into the water where fertilization occurs. Some species scatter millions of eggs in the water where they are left unattended, whereas others lay only a few eggs and provide close parental care. Male sunfish and catfish, for example, aggressively defend their nest and young from predators and intruders. Fish lay their eggs in many places, including in elaborate floating bubble nests, in bank hollows, and in clean bottom gravel. Others build spawning mounds of gravel or attach sticky eggs to aquatic plants, while others lay eggs in underwater caves and cavities. Some fish are live-bearers. Reproduction is internal and embryos develop within the female fish and the young save. The young are born fully formed (Louis and Richard 2003).

The current article demonstrates the effects of these environmental cues on 1) gonadal morphology during periods of sexual activity and inactivity, 2) fecundity, and 3) fertility.

4. iii Action of pollution on reproductive processes:

Disrupted reproduction may result in limited propagation of species and eventually can lead to their extinction.

A. Endocrine disruption:

Environmental endocrine disrupter is defined as an external compound that interferes with or mimics natural hormones that are responsible for the maintenance, reproduction, development, and/or behavior of an organism (U.S. Environmental Protection Agency, 1997). Hypotheses about which chemicals may be endocrine disrupters, about the mechanisms through which they operate, and about which animals may be affected have been discussed in numerous publications (Davis *et al.*, 1992). However, few regional or national studies related to fish populations have been conducted to test these hypotheses.

The National Water Quality Assessment (NWQA) program provided background information on contaminant levels at many of the study sites and cooperated in both the field collection and synthesis of the data. NWQA studies of endocrine disruption have focused on three goals:

1. To determine if endocrine disruption is occurring in fish and, if so, is the phenomenon widespread in the Nation's rivers and streams.
2. To evaluate the relation between endocrine disruption and concentrations of chemical contaminants in water and sediments.
3. To provide information that may guide future monitoring and research related to endocrine disruption in aquatic biota.

The current study was aimed to evaluate the effects of heavy metals and 4- nonylphenol on selected aspects of reproduction and sex differentiation in fish.

A serious threat towards reproduction is posed by heavy metal compounds (mainly heavy metal salts), which are reabsorbed by organisms living in contaminated environments. Heavy metals are known to be cumulated in fish tissues, reaching concentrations up to 20000 fold higher than in surrounding water environment. It has been reported that heavy metals affect both quality and quantity of the gametes as well as the endocrine system, disrupting the gametogenesis (Jezierska and Witeska 2001). Mechanisms of these harmful effects depend on the type of metal and the degree of environmental pollution. For example, it is known that lead's toxicity effects are linked with the disturbance of LH secretion (Buettner, 1993). Cadmium disrupts reproductive function in female fish by reducing the weight of ovaries and arresting their development (Szczerbik *et al.,* 2005). Although zinc and copper are essential for fish at physiological concentrations, these heavy metals may become dangerous at higher concentrations. Disruption of reproductive function by zinc has been described at different stages of fish development (O'Dell BL 1992, Popek *et al.*, 1997and Popek *et al.*, 2003) and it has been shown that copper is detrimental to the development of fish ovaries. The toxic effects of copper can be neutralized by metallothionins (Roch *et al.,* 1982). Action of heavy metals such as mercury and cadmium on spermatozoa may include disturbances in trajectory of sperm movement, inhibition of the enzymes or fragmentation of DNA (Dietrich et *al.,* 2004 & 2005 and Jezierska &Witeska, 2001). As a result of these disturbances, fertilization rate and hatchability are often impaired (Dietrich, *et al*., 2004 and Rurangwa *et al*., 1998).

Natural sexual steroid hormones play a major role in controlling sex differentiation and reproduction in fish. Additionally, physiological concentrations of these hormones are essential for the maintenance of cell proliferation, growth and several other biological activities. Imbalance in hormonal status caused by higher level of endogenous hormones as well as by exposure to exogenous steroid-like compounds is known to produce adverse effects on reproductive processes (Maguire, 1999).

B. Estrogenic pesticides:

Some pesticides act as *endocrine disrupters*, which mean that they interfere with the action of hormones in fishes. Endocrine disrupters can promote development of hormone-responsive tumors, and can interfere with sexual development in fry fish, for examples:

* The insecticide methoxychlor is used on some fruits and vegetables. it is converted within the bodies of birds and mammals to a compound that behaves like the female sex hormone, estrogen.

* The herbicide Roundup is often used in gardens and around homes. A laboratory study has found that Roundup disrupts the production of sex hormones in cells taken from testicular tumors in mice (Denison *et al*., 1981).

Herbicides, insecticides, fungicides, and “Weed and Feed”-type products are all examples of pesticides. Scientific studies link exposure to common lawn care pesticides with an increased risk of several types of cancer, neurological and respiratory diseases, endocrine disruption and birth defects

In several studies, male fish exposed to sewage effluent or estrogenic pesticides produced the yolk-precursor protein, vitellogenin (Denison *et al*., 1981 and White *et al*., 1994)]. Also, fish living below effluent from paper mills processing pine trees were shown to have modulated endocrine function (LeBlanc *et al*., 1997)

Chemicals known to disrupt the endocrine system and act as estrogen-like hormones include: DDT and its degradation products, DEHP (di(2- ethylhexyl)phthalate), dicofol, HCB (hexachloro benzene) kelthane, depone, lindane and other hexachlorocyclohexane congeners, methoxychlor, octachlorostyrene, synthetic pyrethroids, triazine herbicides, EBDC fungicides, certain PCB congeners, 2,3,7,8,-TCDD and other dioxins, 2,3,7,8-TCDF and other furans, cadmium, lead, mercury, tributyltin and other organo-tin compounds, alkyl phenols (non-biodegradable detergents and anti-oxidants present in modified polystyrene and PVCs), styrene dimers and trimers, soy products, and laboratory animal and pet food products (Table 5).

Table 5. Synthetic chemicals that may cause endocrine disruption in wildlife (Smith *et al.*,1997).

POTENTIAL DENDOCRINE DISRUPTERS	
CHEMICAL GROUPS	SPECIFIC CHEMICALS
• Herbicides	atrazine,2,4-D
• Fungicides	Vinclozolin
• Insecticides	DDTs, carbaryl
• Nematocides	Aldicaryl
• Industrial Chemicals	Phenols, PCBs

C. Effect of chemical contamination on fish hormones:

Hormones have been included in this article, as they are measurable in blood and the circulating levels of those hormones can be altered by exposure to xenobiotic chemicals. Most of the information in reference values the effects of xenobiotic chemical. Those values have been collected only during the last 15 years. Measurement of hormones levels can provide additional information on the sublethal effects of many chemicals on fish growth and reproduction. Although significant changes (either increase or decrease) in circulating hormone level may be observed, definitive statement will require additional up take, binding, and clearance studies to identify the cause of the observed changes. In laboratory studies in which both rainbow trout and coho salmon were fed mirex and poly chlorinated bipenyls (PCBs), serum levels of both thyroxine (T4) and Triiodothyronine (T3) were significantly reduced in fish 500 ug mirex /g food (Leatherland and Sonstegrad, 1978). When mature male and female Nile tilapia exposed to sublethal dose of butataf herbcides (0.02- 0.002 mg/l) for 30 days, plasma hormones level (T3, T4 and testosterone) was decreased significantly (Shalaby *et al*., 2007). While the level of estradiol increased significantly (Shalaby *et al*., 2007).These results may be due to that butataf made as estrogenic hormones, where the butataf binds to estrogen receptors and exhibits estrogenic activity (Stephen, 2001), or by direct exposure to sertoli cells resulting in decreased 3- hydroxyl-steroid – dehydrogenase activity that change estroidal to androgen raising estradiol level (Colborn *et al*., 1993 and El-Kashoury *et al*., 2005) reported that pesticides increased deiodinatio and biliary excretion of thyroid hormones which increased the rate of T3 and T4 elimination from the blood.. Moreover, the significant elevation of estradiol in male *O. niloticus* which received high dose of butachlor could be attributed to increase the incidence of hypertrophy and / or vacuolation (empty cavities) of adrenal cortex that enhanced the steroidogenic activity (Pandian and Sheela, 1995). Ankley *et a*l.(2005) found that 17*b*-estradiol in male and female fathead minnows (*Pimephales promelas*) after exposure to fenarimol fungicide.

Wu *et al.* (2003) showed that the serum levels of testosterone, estradiol, and triiodothyronine significantly decreased in common carp (*Cyprinus carpio)* upon chronic exposure to hypoxia. These hormonal changes were associated with retarded gonadal development in both male and female carp, reduced spawning success, sperm motility, fertilization success, hatching rate, and larval survival, indicating that the adverse effects of hypoxia on reproductive performance resulted from endocrine disruption.

D. Effect of pollutants on fish fecundity and gonado-somatic index:

In laboratory studies in which *O aureus* was exposed to chromium, the fecundity and gonado-somatic index decreased significantly. They trend with Al- Hamood *et al*. (1998) found that fertility was reduced in female off spring mice exposed to either trivalent or hexvalent chromium compound, the body weight and weight of ovaries were reduced after exposure to Cr. Fish exposed to butataf sublethal concentrations, exhibited an apparent decrease in gonad weight (ovaries and tests) size resulted in a significant decrease in the gonado-somatic index (G.S.I) and fecundity (Shalaby *et al.,* 2006). Also, Makynen *et al*. (2000) and Ankley *et a*l. (2005) found that the fecundity and gonado- stomatic index were decreased significantly in female fathead minnows (*Pimephales promelas*) after exposure to prochloraz, fenarimol and vinclozolin fungicide. Also, copper sulphate algacides caused highly significantly decrease fecundity, estradiol and testosterone in catfish, *Clarias gariepinus* (Nashwa, 2007).

E. Effect of pollutants on fish sperm:

Mercury and cadmium ions significantly affected sperm motility parameters. Total inhibition of sperm motility by Hg2+ and Cd2+ (at concentrations of 100 mg/l) was observed at all exposure times (Dietrich *et al*., 2004, 2005). Both mercury and cadmium decreased significantly the percentages of sperm and sperm velocities, and both parameters were at dose and time dependent. These results suggest that spermatozoa of sturgeon and salmonid fishes have different sensitivity to negative impact of heavy metals (Popek *et al.*, 2006). Also Shalaby *et al.* (2006) showed a decrease of sperm number and gonado- somatic index in *O aureus* due to chromium toxicity.

A few studies demonstrated that chronic exposure to aqueous solution of 4-nonylphenol (NP) increased significantly the number of female and/or inter sex fish produced (Gray & Metcalfe 1997 and Tanaka & Grizzle 2002). These studies indicated that there are species-specific differences in sensitivity of sperm to adverse effects of heavy metals on sperm fertilizing capacity. Parameters of CASA may be successfully used as predictors of the hatching success. The oral administration of NP resulted in decreases in milt quality and hatching success (Demska-Zakęś and Zakes 1995). The negative impact of (NP)l on milt quality was observed in all treated groups and was characterized by decreased spermatozoa concentration and increased level of DNA fragmentation. The data on the effect of alkylphenols on milt quality are scarce (Demska-Zakęś and Zakes 1995), but corresponding morphological effects including inhibition of testicular growth and spermatogenesis, and pathological changes of germ and Sertoli cells (e.g. fibrosis, vacuolation and atrophy) were reported (Kinnberg *et al.,* 2000 and Weber *et al.,* 2002).

Oral application of NP disrupted reproduction in pikeperch. In juvenile fish, a decrease in the percentage of males and an increase of inter-sex fish was observed in relation to dose of NP and its exposure time. Exposure of adult males to the NP led to the reduction in fecundity, milt quality and fertility (Popek *et al.*, 2006).

Heavy metals added to the diets accumulate in brain tissue of carp, and this accumulation results in inhibition of the secretion of noradrenaline and stimulation of the secretion of dopamine in the hypothalamus ((Popek *et al.,* 2006), these processes results in a disturbance of hormonal equilibrium of the hypothalamo-pituitary system, which can unfavorably influence the efficiency of artificial spawning in fish.

F. The effect of Pollution on fish morphological and histological malformations during fish embryogenesis:

The biotic integrity of an ecological system is reflected by the health of its fauna (Adams *et al.*, 1993) and in aquatic ecosystems, fish are regarded as valuable indicators of environmental pollution. Heavy metals have been a concern for human health and still represent a problem in some countries (Abdel-Nasser *et al.*, 1996). Lead accumulates chiefly in fish bone, scales, gills, kidney, intestine, liver, muscles and to a lesser extent in the brain (Allen, 1995; Chan, 1995 and Vigh *et al.*, 1996) and tissue accumulation depends upon exposure concentration and duration (Canli and Kalay, 1998). Exposure of unfertilized egg was reported to impair fertilization process and exposure of fertilized egg effects early cleavage patterns and hatching time (von Westernhagen, 1988). Generally, larvae display a vast array of gross and minor morphological aberrations after exposure to toxic substances (Jezierska *et al.*, 2000 and Hallare *et al.*, 2005). Morphological aberrations are not pollutant specific and may be caused also by natural stresses (Nguyen and Janssen, 2002). Beside the morphological aberrations, histopathology has been used in sublethal tests in fish ecotoxicology (Ortiz *et al.*, 2001) and can be used to establish toxicity criteria (Ortiz *et al.*, 2001). Although, few ecotxicological studies were carried on embryonic and larval stages of freshwater fish.

Olaifa *et al.* (2003) discussed and describe lead-induced morphological malformations and histopathological changes in embryonic stages of African catfish, *C. gariepinus*. Osman (2007) observed malformations in embryos of the African catfish, *C. gariepinus* in pre-hatching and post-hatching stages after lead exposure. An overall delay in development was reported. Compared to the control embryos

(Fig. 6), morphological and histopathological ((Figs. 7-10)) anomalies in lead exposed embryos were documented.

1. Incubation period and hatching success:

In the control hatching started 40 h after fertilization. The ratio of newly hatched embryos / fertilized eggs was 20 % at the beginning of hatching. The total percentage of hatched embryos / fertilized eggs was 75 % at 48 h post fertilization stage (48h-PFS). Embryos exposed to 100 µg/l lead began to hatch at 40 h post-fertilization (20 % of newly hatched embryos / fertilized eggs). The total percentage of hatched embryos / fertilized eggs was 65 % at 48h-PFS. In the groups exposed to 300 µg/l and 500 µg/l developmental time was prolonged and hatching started at 50 h post-fertilization, with a ratio of 5 % newly hatched embryos / fertilized eggs. The hatching rate was 50 % and 40 % for 300 µg/l and 500 µg/l, respectively. The data indicated that exposure to lead nitrate caused a concentration-dependent delay in hatching and also reduced the percentage of embryos which successfully completed the egg stage period (Osman, 2007).

2. Morphological aberrations in post-hatching stages:

Four major categories of gross morphological abnormalities (irregular head shape, pericardial oedema, yolk sac oedema and notochordal defect) and two minor deformities (finfold defect and reduction of pigmentation) were observed. Some of the affected embryos were recorded with combinations of these abnormalities (Fig 7-10).

2.1. Irregular head shape:

At hatching embryos with an irregular head shape were observed only in the 500 µg /l lead treatment (Fig. 7a, b). The malformed head bears swelling like protrusion on the lateral (Fig. 7a) or anterior (Fig. 7b) side. The malformation was often associated with a branched tail (Fig. 7a) or a collapsed one (Fig. 7b). These malformations were lethal and embryos died shortly after hatching.

2.2. Pericardial oedema:

An enlargement in the pericardial sac or pericardial oedema (Fig. 3c) was observed only in the newly hatched embryos when exposed to 500 µg/l lead. Malformed embryos only survived for few hours (**Osman, 2007)**.

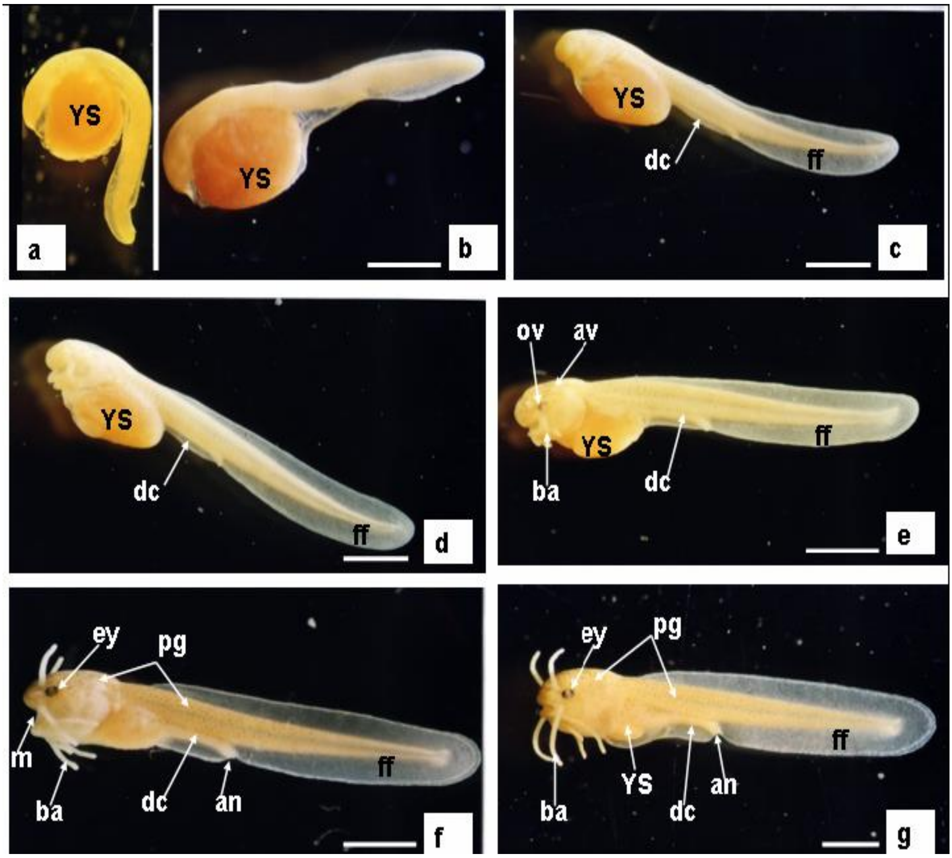

Fig (6): Showed eleutheron-embryonal fixed stages of *Clarias gariepinus* showing (a) newly hatched embryo, (b) 4h-PHS, (c) 24h-PHS, (d) 48h-PHS, (e) 72h-PHS, (f) 96h-PHS, (g) 120h-PHS. ys = yolk sac, ff = fin fold, dc = digestive canal, av = auditory vesicle, op = optic vesicle, ba = barbells, m = mouth, ey = eye, pg = pigments, an = anus, PHS= post hatching stage. Scale bare = 1 mm.

Fig (7): showed deformed newly hatched embryos of *Clarias gariepinus* after exposure to 500 µg/l lead niterate showing (a) embryo with irregular head shape and branched tail, (b) embryo with irregular head shape and collapsed tail (c) embryo with pericardial oedema. YS= yolk sac, S= swelling, PO= pericardial oedema. Scale bare= 1mm.

2.3. Yolk sac oedema:

Yolk sac oedema was observed at 120h and 144h-PFS (Fig. 8a-c) in the groups exposed to 300 and 500 µg/l lead nitrate. Different shapes of yolk sac oedema were observed comprising bag-shape oedema (Fig. 8a), balloon-shape oedema (Fig 8b) and oval-shape oedema (Fig 8c). Malformed embryos were characterized by poorly developed mouth and jaws. Also yolk sac oedema was often associated with notochordal (spinal cord) curvature. Oedematous embryos were usually shorter than the normal ones.

2.4. Notochordal defect:

The most frequently observed gross morphological deformation was a bent body or a notochordal (spinal chord) curvature.

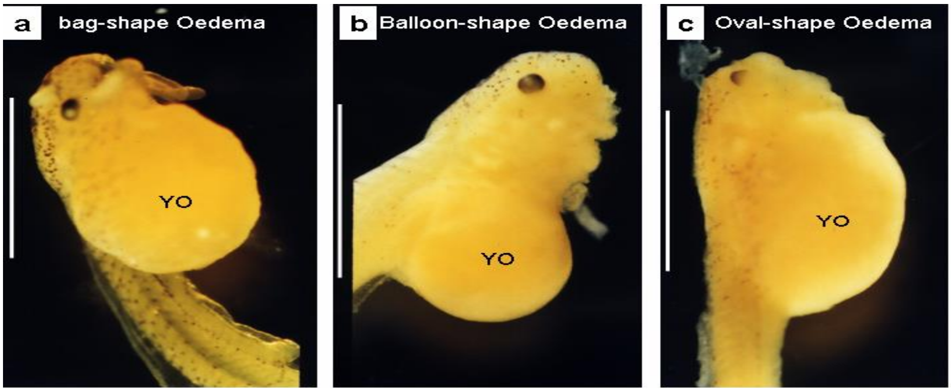

Fig (8): Yolk sac oedema in the embryos of *Clarias gariepinus* after lead exposure (a) bag-shape oedema (144h-PFS exposed to 500 µg/l lead nitrate), (b) Balloon-shape oedema (120h-PFS exposed to 500 µg/l lead nitrate), (c) oval-shape oedema (120h-PFS exposed to 300 µg/l lead nitrate). YO=yolk sac oedema, PFS=post fertilization stage. Scale bare= 1mm.

Fig (9): Showed notochordal abnormality (body curvature) in the embryos of *Clarias gariepinus* after lead exposure (a) lordosis (96h-PFS exposed to 300 μg/l lead nitrate), (b) kyphosis (120h-PFS exposed to 500 μg/l lead nitrate), (c) scoliosis (96h-PFS exposed to 500 μg/l lead nitrate), (d) C-shaped body (96h-PFS exposed to 500 μg/l lead nitrate). PFS=post fertilization stage. Scale bare= 1mm.

2.5. Finfold defects:

Blistering/necrosis of fins was one of the dominant minor deformations (Fig. 10a-c). This abnormality was only observed in these exposition groups (100, 300 and 500 μg /l). In the group exposed to 100 μg/l the blistering was restricted in the posterior part of the tail (Fig 10a).

While the fin was degenerated through the whole tail in the group exposed to 300 µg /l (Fig. 10b). The membranous fin was completely blistered and degenerated in the groups exposed to 500 µg/l (Fig. 10c). The frequency of larvae with finfold defect increased with lead concentration and was often associated with yolk sac oedema and/or notochordal curvature (**Osman, 2007**).

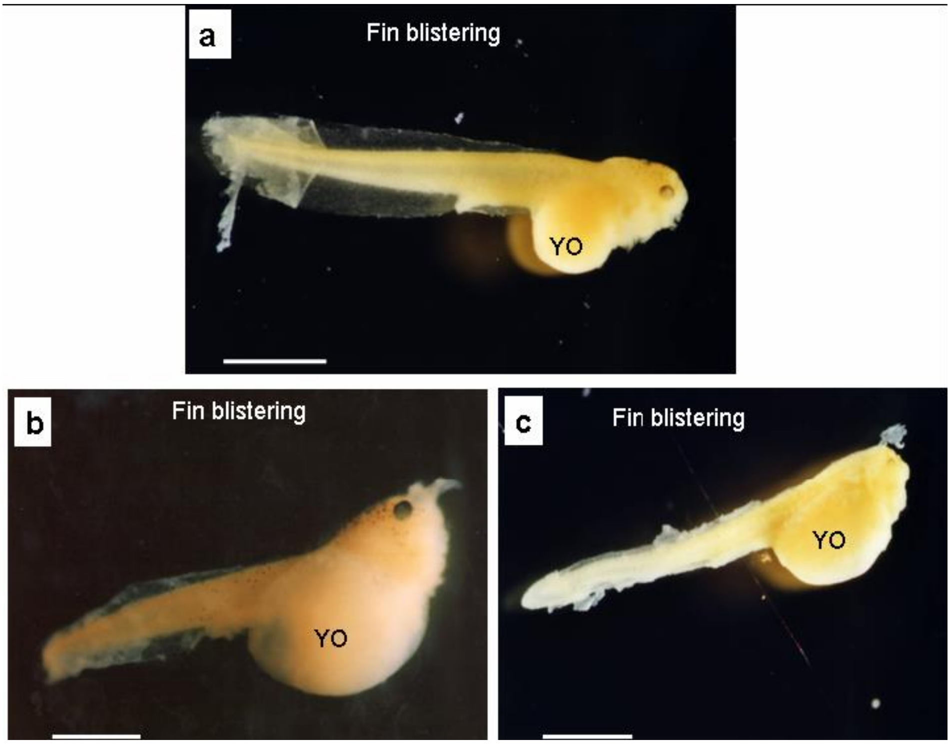

Fig (10): showed fin blistering /necrosis in the embryos of *Clarias gariepinus* after lead exposure (a) blistering of the posterior part of the tail (120h-PFS exposed to 100 µg/l lead nitrate), (b) degeneration of the fin throughout the tail (120h-PFS exposed to 300 µg/l lead nitrate), (c) blistering of the whole fin (120h-PFS exposed to 500 µg/l lead nitrate). YO= yolk sac oedema, PFS=post fertilization stage. Scale bare= 1mm.

Also, **Prane (1999**) showed abnormalities in embryo of rainbow trout (*Oncorhnchus mykiss*) after exposed to heavy metal mixture (Fig. 11) .

Fig (11): Showed morphological effects of HMM on yolk-sac larvae. 1. Control larva (top), 2-5. The malformations of the vertebral axis and retarded yolk-sac resorption

Ovotestes are gonadal tissues with characteristics of both testicular and ovarian structures, and reflect exposure to EDCs that are estrogenic or anti-androgenic. Dissection of fish and gross examination of gonadal tissue for ovotestes is routine. Histological examination of tissue sections for confirmation of ovotestes, as well as for the presence of tumors and apoptosis is useful. Tissue samples for histology can be archived and analyzed at a later date (Fig. 12)

Fig (12): Showed mudsuckers from contaminated sites showed ovotestes where the gonads exhibited both testicular and ovarian tissue (top). Normal females show the typical paired ovaries of equal size (bottom).

Chapter 5: Protect the fish from pollution:

Most of the reference in this review involves Nile tilapia, common carp and cat fish those species are commercially valuable, so much article effort has been expended to increase growth, reduce disease incidence and enhance reproduction. Vitamin E and vitamin C plays important roles in fish health as antioxidants by inactivating damaging free radicals produced through normal cellular activity and from various stressors (Chew, 1995). It has been suggested that the antioxidant function of these micronutrients could enhance immunity by preserving the functional and structural integrity of important immune cells. Ghazaly (1994) reported that ascorbic acid reduced fish mortality, lowered metal content of tissue and prevented the inhibition of blood AST and LDH activities of *Tilapia zillii* after exposure to mercury. Shalaby (2001) and Abdel- Tawwab *et al*. (2004) found that a high level of ascorbic acid (500 mg/kg diet) enhanced the weight gain, specific growth rate and survival rate of Nile tilapia exposed to sublethal dose of mercury. Shalaby (2004) suggests that the level of ascorbic acid (500 mg/kg diet) used in this investigation enhances fish tolerance to environmental stress and reduces ochratoxin toxicity and the probable ameliorative effect of dietary vitamin C on the toxicity of ochratoxin. Abdel- Tawwobe *et al. (*2007). Found that fish Nile tilapia, *Oreochromis niloticus* pre-exposed to 100 mg Ca2+ L−1 for 4 days reduced the harmful effect of Cu against fish, which in turn improves the growth, survival, feed intake and FCR. Also, Ca pre-exposure decreases Cu residues in fish body reducing potential hazardous to human health.

Recently, the removal of metals by synthetic anthropogenic chelating agents has received much attention. The literature reported some chelators that have been used for chelate-induced hyper accumulation (Huang *et al*., 1997). Ethylenediaminetetra acetic acid (EDTA) is the most commonly used chelator due to its strong chelating ability for different heavy metals (Norvell, 1991) The use of EDTA and NTA (nitrilotriacetic acid) has especially been questioned because of their potential for increasing the solubilization and remobilization of heavy metals from aquatic sediments (Muller and Forstner, 1976). Shalaby *et al.* (2006) and Shalaby (2007) concluded that water pollution with chromium and cadmium included significant negative impact in the hematological and biochemical parameters together with marked histopathological alteration impairment of growth and lowering reproductive performance of fish. The addition of EDTA, super phosohate and charcoal could decrease the toxic effects of chromium or cadmium and improve the fish rearing condition and reproduction. Also, Shalaby (2007) found that addition of EDTA to Cd contaminated medium considerably reduced metal absorption and accumulation in fish tissues, while it was increased metals in water and feces the reduction of toxic elements like cadmium in aquatic environments is needed by any acceptable method. The most widely used technique for the removal of toxic elements involves the process of neutralization and metal hydroxide precipitation (Hiemesh and Mahadevaswamy, 1994). Also, Sitohy *et al.* (2006) concluded that adding each of organic peel or rice husk as natural biosorbent materials could be benefit it reducing heavy metals toxicity, which demonstrated in improving fish serum parameter which almost return to control values after applying these materials, in addition to the environmental peneficial of getting rid of a huge amount of wastes.

Suggestion for future research:

Since the knowledge of the physiology of the various stages of fish life cycle is highly essential for successful aquaculture, outmost priority should be accorded for such studies. The researches on protection and reproduction (hormones) especially development of synthetic hormones for breeding, growth and survival are highly essential to make aquaculture economical and affordable by common fish farm. The biotechnology application of hormones for sex control and enhance growth, advanced maturation and multiple breeding requires to be intensified to give new direction to future development in aquaculture system. Studies on the effect of pollutants on gonadal development, larva and adult survival and growth need to be intensified. Physiological mechanisms of cryopreservation of gametes and larva should be taken on priority basis. The results of environmental changes on the physiological mechanisms of aquatic fauna and flora deserve special attention. The studies on the physiology of reproduction, digestion, metabolism, growth and survival and performance need to be counted with vigour for the deeper understanding of the basic mechanisms involved for long term fishery development and nutritional security to the people through higher fish production.

Conclusion and recommendations:

The nature of toxic substances that give rise to chronic poisoning varies ranging from elements, particularly, heavy metals through complex organic and inorganic compounds. Theses substances may be encountered as pesticides, industrial chemicals and pollutants. Generally they constitute a spectrum of substances in a variety of states in a multiplicity of matrices at extremely low concentrations. Thus their ability to bring about pathophysiological changes is not immediately evident. This ensures the early detection of biochemical lesions that are related to subsequent changes in structure and physiology. Thus useful as early indicators of environmental hazards that produce disease in fish and impairment of growth and reproduction.

Acceleration of fish growth and reproduction from other resources of water aquaculture is one of the main objectives of the Egyptian economy. The most important factor that affect fish health, reproduction and production fish is the tremendous amount of wastes and pollutants in the rivers and draining canals in areas of intensive agriculture activities. These harmful substance which contaminates the aquatic environment can be harmful not only by their direct effect on the mortality of the organisms there, but also by causing some disease and development abnormalities. Theses direct and indirect effects may occur more frequently in fish living in these polluted environment, thus with involvement of the environmental stressors as low dissolved oxygen (hypoxia), ammonia and estrogenic activity of pesticides…etc

In all cases the above factors can badly affect not only fish mortality and quality but also fish growth, reproduction and production. Therefore, there is now a real need for more investigations on the interrelationships between the pollution of these environment and their effects on natural fish population, and how we can remove these pollutants from environment.

There are quite a few recommendations and projects by several investigators meant to clean and save the fish farm from the disasters caused by pollution. Although some of these recommendation projects have good impacts on aquaculture, other might have some unfavorable side effect. Following is a review of some recommendation for removing the pollutants from aquaculture and protection the fish from toxic substances. I hope that animal behaviourists will meet this challenge and help to address environmental. As follow:-

(1) "**Pollution evaluations**" to evaluate environmental pollution in waters by:

a. Development of a monitoring system for carcinogenic substances, toxic chemicals and endocrine-disrupting chemicals in the waters, as well as studies on their physiological effects on aquatic organisms.

b. Pollution survey using bio-markers of target organisms and studies of the impact on human bodies.

(2) "**Functional evaluations**" to evaluate food function of organisms to be produced.

a. Detection of new physiologically active substances unique to aquatic organisms.

b. Development of technology for effective use of physiologically active substances.

(3) "**Safety controls**" to ensure safety and reliability

a. Establishment of the HACCP system and traceability including entire process, from fishing to consumers.

Methods of safety control for reducing of pollution:

- Mechanical treatment by means of mechanical screens and aerated grit chamber.

- Biological treatment in a complete mixed activated sludge process performed in an oxidation ditch system.

- D.E. incorporating a selector tank to enhance the formation of non-bulking sludge.

- Sludge treatment by gravity thickening of the aerobically stabilized sludge.

- Dewatering in high-pressure belt presses.

In Agriculture

- In that context, policies with higher prices for chemical and proper information can encourage an appropriate use of fertilizers and pesticides.
- Moreover, using the drip irrigation techniques are recommended where a large source of water is wasted.

In Industry

- Ideally, industries should not reject any waste in the environment.
- In practice, it is a fact that many industrial and agricultural effluents are evacuated into water streams, over their capacity to absorb them.
- Norms and laws exist, defining minimum quality of effluents to achieve before being evacuated in rivers, but in many developing countries, laws are sometimes not constraining and difficult to enforce.
- In that context the main issue is to strengthen create a legal frame, with actual means of control to abate pollution

International Water Managements

- It is necessary to manage water resource with an international vision because the resource ignores boundaries. They can be polluted by acid rains crossing boundaries of other countries.
- In that context, it is necessary to organize a coherent management of water to guarantee that each fundamental functions of water (for health, industry, agriculture, fisheries, navigation and ecosystem) are preserved in a sustainable way.
- This will only be achieved with national policies elaborated in the frame of international cooperation.

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